

Epidemiology of pertussis in children hospitalised with respiratory tract infection

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For he was a shrub among the poplars
Needing more roots
More sap to grow to sunlight,
Thirsting for sunlight,

A low growth among the forest.

Into the soul
The selves extended their branches,
Into the moments of each living hour,
Feeling for audience

Straining thin among the echoes;

And out for the solitude
Voice and soul with selves unite,
Riding the echoes,

Horsemen of the apocalypse;

And crowned with one self
The name displays its foliage,
Hanging low

A green cloud above the forest.

Christopher Ifekandu Okigbo
(Siren Limits II)

Declaration

I, Rudzani Muloiwa, hereby declare that this thesis is my own work, both in concept and execution, apart from the normal guidance received from my supervisors and contributions from others as outlined in the Introduction and the acknowledgement section of each chapter. The assistance I received with study management, data collection, analysis and manuscript review from the co-authors of the publications that form part of this thesis is described for each relevant chapter.

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I present this thesis for examination for the degree of PhD.

Signed:

Signed by candidate

 Dated: 29th December 2020

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Abstract

The availability of an effective vaccine against *Bordetella pertussis* substantially reduced the morbidity and mortality from pertussis, however, in the last decade there appears to have been a substantial increase in pertussis cases as reported mainly in high income countries.

Although it is believed that the greatest burden of pertussis, including deaths, is in low- and middle-income countries (LMICs), there seem to be little data available to back this up.

This thesis set out to find data that will give some insight into the burden of pertussis in a low- and middle-income setting in infants and children with severe lower respiratory tract infection (LRTI). Given the paucity of data in LMICs, the thesis started by systematically searching for existing data that will give some indication of the possible extent of the pertussis problem in these countries. Secondly, a prospective study was conducted at a children's hospital. As hospital admission is a marker of severe disease, these children were targeted as the appropriate population in which to meaningfully conduct a primary study on the burden of pertussis. In addition to quantifying the burden by describing the prevalence of confirmed pertussis in this group of children, the study set out to look for potential factors that may be associated with increased risk of pertussis. LRTI are now commonly known to be associated with identification of multiple organisms in respiratory samples, this study aimed to also look at organisms that are detected with *Bordetella pertussis*; and investigate whether this association was in any way associated with severe disease or negative outcomes. Finally, this study hoped to identify clinical features that could be used to develop a more reliable clinical case definition of pertussis.

Chapter 1 gives a background that justifies the undertaking of this study. In chapter 2 a

systematic review quantifies, using the best available data, the burden of pertussis in LMICs. Chapter 3 clarifies the methods briefly described in the rest of the manuscript. The burden of pertussis due to the two organisms known to cause the disease, *Bordetella pertussis* and *Bordetella parapertussis*, is described in some detail. In both this chapter and the earlier mentioned systematic review (chapter 2), the burden of pertussis is stratified by subgroups to identify potential risk factors. The issue of risk is formally and specifically taken up in the chapter that follows (chapter 5) where potential risk factors are analysed, and the independent impact for some of these factors is established.

The last two results chapters (chapters 6 and 7) deal respectively with the conundrum of finding other respiratory organism in the same specimen with *Bordetella pertussis* and failure to find useful clinical criteria that can help with improved diagnosis of pertussis. While there is no established pattern noted between pertussis and most organisms, a few give signals of being independently associated with *Bordetella pertussis* even if the clinical relevance is not clear at the moment.

In the final chapter of the thesis (chapter 8) I conclude the thesis by making an argument that although there are still knowledge gaps, the thesis gives a clear indication that pertussis remains a serious problem in LMICs especially for some groups that show increased risk of the disease or its severe consequences.

Acknowledgements

Gratitude

To Heather and Greg, the night guides only believed at sunrise

To the duet that hummed incessantly and quietly, "This is not your name"

...and to Mark, Mark who believes that twice-watered cows will calf and give milk

To Mugo and Sizwe and Emmanuel, who sang with me until they were hoarse

Gratitude

To the Physician Partnership Trust

Twice named in a single pulse for the unshattered long hearts in memorium

The pathfinders whose lifting is etched in stone (Maybe not all that is broken turns to dust)

All remembering is indeed honey and gall

To Sanofi with my grandmother's hands,

Her voice straining, not knowing how to cross the ocean:

"You do not live for me"

Gratitude

For the unnumbered gifts of blood and time

Each freely given whilst holding breath

To Chris, Nomawethu and Nezisa, painstakingly counting each ounce of air

Gratitude

To Ngina and Vele for the neglect borne in silence

For the many moons that knew no other life but the distance

To the makers on their bruised knees, chanting my name from infinity to infinity

For the gift of my callused hands: the courage to hold on to my soul

And here I am finally, at the morning-sunset

Each guarded hair accounted for

The yeast is indeed hidden in the dough

Gratitude

Style and abbreviations

The papers that have been included in this thesis comprise manuscripts submitted to both American and British journals. To maintain a sense of consistency through the thesis, all spellings in the manuscripts have been changed to comply with British English spellings, the spelling format most commonly followed in South Africa. The only exception to this has been in the included figures where the text within figures has been retained as submitted to the journals. Although all the journals to which manuscripts have been submitted use Vancouver referencing, they differ slightly in how the style is formatted. To maintain consistency throughout the thesis, a generic Vancouver style template has been adopted and applied throughout. In general, all the manuscripts that contribute to the various chapters have been reproduced in the thesis as submitted to the various journals. Each chapter therefore contains its own relevant literature review and acknowledgements.

Abbreviations:

aP - the acellular vaccine

aRR - adjusted relative risks

ART - antiretroviral treatment

AUC - Area under the curve

CDC - Centre for Disease Control

95% CIs - 95% confidence intervals

DAG - directed acyclic graph

DPT - Diphtheria, Pertussis, Tetanus

EPI - National Expanded Program on Immunisation

GPI - Global Pertussis Initiative

HEU - HIV-exposed uninfected

HICs - High Income Countries

HIV+ - HIV infected

HREC - Human Research Ethics Committee

HUU - HIV-unexposed uninfected

ICH - Institute of Child Health

IQR - interquartile ranges

IS - induced sputum

LMIC - low- and middle-income countries

LRTI - lower respiratory infection

MDI - metered dose inhaler

MeSH - medical subject heading

NHLS - National Health Laboratory Services

NICD - National Institute of Communicable Disease

NP - nasopharyngeal

PCR - polymerase chain reaction confirmed

RCH - Red Cross War Memorial Children's Hospital

ROC - Receiver operating characteristics

RR - relative risks

RTHC - Road to Health Card

UCT - University of Cape Town

WAZ - weight for age Z scores

WHO - World Health Organization

wP - whole cell vaccine

Chapter 1

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Introduction

Epidemiology of pertussis

According to the World Health Organization (WHO), there are more than 20 to 40 million annual cases of pertussis, and 300,000 associated deaths due to the disease; 90% of which are estimated to occur in low- and middle-income countries (LMIC) [1, 2]. Most morbidity and mortality is seen amongst unimmunised or incompletely immunised infants, who have more severe disease and are more likely to have complications.[3] Increasingly, pertussis is also recognised as an important cause of disease in adolescents and adults with waning immunity. Older individuals have less severe disease and fewer complications, but substantial economic costs are associated with unrecognised infection in these individuals who also serve as an important source of infection for non-immune infants. [4]

Pertussis is a notifiable disease in South Africa (SA). Notification may be on clinical suspicion alone and does not require laboratory confirmation, but laboratory tests should be performed where available. At present there is no active surveillance for pertussis and the introduction of such surveillance is not currently a priority. Lack of surveillance is not a uniquely South African problem, most LMICs lack resources for surveillance.[4, 5] Only 60 cases of pertussis were notified to the Department of Health from January 2000 to September 2004, which is likely a substantial underestimate of the true prevalence of disease in South Africa. Anecdotal reports from concerned paediatricians and general practitioners indicate that the incidence of the disease is possibly much higher than the statistics reflect.

26 A retrospective folder review of children at the Red Cross War Memorial Children's
27 Hospital in Cape Town, South Africa revealed that 61 out of 75 (81%) children with
28 polymerase chain reaction confirmed (PCR) pertussis seen between 2008 and 2012 were
29 never notified. [6] These were children who were ill enough to warrant an investigation by
30 the attending physician. The epidemiology of pertussis in children presenting with less
31 severe respiratory disease who do not warrant admission is still unknown and further
32 study needs to be done in this regard.

33

34 **Pathogenesis**

35 Pertussis is an acute, communicable infection of the respiratory tract caused by *Bordetella*
36 *pertussis* and occasionally by *Bordetella parapertussis*. Both organisms are strict human
37 pathogens with most of the burden attributable to *Bordetella pertussis*. Other *Bordetella*
38 species have been known to cause human disease, but it is *Bordetella holmesii* that in
39 addition to *Bordetella pertussis* and *Bordetella parapertussis* has been recognised as a
40 cause of pertussis-like illness. [7] Pertussis is spread by droplets from person to person and
41 patients are infectious from 7 days after exposure to 3 weeks after the onset of
42 paroxysms. [8] The organism does not invade systemically but attach to ciliated epithelial
43 cells of the airways causing ciliastasis, local tissue damage and interference with
44 phagocytic cell functioning. Viscous secretions and sloughed cells may accumulate in the
45 airways and cause obstruction. Complications include apnoea, bronchopneumonia, otitis
46 media, atelectasis, pneumothorax, hypoxic seizures, encephalopathy, feeding difficulties,
47 and vomiting. Pressure-related complications include rectal prolapse, petechiae, hernias,
48 epistaxis, subconjunctival and intracranial haemorrhages. [4]

49

50 Central to the pathogenesis are the many toxins that are produced by the organism. [9] The
51 most important of these is the pertussis toxin that seems crucial in the pathogenesis of

52 severe disease and death.[10] Although *Bordetella pertussis* and *Bordetella parapertussis*
53 cause a clinically indistinguishable disease, the latter does not produce the pertussis
54 toxin.[7]

55

56 **Diagnosis**

57 Diagnosis of *Bordetella pertussis* illness in infants and young children is difficult as
58 clinical presentation is variable and non-specific. Diagnosis is made on the basis of the
59 clinical picture (mild cough, coryza and fever, progressing to paroxysmal cough,
60 precipitated by crying, eating or drinking, “whooping”, vomiting, cyanosis, sweating,
61 prostration and exhaustion).

62

63 In the absence of access to laboratory confirmation, most low and-middle income
64 countries rely exclusively on clinical criteria to diagnose pertussis. The two commonly
65 used diagnostic criteria are those defined by WHO and the Centers for Disease Control
66 and Prevention (CDC). Both WHO and CDC criteria include presence of a cough for at
67 least 14 days characterized by one of paroxysms, inspiratory whoop or post-tussive
68 vomiting.[11] In addition to the three clinical features CDC also includes presence of
69 apnoea in its criteria.[12] Disease presentation may be modified by age, previous
70 immunisation or infection, antibiotic exposure and concurrent infection with other
71 pathogens. As a result, the presentation of pertussis is frequently atypical, especially in
72 very young infants and adults. Supporting laboratory investigations are also important
73 (leucocytosis and lymphocytosis, isolation of *B pertussis* from nasopharyngeal
74 secretions).[13]

75

76 A conference poster presentation of a review of PCR 115 confirmed pertussis cases from
77 the area of Bloemfontein, South Africa, found a much shorter duration of cough (Median

78 6 days, interquartile range 3 -12days) that might not have met most clinical criteria for
79 suspicion of pertussis disease. Lobar pneumonia was a finding in 62% of the patients in
80 this cohort.[14]

81

82 Culture of the organism, which has been regarded as the gold standard, is possible but
83 difficult. Although culture is highly specific, it has poor sensitivity, particularly late in
84 disease. Culture is most likely to be positive during the catarrhal phase (5 to 14 days from
85 the onset of illness).[4] Most cases of pertussis are only recognised once they have a
86 paroxysmal cough. Unfortunately - especially when classical case definitions based on
87 long duration of cough symptoms are employed - the sensitivity of culture is very poor by
88 the time the diagnosis is suspected. Sensitivity is higher in infants than in adolescents and
89 adults and is influenced by quality and timing of specimen collection and laboratory
90 expertise.[4] Specimens should include a properly performed nasopharyngeal aspirate or
91 nasopharyngeal swab, ideally inoculated directly onto a suitable culture medium (e.g.:
92 Regan Lowe medium) at the bedside. The organism is fastidious and grows slowly.
93 Culture plates must be incubated for at least 7 days before reported negative. Culture
94 allows for surveillance of antibiotic resistance and molecular epidemiological typing in
95 outbreak situations.[4]

96

97 PCR has greatly improved the ability to confirm pertussis cases and is increasingly used
98 for diagnosis on clinical specimens. There are many advantages associated with the use of
99 PCR: results are rapid, less dependent on delays in transport and even non-viable
100 organisms may be detected. PCR targets include IS481, the common *Bordetella* target that
101 includes *Bordetella pertussis* and IS1001 for *Bordetella parapertussis*. The qualitative
102 PCR using these gene targets can be performed on a nasopharyngeal swab (Dacron not
103 calcium alginate swabs as the latter may inhibit PCR) and/or aspirate specimens. The

104 IS481 target is also found in other *Bordetella* species such as *Bordetella holmesii* and
105 *Bordetella brochiseptica*. [15] IS481 is therefore not specific to *Bordetella pertussis*. A
106 published study demonstrated that up to 20% of patients initially diagnosed as pertussis
107 using the IS481 target were in fact *Bordetella holmesii* infected [16]. If IS481, a target
108 with high sensitivity is used to screen cases, it remains important to test the positive
109 specimen further for the presence of pertussis toxin promoter gene sequences, as these are
110 specific for *Bordetella pertussis*. [15] Ideally two targets should be used for diagnostic
111 consensus. The sensitivity of PCR is highest earlier in the course of disease and declines
112 with time from the onset of symptoms. PCR is more sensitive than culture and remains
113 positive even once treatment is commenced. It is therefore still useful for persons
114 presenting as late as three weeks since onset of illness. [17]

115

116 In a prior unpublished study [18] to compare a standard nested PCR and a LightCycler-
117 based real-time assay with culture for confirming the diagnosis of pertussis, 48 children
118 presenting to Red Cross War Memorial Children's Hospital with clinical features of
119 pertussis were sequentially enrolled, nasopharyngeal aspirates were collected, and
120 inoculated onto charcoal agar for isolation of *Bordetella pertussis*. The identity of colonies
121 morphologically resembling *B. pertussis* was confirmed by amplification of the pertussis
122 toxin promoter gene (*ptxA-Pr*). A nested PCR assay targeting the IS481 sequence was
123 performed directly on the NPA samples. In addition, a real-time PCR assay for detection
124 of the pertussis toxin promoter gene was performed on all samples. *Bordetella pertussis*
125 was cultured from five (10%) patients. However, the nested PCR assay for IS481 was
126 positive in 31 (65%) patients, and the toxin promoter gene was present in 17 (55%) of
127 these 31. No patient with a negative IS481 assay was culture positive or PCR positive for
128 the toxin promoter. These initial results suggest that PCR is a more sensitive method of
129 detecting *Bordetella pertussis* than culture. But it also shows that *ptxA-Pr*, even though

130 more specific for *Bordetella pertussis*, may lack the requisite sensitivity for case
131 confirmation. In the absence of double assays, PCR for the *IS481* target, even though less
132 specific, may offer the best diagnostic confirmation support for clinically suspected cases
133 of pertussis.

134

135 Serological diagnosis using ELISA is available, quick and easy to perform in the
136 laboratory. As the measured response is uses antibodies against common vaccine
137 components, in patients recently vaccinated, it is not easy to distinguish between acute
138 infection and recent infection on a single serum sample.[19] Acute and convalescent
139 paired sera can be taken and the change in titres used for diagnostic purposes. Serology
140 suffers a number of drawbacks: only anti-PT (pertussis toxin) serology has been well-
141 standardized and validated, and only anti-PT IgG ELISA testing is recommended for use
142 in the diagnosis of pertussis. Appropriate cut-offs have not yet been determined in many
143 instances, making interpretation, especially for unpaired testing, difficult. IgG titres need
144 to be interpreted in the context of a diagnostic cut-off determined by local sero-
145 epidemiological surveys. A recommendation for considering anti-PT IgG titres between
146 50—120 international units per millilitre (IU/ml) as highly suggestive of recent pertussis
147 has been proposed by a collaboration of European laboratories, but this is based on sero-
148 epidemiological surveys in Western Europe.[20] No sero-epidemiological data exists for
149 SA or other countries in Sub-Saharan Africa from which we could infer appropriate cut-
150 off levels; this limits the usefulness of such tests in our setting at present.

151 Direct fluorescent antibody tests for direct antigen detection have poor sensitivity and
152 specificity and should not be relied on.[21]

153

154 The current recommendation from the South African National Institute of Communicable
155 Disease (NICD) is that suspected cases of pertussis in South Africa (SA) should have the

156 following tests performed for detection of *B. pertussis*:

- 157 • ideally: a nasopharyngeal swab or aspirate for PCR detection of *B. pertussis*.
- 158 • a nasopharyngeal swab or aspirate for culture of *B. pertussis* if PCR testing is not
- 159 feasible or available. [22]

160

161 The advantages and disadvantages of the available diagnostic methods are summarised in

162 Table 1 [22].

163

Table 1: Comparison of diagnostic methods for *Bordetella pertussis*

Method	Advantage	Disadvantage
Culture	Highly specific – if positive it confirms the diagnosis. Can be done by most diagnostic microbiology laboratories provided media and SOPs are available for processing. Relatively cheap.	Poor sensitivity – highest in first two weeks (catarrhal phase) and is reduced following treatment. Higher sensitivity for infants than for adolescents and adults. Requires selective media and prolonged incubation (at least 7 days). Ideally culture medium should be inoculated at the bedside.
Molecular techniques (PCR)	Highly sensitive. Can detect <i>B. pertussis</i> DNA even after treatment has commenced and remains positive late in the disease (≤ 3 weeks). Rapid results. At present the recommended diagnostic test of choice if available.	Specificity can be a problem. False positives do occur especially if only a single target PCR is used e.g. IS481. Requires molecular expertise and equipment. Relatively expensive.
Serology	Relatively cheap and rapid test.	Only use of a fast standardized anti-PT IgG ELISA test is used (other antibodies lack sensitivity and specificity); even then, local cut-offs have not been determined. Serology can NOT be used for diagnosis of pertussis in children or adults who have received acellular pertussis vaccine in the previous year, if not longer. Not recommended alone for routine diagnosis.
Direct fluorescent antibody detection (DFA)	Rapid results.	Poor sensitivity and specificity - many false negatives and false positives. Slides are difficult to interpret and prone to reader error. No longer recommended for routine diagnosis.

164

165 Accurate diagnosis of pertussis is important for the timely institution of optimal treatment

166 (a macrolide antibiotic) and infection control measures, especially in-hospital, and for

167 appropriate treatment of household contacts.

168

169 Recent evidence has shown that the finding of a pathogen or pathogens in respiratory

170 specimens does not always indicate a causal relationship. Organisms whose presence used

171 to be regarded as being of pathogenic significance are now found in otherwise healthy

172 subjects using new more sensitive molecular diagnostic tests. This has highlighted the
173 importance of the use of controls in studies looking at aetiological causes of respiratory
174 disease.[23]

175

176 **Pertussis vaccine**

177 The availability of an effective vaccine against *Bordetella pertussis* since the 1940's has
178 substantially reduced the morbidity and mortality from this disease, preventing an
179 estimated 760 000 deaths annually. In many countries the original whole cell vaccine (wP)
180 has since been replaced by various formulations of the acellular vaccine (aP). However,
181 despite adequate vaccine coverage in many parts of the world, pertussis continues to
182 contribute a substantial burden of disease in un-immunised infants and increasingly
183 recognised infection and/or disease in adolescents and adults.[24] In the last decade there
184 appears to have been a substantial increase in pertussis cases amongst immunised
185 populations. The reasons for this are not fully elucidated but are in part due to improved
186 case detection and laboratory diagnostic procedures.[25] Recent evidence indicate that due
187 to the immune responses that aP vaccines induce that involve largely Th2 responses, they
188 may be less effective than wP vaccines that induce Th1 and Th17 responses. In addition,
189 the duration of protective immunity induced by aP vaccines is shorter than that induced by
190 wP.[26] South African infants were routinely immunised with the whole cell vaccine at 6,
191 10 and 14 weeks and boosted at 18 months of age as part of the National Expanded
192 Program on Immunisation (EPI) until April 2009 when this was changed to aP.[21]

193

194 In the Western Cape Province of South Africa, vaccine coverage in 2005 was found to be
195 80%, 77% and 48% for vaccines due by 14 weeks, 9 months and 18 months respectively.
196 Thus, a substantial number of children did not receive their early vaccines, while a large
197 proportion of children did not receive full courses of Diphtheria, Pertussis, Tetanus (DPT)

198 and measles vaccines. Children in the Boland region were significantly less likely to have
199 received vaccines due by both 14 weeks and 9 months compared to those in the Cape
200 Town Metro region.[27] Another study found vaccine coverage rates of 100%, 99% and
201 94% at 6, 10 and 14 weeks respectively in the Paarl area of the Western Cape between
202 2006 and 2008.[28] In another study, vaccine coverage had declined to 53% by the time of
203 the pertussis vaccine booster dose at 18 months.[29] There are not available reliable and
204 recent data – a survey is currently underway to collect this data.

205

206 The relative effectiveness of the vaccine in HIV-infected and HIV-exposed but uninfected
207 infants and children compared to HIV unexposed children is uncertain. In one
208 Cameroonian study, levels of antibodies against pertussis fimbrial antigens were
209 substantially lower in HIV-infected than in HIV-exposed but uninfected children and there
210 was a high risk of low antibody levels in response to the DTwP vaccine in those HIV-
211 infected children with severe immunodeficiency (CD4 T-cell level, <25%).[30, 31] The
212 concentrations of antibodies induced by the DTwP vaccine were lower in HIV-infected
213 children than in uninfected children. Likewise the quality and duration of immunity to
214 pertussis in HIV infected children once they are started on HAART is uncertain.[32] In a
215 cohort study conducted in Khayelitsha, Western Cape Province, South Africa that
216 included a review of antibodies against pertussis, the authors concluded that “Among
217 South African infants, antenatal HIV exposure was associated with lower specific
218 antibody responses in exposed uninfected infants compared with unexposed infants at
219 birth, but with robust responses following routine vaccination.”[33]

220

221 **Surveillance Challenges**

222 Potential obstacles to surveillance include a lack of standardised clinical case definitions,
223 making inter-country comparisons difficult, a lack of accurate diagnostic facilities for

224 confirmation of *Bordetella pertussis* in many developing countries (only two public health
225 laboratories currently offer the PCR diagnostic in South Africa), inadequate recognition
226 and reporting of cases by health care workers, particularly in adults and adolescents, and
227 the fact that passive notification systems significantly underestimate disease burden.

228

229 **Rationale for the study**

230

231 **Hypothesis**

232 We hypothesised that a substantial number of cases of severe childhood acute respiratory
233 infection in a South African hospital were due to *Bordetella pertussis* and *Bordetella*
234 *parapertussis* infection.

235

236 **The aims:**

- 237 1. To determine the burden of pertussis in infants and children with severe LRTI
- 238 2. To determine factors that are associated with increased risk of pertussis in children
239 with severe LRTI.
- 240 3. To determine the prevalence and type of respiratory co-infection in children
241 infected with confirmed pertussis
- 242 4. To develop a reliable clinical case definition of pertussis.
- 243 5. To conduct a systematic review of the epidemiological patterns of confirmed
244 pertussis in low- and middle-income countries since the inception of EPI in 1974.

245

246 **A brief description of the cohort of participants used to answer the aims of the thesis**

247

248 From September 2012 the study recruited children admitted for a lower respiratory tract
249 infection to the acute admission ward of the Red Cross War Memorial Children's Hospital

250 in Cape Town, South Africa. The children were sequentially enrolled to a maximum of
251 four children per day over a full one-year period. Inclusion criteria were WHO-defined
252 age-specific tachypnoea or lower chest indrawing, apnoea. The children were less than 13
253 years (the age limit for children coming to the hospital). Children could only be enrolled if
254 parents were willing to sign informed consent.

255

256 We excluded participants if they had a previous admission to a health care facility in the
257 preceding two weeks. The reason for this was to minimise health care-associated infection
258 as study wanted primarily to assess community acquired pertussis.

259

260 A detailed history and clinical examination were done, especially noting the presence of
261 cough, apnoea, duration of symptoms and use of antibiotics prior to admission. History of
262 HIV exposure, infection and where relevant, antiretroviral treatment (ART) were
263 recorded. Information on immunisation was abstracted from the Road to Health Card
264 (RTHC), and the date and type of each vaccine recorded. The RTHC is a standardized
265 national record for each child.

266

267 HIV testing was done as appropriate for the age of the child and the children's status
268 classified accordingly. Specific descriptions of both testing and classification appear in
269 each chapter where this is relevant.

270

271 Two nasopharyngeal (NP) swabs followed by an induced sputum (IS) specimen were
272 collected from each child and sent to the laboratory for both culture and PCR testing for
273 pertussis. In addition, a multiplex PCR for other respiratory pathogens was also
274 performed. As with HIV, descriptions of specific tests used, and their interpretations
275 appear in each relevant chapter.

276

277 As we also needed to assess the risk of pertussis posed by a close family member carrying
278 *B. pertussis* in their nasopharynx, the caregiver bringing the child was also enrolled for the
279 study. As with the child, the caregiver's previous medical history (including HIV related
280 data) and history of recent symptoms were taken. An NP sample was taken from the
281 caregiver to be likewise tested for pertussis.

282

283 The study enrolled 460 child-caregiver pairs into the study. The data collected from these
284 enrolled participants were used to answer Aims 1 to 4 of the thesis as stated above. Brief
285 composition of the recruited participants is shown in Table 2.

286

Table 2: Baseline characteristics of enrolled participants (N=460)

Children	Frequency n (%)
Age	
< 2 months old	41 (8.9)
≥ 2 months old	419 (91.1)
Median (interquartile range)	7.8 (3.6-17.8) months
Range	3.9 weeks - 12.7 years
Gender	
Female	202 (43.9)
Male	258 (56.1)
Number of samples	
Nasopharyngeal Swabs	460 (100.0)
Induced sputa	454 (98.7)
Caregivers	
Age	
Median (Interquartile range)	28 (24 - 33) years
Range	15 - 52 years
Relationship to child	
Mother	450 (97.8)
Father	2 (0.4)
Grandmother	5 (1.1)
Other	3 (0.7)

287

288

289

290 **Outline of the thesis**

291

292 **General statement on the structure of the thesis**

293 With the exception of Chapter 1, the introduction chapter, and Chapter 7, the conclusion
294 chapter, all the chapters in the thesis take the form of manuscripts that are either
295 published (in the case of Chapter 3) or undergoing peer review in various journals. Each
296 chapter contains its own literature review, methods and discussion sections, each relevant
297 to the specific aim of the thesis addressed by that chapter. As a result, the thesis does not
298 contain standalone literature review, methods, or discussion chapters. The thesis does
299 however contain a systematic review and metanalysis as detailed below. In addition, the
300 thesis has a short conclusion chapter, highlighting the findings of the thesis.

301

302 Each aim is dealt with separately in its own chapter with only the data necessary to
303 answer the aim specified for each chapter utilised as required in each instance. As a
304 result of this approach, numerators and denominators as well as summarised data, are
305 not always the same across all chapters, even when involving the same variables. This is
306 not an error. As an example, Chapter 3 which answers Aim 1 of the thesis includes both
307 *Bordetella pertussis* and *Bordetella parapertussis* confirmed cases in its numerator and
308 all 460 children in its denominator while Chapter 4, which is concerned with the risk of
309 pertussis due to *Bordetella pertussis*, and thus uses only confirmed *Bordetella pertussis*
310 its numerator to allow for comparison with other studies. Similarly, in Chapter 6, the
311 data of the few children above 9 years of age are excluded as they fell outside the ranges
312 of ages being considered for the diagnostic criteria considered in the analyses.”

313

314

315

316 **Chapter 1**

317 The background, rationale and outline of the thesis is presented. In the background,
318 the burden of pertussis is briefly described in the context of its changing epidemiology,
319 diagnostic and notification challenges, as well as vaccine coverage. South African
320 specific data is highlighted, indicting paucity thereof. In addition, a brief summary of
321 the methods and the participants is given. The chapter also includes an outline of the rest
322 of the chapters in the thesis. As each chapter contains its own literature review, the
323 chapter does not contain extensive literature review, but only what is essential to
324 establish grounds for this research.

325

326 **Chapter 2**

327 The chapter contains a formal systematic review on the burden of pertussis in LMICs.
328 The prevalence of pertussis is described stratified by geographic location, diagnostic
329 method, age categories as well as the period over which the cases were detected. The
330 chapter highlights the high case fatality rate in young infants as well as the increased
331 risk of pertussis burden posed by HIV infection and in utero exposure to HIV. The
332 systematic review describes both laboratory-confirmed *Bordetella pertussis* and
333 *Bordetella parapertussis*.

334

335 **Chapter 3**

336 In this chapter, the burden of pertussis in children admitted with acute lower respiratory
337 tract infections as defined by WHO is described. The chapter highlights the shorter
338 duration of symptoms at the time of diagnosis and describes an increased yield in
339 confirmed cases secondary to the use of a second specimen collected following induced
340 sputum. Also noted in this study is association of high risks of pertussis with HIV
341 exposure and infection.

342 **Chapter 4**

343 In this chapter, factors that flagged as potential risk factors in the previous
344 descriptive chapter are taken up and analysed further in more detail. A major
345 finding reported in this chapter is the high risk of confirmed pertussis in children whose
346 mothers have *Bordetella pertussis* isolated from a nasopharyngeal specimen. In
347 addition, the study analyses further the association with both HIV infection as well as
348 in-utero exposure to HIV uninfected children noted in the previous chapter by
349 quantifying the level of risk and establishing independence of risk. Additionally, the
350 study confirms in this African study, the well-known increased risk to pertussis
351 associated with incomplete immunisation, early infancy and poor nutritional status.

352

353 **Chapter 5**

354 The manuscript deals with bacterial and viral co-infections that occur with
355 pertussis. Here we describe the frequency of specific viral and bacterial
356 organism that are found in the lower respiratory tract of children investigated for
357 pertussis. The analysis includes correlating confirmed pertussis with the overall
358 number of coexisting potential pathogens as well as assessing the association
359 between pertussis and specific organisms. The importance of associated
360 respiratory pathogens detected with *Bordetella pertussis* are analysed with
361 respect to severity of respiratory symptoms.

362

363 **Chapter 6**

364 This manuscript assesses the sensitivity and specificity of clinical features compared to
365 PCR as reference standard in the diagnosis of pertussis. The chapter shows the poor
366 diagnostic accuracy of clinical case definitions and the limitation of these in both
367 clinical use and surveillance of pertussis. The addition of lymphocytosis to clinical
368 definitions is shown to be of limited value in improving diagnostic accuracy.

369 **Chapter 7**

370 As all relevant discussions are contained in each manuscript chapter, this is a short chapter
371 that reflects on findings and conclusions of the thesis. The chapter discusses the significance
372 of the findings from the systematic review and the four chapters reporting results from the
373 primary study. The resurgence of pertussis, highlighting the high mortality in young infants
374 and identified risk factors for pertussis are reflect on. The discussion brings into focus the
375 need for more awareness and need for improved diagnosis of pertussis. Finally, the focus
376 falls on the need for improved immunisation programs to control pertussis, especially
377 targeting high risk groups in the population.

378

379 **Appendices:** The following three documents have been appended to the end of the thesis:

380 Appendix A: Informed consent form

381 Appendix B: Ethical approval HREC 371/2011

382 Appendix C: Case Report Form

383

384 **Author contributions to included manuscripts**

385

386 The contributions to the manuscripts have been endorsed by my co-supervisors,
387 Professors Heather Zar and Gregory Hussey. All six manuscripts (published or under
388 review) have been approved by the University of Cape Town (UCT) doctoral degrees
389 board and UCT Vice chancellor as being appropriate for inclusion in the thesis as per
390 UCT policy. Permissions to include these manuscripts have be sought from and granted by
391 each of the co-authors involved in each manuscript.

392

393 **1. The burden of laboratory confirmed pertussis in low- and middle-income countries**
394 **since the inception of the Expanded Programme on Immunisation (EPI) in 1974: a**
395 **systematic review and metanalysis.** Muloiwa R, Kagina BM, Engel ME, Hussey

396 [Under review BMC Medicine]

397

398 Building on the published study protocol, I implemented the literature search strategy,
399 extracted the data and analysed it. B. Kagina assisted with the quality assurance required
400 for a systematic review study as per the study design and protocol. I analysed the data and
401 drafted the first manuscript. M. Engel reviewed the statistical analysis plan and results. G.
402 Hussey supervised all the aspects of the design and in the editing of the manuscript. The
403 final manuscript was approved by all the authors. This manuscript addresses Aim 5 of the
404 thesis.

405

406 **2. Incidence and Diagnosis of Pertussis in South African Children Hospitalised With**

407 **Lower Respiratory Tract**, Muloiwa R, Dube FS, Nicol MP, Zar HJ, Hussey GD. *The*
408 *Pediatric infectious disease journal* 2016; **35**(6): 611-6.

409

410 I did the epidemiology study design for the project, including the analysis plan. G.
411 Hussey sourced the funding for the study. I managed the field data collection supervised
412 by H. Zar. F. Dube did the laboratory analysis of the specimens under supervision of M.
413 Nicol. I did all the data analysis and wrote the first draft of the paper, integrating
414 contributions from the co-authors. H. Zar and G. Hussey co-supervised the writing. All
415 authors provided contributions to the published manuscript. This manuscript talks to
416 Aim 1 of the thesis.

417

418 **3. Impact of HIV status and maternal carriage on risk of childhood *Bordetella***

419 ***pertussis* disease**. Muloiwa R, Dube FS, Nicol MP, Hussey GD, Zar HJ [Under review
420 Plos One]

421

422 I designed the analysis plan to answer the question addressed by this manuscript using

423 the laboratory data supplied by F. Dube under supervision of M. Nicol. I did all the
424 data analysis and wrote the first draft of the paper, integrating contributions from the
425 co-authors. H. Zar and G. Hussey supervised and reviewed the manuscript. All authors
426 provided contributions to the published manuscript. The manuscript answers to Aim 2 of
427 the thesis.

428

429 **4. Co-detection of *Bordetella pertussis* and other respiratory organisms in children**
430 **hospitalised with lower respiratory tract infection.** Muloiwa R, Dube FS, Nicol MP,
431 Hussey GD, Zar HJ [Under review Scientific Reports]
432

433 I designed the analysis plan to answer the question addressed by this manuscript using
434 the laboratory data analysed by F. Dube under supervised by M. Nicol. I did all the
435 data analysis and wrote the first draft of the paper, integrating contributions from the
436 co-authors. H. Zar and G. Hussey supervised and reviewed the manuscript. All authors
437 provided contributions to the published manuscript. This manuscript address Aim 3 of
438 the thesis.

439

440 **5. Diagnostic limitations of clinical case definitions of pertussis in infants and children**
441 **with severe lower respiratory tract infection.** Muloiwa R, Nicol MP, Hussey GD, Zar
442 HJ [Under review Plos One]
443

444

444 I designed the analysis plan to answer the question addressed by this manuscript. I did
445 all the data analysis and wrote the first draft of the paper, integrating contributions from
446 the co-authors. M Nicol reviewed the manuscript while the final supervision was done
447 by H. Zar and G. Hussey. All authors provided contributions to the published
448 manuscript. Aim 4 of the thesis is addressed by this manuscript.

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Chapter 2

1 **The burden of laboratory-confirmed pertussis in low- and middle-income countries**
2 **since the inception of the Expanded Programme on Immunisation (EPI) in 1974: a**
3 **systematic review and meta-analysis**

4
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37

38 **Abstract**

39

40 **Background:** An effective vaccine against *Bordetella pertussis* was introduced into the
41 Expanded Programme on Immunisation (EPI) by WHO in 1974; leading to substantial
42 global reduction in pertussis morbidity and mortality. In Low and Middle-Income
43 Countries (LMICs), however, the epidemiology of pertussis remains largely unknown.
44 This impacts negatively on pertussis control strategies in these countries. This study aimed
45 to systematically and comprehensively review published literature on the burden of
46 laboratory-confirmed pertussis in LMICs over the 45 years of EPI.

47

48 **Methods:** Electronic databases were searched for relevant literature (1974 to December
49 2018) using common and MeSH terms for pertussis. Studies using PCR, culture or paired
50 serology to confirm *Bordetella pertussis* and *parapertussis* in symptomatic individuals
51 were included if they had clearly defined numerators and denominators to determine
52 prevalence and mortality rates.

53

54 **Results:** Eighty-two studies (49 167 participants) made the inclusion criteria. All six
55 WHO regions were represented with most of the studies published after 2010 and
56 involving mainly upper-middle income countries ((n=63; 77%). PCR was the main
57 diagnostic test after the year 2000.

58

59 The overall median point prevalence of PCR-confirmed *Bordetella pertussis* was 11%
60 (Interquartile range, 5-27%), while culture-confirmed was 3% (IQR 1-9%) and paired
61 serology a median of 17% (IQR 3-23%) over the period. On average, culture
62 underestimated prevalence by 85% (RR=0.15, 95% CI, 0.10-0.22) compared to PCR in
63 the same studies.

64 Higher proportions of pertussis were associated with HIV exposure [RR, 1.4 (95% CI,
65 1.0-2.0)] and infection [RR, 2.4 (95% CI, 1.1-5.1)]. HIV infection and exposure were
66 also related to higher pertussis incidences, higher rates of hospitalisation and pertussis
67 related deaths.

68

69 In studies reporting deaths, the case fatality rate was 6.5% (95% CI, 4.0-9.5%). Most
70 deaths occurred in infants less than six months of age.

71

72 **Conclusions:** Despite widespread use of pertussis vaccines, prevalence of pertussis
73 remains high in LMIC over the last three decades. There is a need to increase access to
74 PCR based diagnostic confirmation in order to improve surveillance. Disease control
75 measures in LMICs must take into account the persistent significant infant mortality and
76 increased disease burden associated with HIV infection and exposure.

77

78

79 **KEYWORDS**

80 pertussis, burden, prevalence, incidence, mortality, case fatality, HIV, low and middle-
81 income countries (LMIC)

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90 **Background**

91

92 Pertussis is a highly infectious respiratory illness caused by *Bordetella pertussis* or
93 *Bordetella parapertussis*. The World Health Organization (WHO) estimates that 90% of
94 the 20 to 40 million annual cases of pertussis, and 300,000 associated deaths due to the
95 disease, occur in low and middle-income countries (LMIC) [1, 2]. While there are good
96 surveillance data to support the re-emergence of pertussis in High Income Countries
97 (HICs), the disease trends are unknown in LMICs due to paucity of epidemiological data
98 in these settings [3, 4]. A non-systematic review of available data for the African
99 continent was published recently by the Global Pertussis Initiative (GPI).[5]

100

101 The high HIV prevalence estimates in LMICs coupled with suboptimal vaccines uptake
102 are modifiable risk factors that can fuel pertussis epidemics in these settings [6, 7]. The
103 pertussis resurgence reported lately in HICs, has resulted in the review of disease control
104 strategies in these countries [3, 8]. A review of existing pertussis control programs in
105 LMICs is yet to be undertaken.

106

107 The availability of an effective vaccine against *Bordetella pertussis* since the 1940s has
108 led to a substantial global reduction in the morbidity and mortality caused by pertussis [9].
109 In 1974, WHO included the whole cell vaccine (wP) in the Expanded Programme on
110 Immunisation (EPI) adopted in several countries. Although wP is still widely used in
111 many LMICs, many HICs have replaced wP with various formulations of the acellular
112 vaccine (aP) [10]. Epidemiological data from HICs show that despite high vaccine
113 coverage with aP, the pertussis burden has increased in non-immunised, partially-
114 immunised infants, as well as in previously immunised adolescents and adults [3, 8, 11-
115 13]. The reported pertussis resurgence has been linked to several factors such as reduced

116 efficacy of aP vaccines, genetic evolution of the pertussis bacteria as well as improved
117 diagnosis and reporting of the disease [4].

118

119 A sound understanding of trends in the burden of pertussis is required to assess the impact
120 of current pertussis control strategies as well as to decide on future policy. We conducted
121 a comprehensive systematic review to address the knowledge gap in the longitudinal
122 epidemiology of pertussis in LMICs for the 45 years starting in 1974 to 2018, inclusive.
123 Primarily, our systematic review aimed to review available published literature on the
124 prevalence and/or incidence of laboratory confirmed pertussis in LMICs since the
125 inception of the EPI and to determine the trend in the burden of pertussis in LMICs from
126 1974. For secondary objectives, we sought to determine the mortality and case fatality
127 rates ascribed to pertussis in LMICs as well as to investigate the impact of vaccine choice
128 (wP or aP), and HIV infection and *in utero* exposure on the burden of pertussis in LMICs
129 over the review period.

130

131 **METHODS**

132

133 **Search strategy and criteria for selecting studies**

134 The protocol for the systematic review was registered with PROSPERO International
135 Prospective Register of systematic reviews (<http://www.crd.york.ac.uk/PROSPERO>), with
136 registration CRD42015015159. The methods employed in conducting this review have
137 been previously published [14]. The following electronic databases were searched for
138 qualifying literature: MEDLINE, Scopus, Africa-Wide, PDQ-Evidence, WHOLIS,
139 CINAHL, CENTRAL and Web of Science. Search terms used included “pertussis,”
140 “*Bordetella pertussis*”, “*Bordetella parapertussis*,” and “whooping cough” combined with

141 “burden”, “epidemiology”, “incidence”, “prevalence”, and “case”. These were used
 142 together with the specific names of all LMICs as classified by the World Bank [15, 16].
 143 The search strategy as used in MEDLINE via Pubmed is shown in Table 1. The search
 144 was carried out in April 2015, March 2018 and updated in January and April 2019. The
 145 World Bank groupings reflect the status at last search.
 146

Table 1: Strategy used to search for literature in MEDLINE (Via Pubmed)

Query No.	Search term
#1	Pertussis (MeSH) OR whooping cough (MeSH)
#2	Bordetella pertussis OR B. pertussis OR Bordetella parapertussis OR B. parapertussis
#3	#1 OR #2
#4	Burden OR epidemiology OR incidence OR prevalence OR case*
#5	#3 AND #4
#6	(Afghanistan OR Albania OR Algeria OR American Samoa OR Angola OR Armenia OR Armenian Azerbaijan OR Bangladesh OR Belize OR Benin OR Byelarus OR Byelorussian OR Belarus OR Belorussian OR Belorussia OR Bhutan OR Bolivia OR Bosnia OR Herzegovina OR Hercegovina OR Botswana OR Republic of Botswana OR Brazil OR Brasil OR Bulgaria OR Burkina Faso OR Burkina Fasso OR Upper Volta OR Burundi OR Urundi OR Cambodia OR Khmer Republic OR Kampuchea OR Cameroon OR Camerons OR Cameron OR Camerons OR Cape Verde OR Cabo Verde OR Central African Republic OR Chad OR China OR Colombia OR Comoros OR Comoro Islands OR Comores OR Mayotte OR Congo OR Republic of Congo OR Zaire OR Costa Rica OR Cote d'Ivoire OR Ivory Coast OR Cuba OR Djibouti OR French Somaliland OR Democratic Republic of Congo OR DRC OR Zaire OR Dominica OR Dominican Republic OR East Timor OR East Timor OR Timor Leste OR Ecuador OR Egypt OR United Arab Republic OR El Salvador OR Eritrea OR Equatorial Guinea OR Ethiopia OR Fiji OR Gabon OR Gabonese Republic OR Gambia OR Georgia Republic OR Georgian Republic OR Georgia)
#7	(Ghana OR Gold Coast OR Greece OR Grenada OR Guatemala OR Guinea OR Guinea-Bissau OR Guiana OR Guyana OR Haiti OR Honduras OR India OR Maldives OR Indonesia OR Iran OR Iraq OR Jamaica OR Jordan OR Kazakhstan OR Kazakh OR Kenya OR Kiribati OR Korea OR Kosovo OR Kyrgyzstan OR Kirghizia OR Kyrgyz Republic OR Kirghiz OR Kirgizstan OR Lao PDR OR Laos OR Lebanon OR Lesotho OR Basutoland OR Liberia OR Libya OR Macedonia OR Madagascar OR Malagasy Republic OR Malaysia OR Malay OR Malay OR Sabah OR Sarawak OR Malawi OR Nyasaland OR Mali OR Marshall Islands OR Mauritania OR Mauritius OR Agalega Islands OR Mexico OR Micronesia OR Moldova OR Moldavia OR Moldovan OR Mongolia OR Montenegro OR Morocco OR Ifni OR Mozambique OR Myanmar OR Myanma OR Burma OR Namibia OR South-West Africa OR Nauru OR Nepal OR Nicaragua OR Niger OR Nigeria OR Pakistan OR Palestine OR Papua New Guinea OR Paraguay OR Peru OR Philippines OR Philipines OR Phillipines OR Phillipines OR Romania OR Rumania OR Roumania)
#8	(Rwanda OR Ruanda OR Saint Kitts OR St Kitts OR Nevis OR Saint Lucia OR St Lucia OR Saint Vincent OR St Vincent OR Grenadines OR Samoa OR Samoan Islands OR Navigator Island OR Navigator Islands OR Sao Tome OR Sao Tome and Principe OR São Tomé OR Senegal OR Serbia OR Montenegro OR Sierra Leone OR Sri Lanka OR Ceylon OR Solomon Islands OR Somalia OR Somaliland OR Sudan OR South Sudan OR South Africa OR Republic of South Africa OR Suriname OR Surinam OR Swaziland OR Syria OR Syrian Arab Republic OR Tajikistan OR Tadjikistan OR Tadjikistan OR Tadjik OR Tanzania OR United Republic of Tanzania OR Thailand OR Tuvalu OR Togo OR Togolese Republic OR Tonga OR Tunisia OR Turkey OR Turkmenistan OR Turkmen OR Uganda OR Ukraine OR USSR OR Soviet Union OR Union of Soviet Socialist Republics OR Russian Federation OR Russia OR Uzbekistan OR Uzbek OR Vanuatu OR New Hebrides OR Venezuela OR Vietnam OR Viet Nam OR West Bank OR West Bank and Gaza OR Gaza OR Yemen OR Yugoslavia OR Zambia OR Zimbabwe OR Rhodesia)
#9	#6 OR #7 OR #8
#10	#5 AND #9

147
 148 The search was limited to studies published from 1974, the year that the Expanded
 149 Programme on Immunisation (EPI) was introduced, until December 2018. Titles and
 150 abstracts of the search outputs and references were screened, and the full texts of
 151 potentially relevant articles were independently assessed by two reviewers (RM and BK)

152 using a standardized score sheet. Disagreements on final inclusions were resolved by
153 consensus following discussions involving a third reviewer (GH). Authors and publishers
154 were contacted for full texts not available online or via our collaborative networks.

155

156 Studies were included if the study populations were from LMICs. While the diagnosis of
157 pertussis is largely made on the basis of clinical parameters, it is well-known that clinical
158 presentation may be modified by age, previous immunisation or infection, antibiotic
159 exposure and concurrent infection with other pathogens [9]. This makes the presentation
160 of pertussis frequently atypical, thus requiring laboratory confirmation of cases by
161 serology, culture or polymerase chain reaction (PCR). Therefore, laboratory confirmation
162 by either PCR, culture or paired serological assays was also an inclusion criterion.

163

164 Studies that failed to provide a numerator (number of participants testing positive) or
165 denominator (number of participants tested for pertussis), as well as those that failed to
166 specify the laboratory diagnostic method utilized, were excluded. Studies on sero-
167 epidemiological and laboratory diagnostic methods in the absence of clinical disease were
168 also excluded.

169

170 **Data extraction**

171 The denominator and numerator were extracted from each study to determine prevalence
172 for each diagnostic method. We defined prevalence as proportions with confirmed
173 laboratory diagnosis from all participants suspected and tested for pertussis. *Bordetella*
174 *pertussis* prevalence data were stratified by WHO region, diagnostic method (culture,
175 paired serology or PCR), clinical setting (hospital or population based) and age category
176 of the study participants. Prevalence was further stratified by HIV status, that is HIV

177 infected (HIV+), HIV-exposed uninfected (HEU) and HIV-unexposed uninfected (HUU).
178 HEU was used in reference to infants.

179

180 Incidence data were extracted as reported by the authors.

181

182 The epidemiology of *Bordetella parapertussis* was separately assessed. Data on the type
183 of pertussis vaccine (wP or aP) used, clinical diagnostic criteria (e.g. WHO, CDC, etc.),
184 and the study design were captured.

185

186 Non-English language articles were reviewed and data extracted with the assistance of
187 online translation programs and native speakers [17, 18].

188

189 **Data analysis and reporting**

190 Percentage point-estimates together with their 95% confidence intervals (CIs) were
191 calculated to represent prevalence of laboratory-confirmed pertussis for all outcomes. The
192 Mantel-Haenszel method was used to pool together prevalence data from individual
193 studies using random-effects meta-analysis. Heterogeneity was evaluated both visually by
194 assessing forest plots and formally using the χ^2 -based Q and I² statistics [19]. Where a
195 meta-analysis was not feasible, either because data were too heterogeneous or insufficient
196 to allow for meaningful pooling, narrative reporting was used. Narratively reported
197 frequencies were summarized using medians and interquartile ranges (IQR) of prevalence
198 point estimates and graphically represented using forest-like plots that omitted pooled
199 data. Instead dotted lines were used to indicate where group averages would lie without
200 emphasizing their meaning. The Kruskal-Wallis test was used to compare point
201 prevalence between groups.

202

203 Incidence of pertussis could not be independently estimated as the requisite data was not
204 available. Incidence was narratively reported per 100 000 as reported by the authors
205 themselves.

206 All statistical analyses were done on STATA software version 14 (STATA Corporation,
207 College Station, TX). The command *metaprop_one* was used to generate pooled
208 prevalence forest plots after Freeman-Tukey transformation and *metan*, for comparative
209 effect forest plots showing relative risks (RR) and their 95% CI, respectively.

210 The study utilized the guidelines for reporting systematic reviews as set down by the
211 revised 2009 PRISMA Statement [20].

212

213 **Quality of included studies**

214 An adaptation of the tool developed by Wasserman *et al.*, was used to assess the risk of
215 bias as well as the quality of the included studies [21]. The quality assessment criteria
216 examined specific variables to make judgement on the studies, taking into account
217 methodological aspects discussed by Hoy *et al.* pertaining to internal and external validity
218 of prevalence studies [22].²²

219

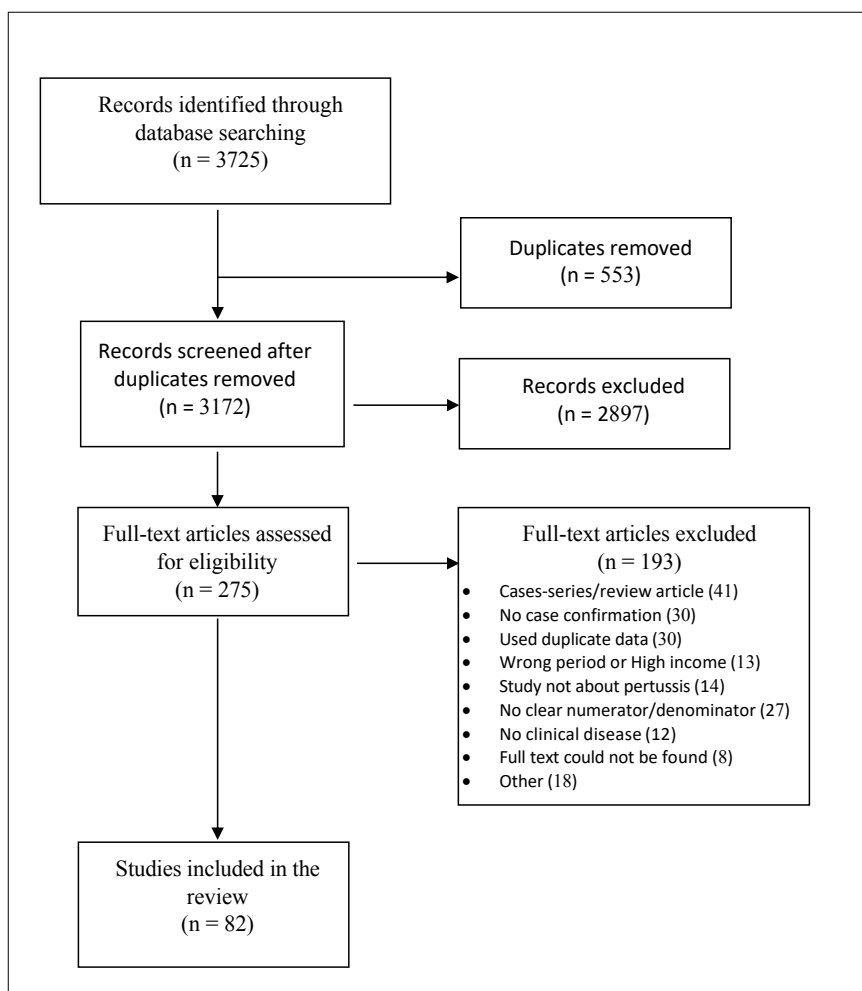
220 **RESULTS**

221

222 **Characteristics of the included studies**

223 The search strategy returned 3 725 studies which reduced to 3172 after excluding
224 duplicates. Following screening of abstracts and titles, 275 articles were deemed
225 potentially relevant and subjected to full-text evaluation. Eighty-two studies (n=49 167)
226 met the final criteria for inclusion into the systematic review; Figure 1. Amongst others,
227 studies were excluded if they did not report clinical cases such as in laboratory studies,
228 animal studies, economic evaluation and modelling studies. The included studies

229 involved symptomatic individuals meeting WHO and CDC (n=52 and n=8 respectively)
 230 clinical criteria. The remaining studies (n=22) used clinical definitions derived from
 231 modifications of the criteria set by WHO or CDC. Two studies were multinational (two
 232 and seven countries in each) so that in the end the final 82 studies included, represented
 233 88 unique populations.



234

235 **Figure 1: Studies included in the systematic review**

236

237 Table 2 shows the characteristics of the included studies by WHO region. A large
 238 proportion of the studies (n=63; 77%) was published between 2010 and 2018. Sixty-eight
 239 studies (83%) involved hospital-based participants. Forty-seven studies (57%) used one
 240 laboratory diagnostic confirmatory test while 32 (39%) and three (4%) studies used two
 241 and three methods for pertussis diagnosis, respectively.

Table 2: Characteristics of studies included in the systematic review

Region & Study	Design	Setting	Diagnosis	Country	Vaccine	Period	Sample (cases)
Africa							
Voorhoeve# (1978)[23]	Surveillance	Population	C, S	Kenya	wP	1974-77	1078 (138)
Ramkissoon (1991)[24]	Clinical trial	Population	S	South Africa	wP	1988	112 (3)
Strebel (1991)[25]	Surveillance	Hospital	C, S	South Africa	wP	1989	34 (3)
Simondon (1997)[26]	Clinical trial	Population	C, P, S	Senegal	wP & aP	1990-95	3619 (193)
Anukam# (2004)[27]	Cross-Sectional	Hospital	C	Nigeria	wP	1997-00	296 (22)
Lassmann (2008)[28]	Cross-Sectional	Hospital	P	Gabon	wP	2003-04	99 (6)
Jusot# (2014)[29]	Cross-Sectional	Hospital	C, P	Niger	wP	2010-11	305 (34)
Kayina (2016)[30]	Cross-Sectional	Hospital	P	Uganda	wP	2013	449 (67)
Barger-Kamate (2016)[31]	Cross-Sectional	Hospital	P	Multinational	wP & aP	2011-14	3451 (52)
Gill (2016)[32]	Cohort	Population	P	Zambia	wP	2015	775 (10)
Hallbauer# (2016)[33]	Cross-Sectional	Hospital	P	South Africa	aP	2008-15	1259 (183)
Muloiwa# (2016)[34]	Cross-Sectional	Hospital	C, P	South Africa	aP	2011-12	460 (41)
Nunes (2016)[35]	Clinical trial	Population	P	South Africa	aP	2011-12	1644 (79)
Soofie (2016)[36]	Cross-Sectional	Hospital	P	South Africa	aP	2015	1839 (42)
Zar (2016)[37]	Cohort	Population	P	South Africa	aP	2012-14	284 (16)
du Plessis# (2018)[38]	Surveillance	Hospital	C, P	South Africa	aP	2013-15	990 (76)
Eastern Mediterranean							
Al-Bargish# (1999)[39]	Cross-Sectional	Hospital	C	Iran	wP	1996	133 (67)
Kakar (2009)[40]	Surveillance	Hospital	C	Afghanistan	wP	2006-07	203 (7)
Ghanaie# (2010)[41]	Cross-Sectional	Population	C, P	Iran	wP	2007-08	328 (27)
Bokhari# (2011)[42]	Cross-Sectional	Hospital	C, P	Pakistan	wP	2005-09	802 (64)
Hajia (2012)[43]	Cross-Sectional	Hospital	P	Iran	wP	2008-11	138 (12)
Mughal (2012)[44]	Cross-Sectional	Hospital	C	Pakistan	wP	2004-06	700 (22)
Zouari# (2012)[45]	Cross-Sectional	Hospital	C, P	Tunisia	wP	2007-11	599 (120)
Bahari (2013)[46]	Cross-Sectional	Hospital	C	Iran	wP	2008-12	156 (7)
Nikbin (2013)[47]	Cross-Sectional	Hospital	C, P	Iran	wP	2009-10	779 (100)
Saffar (2014)[48]	Cross-Sectional	Hospital	P	Iran	wP	2008-12	518 (43)
Sedaghat# (2014)[49]	Cross-Sectional	Hospital	C, P	Iran	wP	2004-08	347 (30)
Benamrouche (2016)[50]	Surveillance	Hospital	C, P	Algeria	wP	2012-13	246 (123)
Ghorbani (2016)[51]	Surveillance	Hospital	P	Iran	wP	2011-13	3629 (239)
Omer (2016)[52]	Surveillance	Hospital	P	Pakistan	wP	2015-16	2021(8)
Katfy# (2017)[53]	Cross-Sectional	Hospital	C, P	Morocco	wP	2013-15	156 (88)
Ben Fraji# (2018) [54]	Cross-Sectional	Hospital	C, P	Tunisia	wP	2007-17	1844 (306)
Dumaidi (2018)[55]	Cross-Sectional	Hospital	P	West Bank	wP	2004-08	267 (130)
Mohammadzadeh (2018)[56]	Cross-Sectional	Hospital	C, P	Iran	wP	20015-16	184 (43)
Europe							
Lukić-Gričić# (1999)[57]	Cross-Sectional	Hospital	C, S	Croatia	wP	1988-94	201(2)
Dağla (2004)[58]	Cross-Sectional	Hospital	C	Turkey	wP	2001-03	66 (2)
Aksakal (2007)[59]	Cross-Sectional	Population	S	Turkey	wP	2004	307 (51)
Yıldırım (2008)[60]	Cross-Sectional	Hospital	P, S	Turkey	wP	2005-06	148 (16)
Medkova (2010)[61]	Cross-Sectional	Unclear	C, P	Russian Fed.	wP	Unknown	172 (81)
Gürsel (2012)[62]	Cross-Sectional	Hospital	C, P, S	Turkey	aP	2009-10	51 (6)
Karlı (2013)[63]	Cross-Sectional	Hospital	C, P	Turkey	aP	2008-12	40 (6)
Uslu (2013)[64]	Cross-Sectional	Hospital	P	Turkey	aP	2012	173 (48)
Dinu (2014)[65]	Cross-Sectional	Hospital	C, P, S	Romania	aP	2012-13	51 (14)
Karagül (2014)[66]	Cross-Sectional	Hospital	C, P	Turkey	aP	2010-11	214 (26)
Öksüz# (2014)[67]	Cross-Sectional	Hospital	C, P	Turkey	aP	2010-13	410 (106)
Aslan (2016)[68]	Cross-Sectional	Hospital	P	Turkey	aP	2013-14	101 (20)
Goktas (2016)[69]	Cross-Sectional	Hospital	P	Turkey	aP	2014-15	845 (15)

Gökçe (2018)[70]	Cross-Sectional	Hospital	P	Turkey	aP	2013-16	172 (44)
South-East Asia							
Singh (1987)[71]	Cross-Sectional	Hospital	C	India	wP	c.1986	560 (20)
Dahiya (2009)[72]	Cross-Sectional	Hospital	C, P	India	wP	2007-07	21 (2)
Barger-Kamate (2016)[31]	Cross-Sectional	Hospital	P	Multinational	wP	2011-14	749 (1)
Das (2016)[73]	Cross-Sectional	Hospital	P	India	wP	2013-14	180 (7)
Siriyakorn (2016)[74]	Cross-Sectional	Hospital	P	Thailand	wP	2010-11	76 (14)
Hughes# (2017)[75]	Cohort	Population	P	Nepal	wP	2011-14	2026 (17)
Chinthate (2018)[76]	Cross-Sectional	Hospital	P	Thailand	wP	2016-17	70 (7)
The Americas							
Cooper (1983)[77]	Surveillance	Hospital	C, S	St Lucia	wP	1981	10 (2)
Baptista (2006)[78]	Cross-Sectional	Hospital	C	Brazil	wP	2003	287 (51)
Kowalzik (2007)	Cross-Sectional	Hospital	P	Multinational	wP & aP	2001-4	181 (19)
Sandoval (2008)[79]	Cross-Sectional	Population	P	Mexico	wP	2002-03	61 (20)
Nieto Guevara (2010)[80]	Surveillance	Hospital	C, P	Panama	wP & aP	2001-08	759 (178)
Astudillo (2011)[81]	Cross-Sectional	Hospital	C, P	Colombia	wP	2006-07	133 (45)
Leite (2012)[82]	Surveillance	Hospital	C	Brazil	wP	2006-08	652 (132)
Ferronato (2013)[83]	Cohort	Hospital	C, P	Brazil	wP	2009-12	57 (25)
Ochoa-Perez (2014)[84]	Surveillance	Hospital	C, P	Mexico	aP	2011-12	147 (59)
Vaz-de-Lima (2014)[85]	Surveillance	Hospital	P	Brazil	wP	2009-12	503 (66)
Castillo (2015)[86]	Cross-Sectional	Hospital	P	Peru	wP	2010-12	392 (155)
Pavic-Espinoza (2015)[87]	Cross-Sectional	Hospital	P	Peru	wP	2009-10	596 (114)
Pimentel (2015)[88]	Cross-Sectional	Hospital	C, P	Brazil	wP	2010-11	192 (10)
del Valle-Mendoza (2015)[89]	Cross-Sectional	Hospital	P	Peru	wP	2010-13	133 (51)
Bailon# (2016)[90]	Cross-Sectional	Hospital	C, P	Peru	wP	2012	840 (191)
Aquino-Andrade# (2017)[91]	Cross-Sectional	Hospital	P	Mexico	aP	2011-14	286 (192)
Phadke (2018) # [92]	Cross-Sectional	Hospital	P	Guatemala	wP	2009-12	301 (11)
del Valle-Mendoza (2018)[93]	Cross-Sectional	Hospital	P	Peru	wP	20016-17	88 (18)
Western Pacific							
Ong (1978)[94]	Cross-Sectional	Hospital	C	Malaysia	wP	1974	65 (1)
Lin (2010)[95]	Cross-Sectional	Hospital	C, P	China	wP	2008-09	1001 (99)
Mi (2013)[96]	Cross-Sectional	Hospital	P	China	wP	2011-12	176 (51)
Ting (2013)[97]	Cross-Sectional	Hospital	C, P	Malaysia	wP	2011	707 (275)
Huang (2014)[98]	Surveillance	Population	P	China	wP & aP	2010-12	1022 (113)
Liu (2014)[99]	Cross-Sectional	Hospital	C, P	China	wP	2013	148 (101)
Wang (2014)[100]	Cross-Sectional	Hospital	C, P	China	wP & aP	2012-13	313 (122)
Hu (2015)[101]	Cross-Sectional	Hospital	P	China	aP	2013-14	2536 (247)
Moriuchi (2017)[102]	Cross-Sectional	Unclear	P	Cambodia	wP	2008-16	651 (82)
Sadiasa (2017)[103]	Cross-Sectional	Hospital	P	Philippines	wP	2012-15	1152 (34)

NB. "Sample" refers to all individuals tested for pertussis in each study; C=culture; P=polymerase chain reaction, S=paired serology; wP= whole cell vaccine; aP=acellular vaccine; # includes *Bordetella parapertussis*

242

243 Study designs included three clinical trials, four cohort, 62 cross-sectional and 13

244 surveillance studies. There were 71 studies published in English, four in Mandarin, three

245 each in Spanish and Turkish, and one in Persian. Three studies by Strebel et al, Al-Bargish

246 et al, and Cooper and Fitch, were conducted in outbreak settings [25, 39, 77].

247

248 In total, the studies originated from 37 countries, representing all six WHO regions;
249 Additional file 1. Nineteen (51%) of the countries represented were upper-middle income,
250 while 11 (30%) and seven (19%) were lower-middle and low-income countries,
251 respectively. Five countries contributed 28 (49%) of the studies (Turkey=11, Iran=9,
252 South Africa=8, China=6 and Brazil=6). Sixty-four (78%) studies had epidemiological
253 data for *Bordetella pertussis* only while 18 (22%) studies investigated for both *Bordetella*
254 *pertussis* and *Bordetella parapertussis*. The most frequently used vaccine over the period
255 the included studies were conducted was wP in 72/88 (82%) settings either on its own
256 (n=66) or in combination with aP (n=6). In 16 (18%) settings aP was the only vaccine in
257 use.

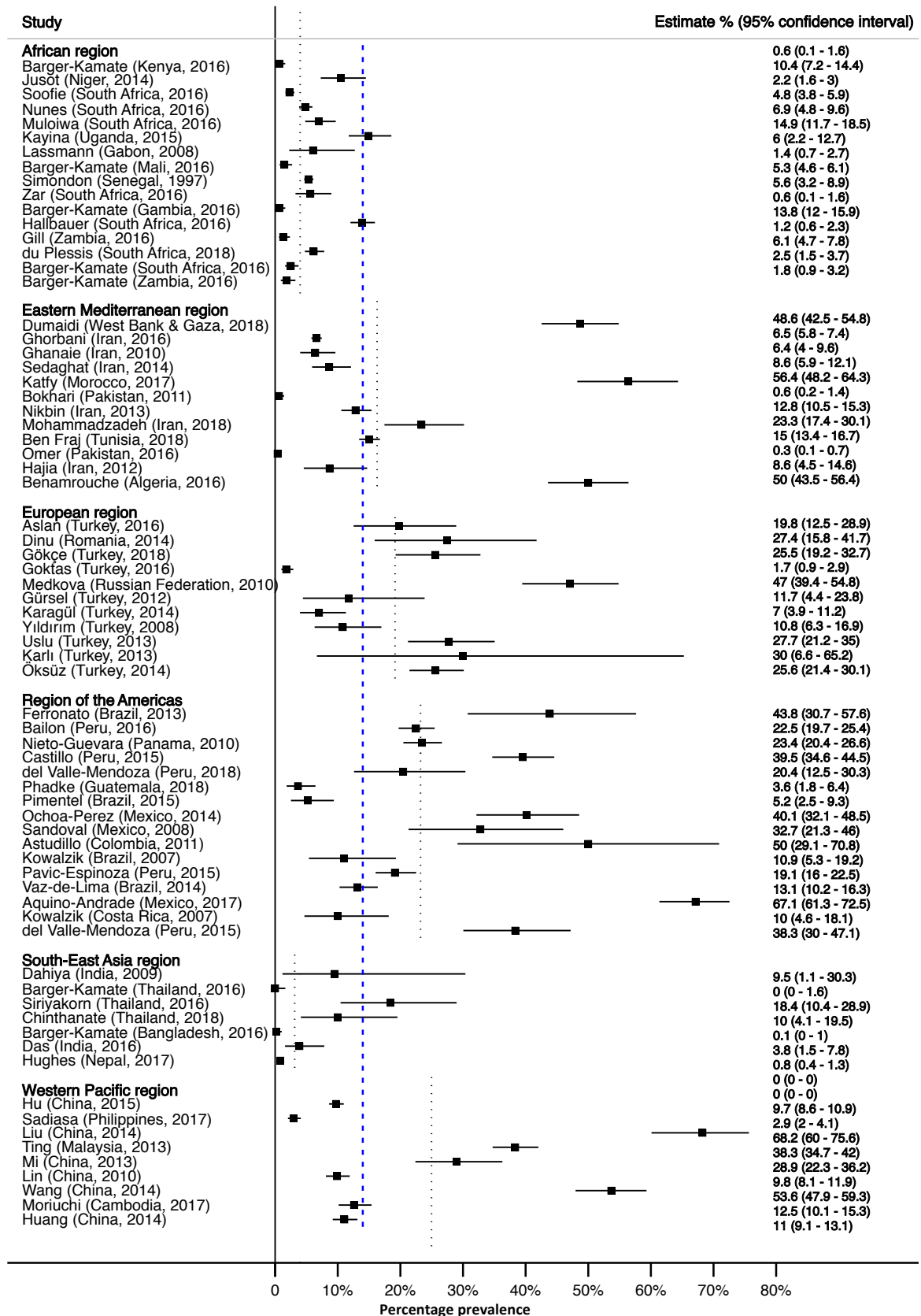
258

259 Data from a study by Zouari *et al.* was considered only for the purpose of estimating
260 mortality but not for estimation of disease burden as its data overlapped with that of Ben
261 Fraj *et al.* who reported cases over a longer period but did not report on mortality [45, 54].

262

263 **Prevalence of pertussis**

264 The median prevalence of PCR- confirmed disease due to *Bordetella pertussis* was 11%
265 (IQR, 5-27%; n=43 696, 64 studies). Figure 2 and Additional file 2. PCR prevalence
266 differed across WHO regions ranging from a median of 4% (IQR 4-10%) in South-East
267 Asia to median of 22% (IQR 12- 40%) in the Region of the Americas; P=0.001. In one
268 multinational study, conducted in countries in the Africa and South-East Asia regions,
269 Barger-Kamate *et al.*, found an increased risk for pertussis in African countries with an
270 adjusted odd ratio of 8.8 (P = 0.03)[31].

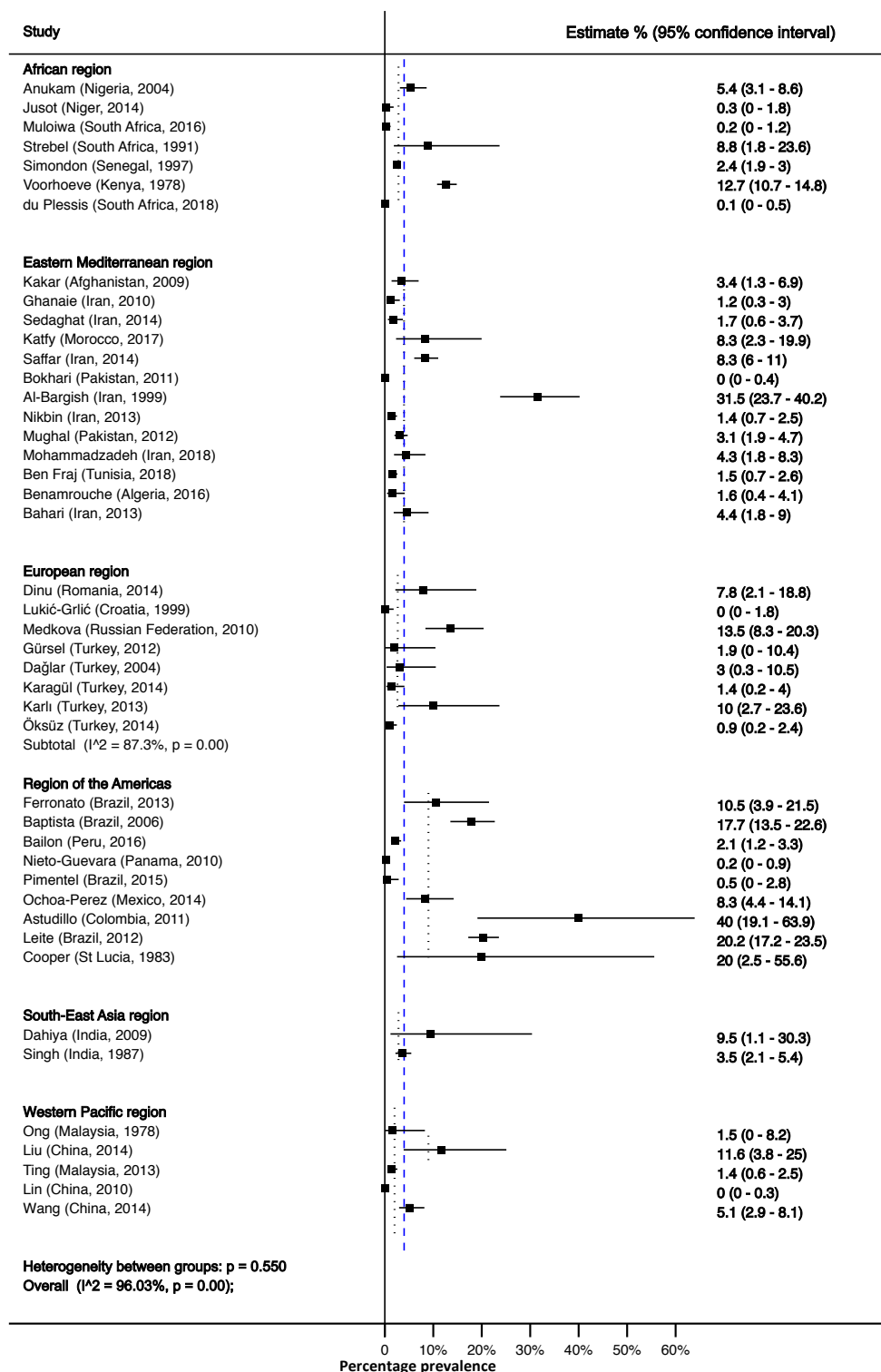


271

272 **Figure 2. Prevalence (proportion testing positive) of polymerase chain reaction**
 273 **confirmed *Bordetella pertussis*** Dotted lines show subgroup and whole group average
 274 estimates
 275

276 The median prevalence of culture-confirmed *Bordetella pertussis* was 3% (IQR 1-9%);
 277 n=18 868, 44 studies). The point prevalence was similar across WHO regions; P=0.1380.

278 Figure 3 and Additional file 2.



279

280 **Figure 3. Prevalence (proportion testing positive) of culture confirmed *Bordetella***
 281 ***pertussis*.** Dotted lines show subgroup and whole group average estimates.

282 Confirmation of *Bordetella pertussis* using paired serology showed a median of 17% (IQR
283 3-23%; n=4 912, 9 studies). Only three WHO regions were represented and median
284 prevalence was 3% (IQR 3-13%) in the African region and 17% (95% CI, 13-26%) in the
285 European region, while the one country, St Lucia, representing the Region of the
286 Americas had a prevalence of 44% (95% CI, 14-79%); P=0.1309. Additional file 2 and
287 Additional file 3.

288

289 The prevalence of confirmed *Bordetella parapertussis* infection using any of the three
290 confirmatory methods was 1% (IQR, 0-2%; n=12 062, 18 studies). The Eastern
291 Mediterranean region was noted to have the highest prevalence with a median 2% (IQR 0-
292 7%) with one study from the same region having a prevalence of 19% (95% CI 13-
293 26%),[39] . Additional file 4.

294

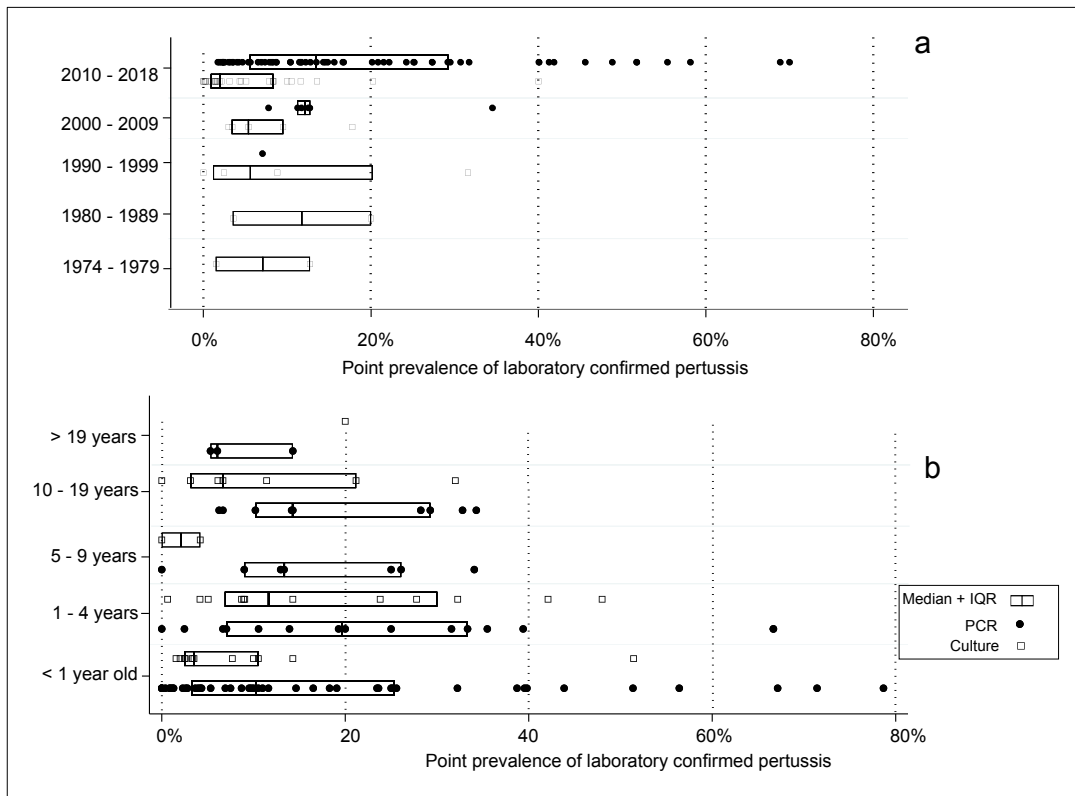
295 Prevalence of *Bordetella pertussis* differed in the same population depending on the
296 method of laboratory confirmation used. On average, culture underestimated prevalence
297 by 85% (RR=0.15, 95% CI, 0.10-0.22) compared to PCR in the 29 (n=14 315) studies that
298 used both methods. (Additional file 5).

299

300 Pertussis prevalence declined in the 1990's from the levels seen in the 70's and 80's. A
301 slight increase was noted since the period after 2000. Figure 4a. Huang *et al.*, reported a
302 26-fold increase in confirmed adult pertussis between 2010 and 2014 in China [98]. There
303 was sufficient information in 48 studies to estimate age prevalence by age group. The
304 lowest prevalence was noted in individuals older than 19 years with median prevalence of
305 6% (IQR 5 -14%). After the high prevalence noted below five years of age, the risk
306 declined in late childhood (6 to 10 years) but increased again in adolescents who showed

307 the highest prevalence of all groups with a median prevalence of 20% (IQR 14 - 32%).

308 Figure 4b.



309

310 **Figure 4. Distribution of point prevalence (proportion testing positive) of polymerase**
311 **chain reaction and culture confirmed pertussis by period (a) and age group (b)**
312

313 Pertussis prevalence was also stratified by the study setting (hospital versus population-
314 based) as well as by the type of vaccine used in settings where the included studies were
315 conducted. The prevalence of pertussis in hospital-based studies had a median of 10%
316 (IQR 4-25%) compared to population-based studies which reported median prevalence of
317 6% (IQR 3-13%). There was an overlap in the distribution of prevalence of confirmed
318 pertussis in populations using aP only [Median 10% (IQR 6-28%)] compared to
319 populations using some wP [Median 10% (IQR 3-20%)]. The overlap remained even
320 when prevalence was stratified by diagnostic method. In the only included clinical trial
321 comparing the two vaccine types, Simondon *et al.*, found aP vaccine efficacy against all
322 confirmed pertussis to be 85% (95% CI, 66-93%) and that of wP to be 96% (95% CI, 86-
323 99%)[26].

324 **Incidence of pertussis**

325 Population level incidence rates of pertussis and hospitalisation were reported by some of
 326 the authors, but these could not be independently verified as the requisite population data
 327 were not available; Table 3. In addition, a majority of the authors reported point estimates
 328 incidences with no confidence intervals. Where data was available for different age groups
 329 [Voorhoeve *et al*, Nieto Guevara *et al*, Saffar *et al* and Ochoa-Perez *et al*] the incidence
 330 was always highest in infancy.[23, 48, 80, 84] The highest incidence of 15 900/100 000
 331 was reported in Kenyan infants between 1974 and 1977 before the introduction of wP.
 332 [23] In addition to *Bordetella pertussis* incidence, Ghanaie *et al* reported a separate
 333 incidence of 2 per 100 000 for *Bordetella parapertussis*.[41] Contacts were reported to
 334 have an incidence of 0.69/100 000 in Benamrouche *et al*'s study.[50].

335

Table 3: Population and hospitalisation incidence rates of *Bordetella pertussis*

Study (Year)	Incidence	Age ranges	Country
Voorhoeve (1978)[23]	3800/100 000	All ages	Kenya
Strebel# (1991)[25]	187/100 000	6 months to 5 years	South Africa
Simondon (1997)[26]	119/100 000	2 months to 15 years	Senegal
Sandoval (2008)[79]	500/100 000	12 years to 15 years	Mexico
Ghanaie (2010)[41]	318/100 000	6 years to 14 years	Iran
Nieto Guevara# (2010)[80]	144/100 000	All ages	Panama
Uslu (2013)[64]	0.9/100 000	< 5 years	Turkey
Huang (2014)[98]	23.52/100 000	All ages	China
Jusot (2014)[29]	820/100 000	< 5 years	Niger
Ochoa-Perez (2014)[84]	2.3/100 000	0 year to > 18 years	Mexico
Saffar (2014)[48]	4.92/100 000	0 month to 25 years	Iran
Benamrouche (2016)[50]	1.04/100 000	All ages	Algeria
Gill (2016)[32]	520/100 000	< 1 year	Zambia
Muloiwa# (2016)[34]	526/100 000	< 13 years	South Africa
Omer (2016)[52]	247/100 000	< 1 year	Pakistan
Soofie (2016)[36]	220/100 000	< 1 year	South Africa
Giayetto (2017)[104]	4.53/100 000	All ages	Argentina
Ben Fraj (2018)[54]	134/100 000	< 5 years	Tunisia

Incidence represents hospitalisation rates for pertussis

336

337 Time-denominated rates were recorded for three community-based studies. Gill *et al.*
338 reported a rate of 2.4 (95% CI, 1.2-4.2) cases per 1000 infant-months with the highest rate
339 of 3.5 cases/1000 infant-months noted between birth and six weeks while Hughes *et al*
340 reported a rate of 13.3(95% CI, 7.7-21.3) cases per 1000 infant-years.[32, 75] Nunes *et*
341 *al.*'s data gave an overall rate of 4.9 per 1000 person-months, which differed between
342 infants (5.7/1000) and mothers (4.3/1000) as well as by HIV status as reported below.[35]

343

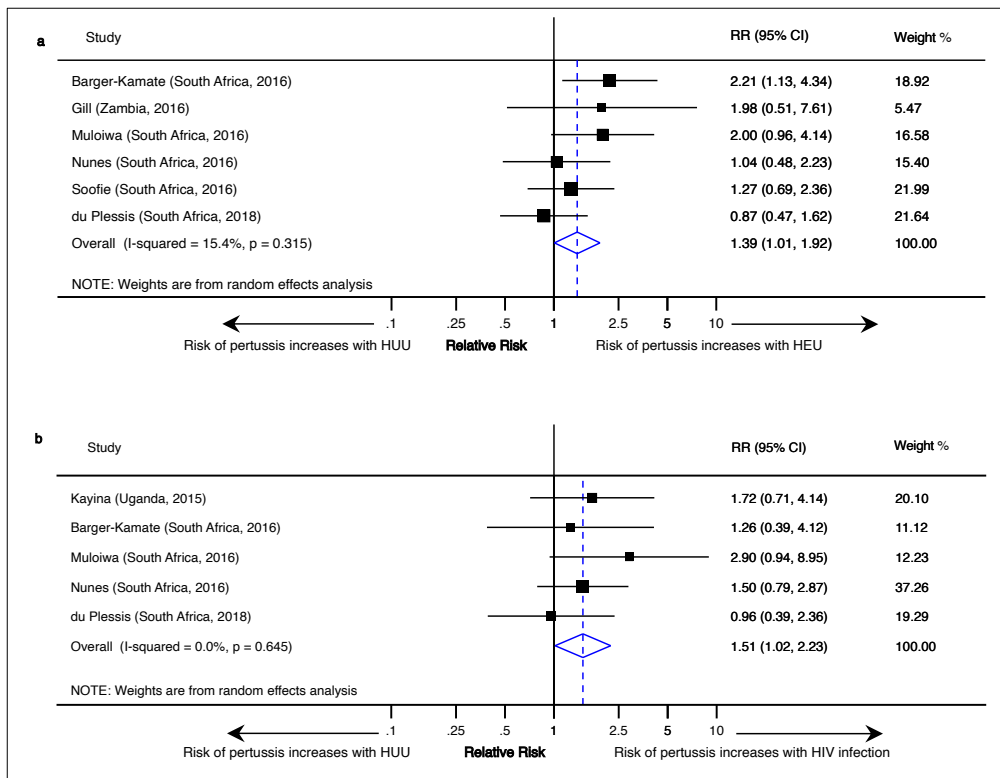
344 **Risk of pertussis in HIV exposed and infected**

345 Ten studies, all from the Africa region, investigated the impact of HIV status on the risk
346 of pertussis. The incidence rate of pertussis was 7.4/1000 infant-months in HEU infants
347 and 5.5/1000 in HUU infants in the study by Nunes *et al.* while the rates in HIV+ and HIV
348 uninfected mothers were 6.8 and 3.9/1000 respectively.[35] Gill *et al.*, reported RR 1.8
349 (95% CI, 95% CI 0.5 - 6.9) in HEU infants compared to HUU. The incidence of
350 *Bordetella pertussis*-associated hospitalisation was 2.9 (95% CI, 1.8-4.5) and 1.9 (95% CI,
351 1.3-2.6) per 1000 in HIV-exposed and HIV-unexposed infants, respectively in a study by
352 Soofie *et al.* The reported 4.8% case fatality rate in Soofie *et al* was only due to deaths in
353 HIV-exposed infants.[36] In the study by Hallbauer *et al.*, there was insufficient data to
354 estimate stratum specific rates, but HIV+ cases, who made 14% of the study sample,
355 accounted for 22 (19%) of the 113 pertussis cases with known HIV status.[33] A gradual
356 increase in risk of pertussis was reported in a study by Muloiwa *et al* in which the risk of
357 pertussis was 5.4% in HUU, 10.9% in HEU and 15.8% in HIV+.[34]

358

359 There was sufficient data to do a metanalysis comparing risk of pertussis in HUU with
360 HEU and HIV+ in six and five studies, respectively. Compared to HUU, HIV+ and HEU
361 individuals had a RR 1.51 (95% CI, 1.02-2.23) and RR 1.4 (95% CI, 1.01-1.92) for
362 confirmed pertussis, respectively. Figure 5. The highest risk of pertussis was reported by

363 Anukam *et al.*, in a cohort of wP vaccinated HIV infected adolescents who were not on
 364 antiretroviral therapy with RR 22.8 (95% CI, 6.9-75.1).[27]. This study was not included
 365 in the HIV metanalysis as it was an obvious outlier composed of individuals not on
 366 treatment which seemed to show excessive risk for pertussis. All other studies involved
 367 HIV+ individuals on antiretroviral therapy.

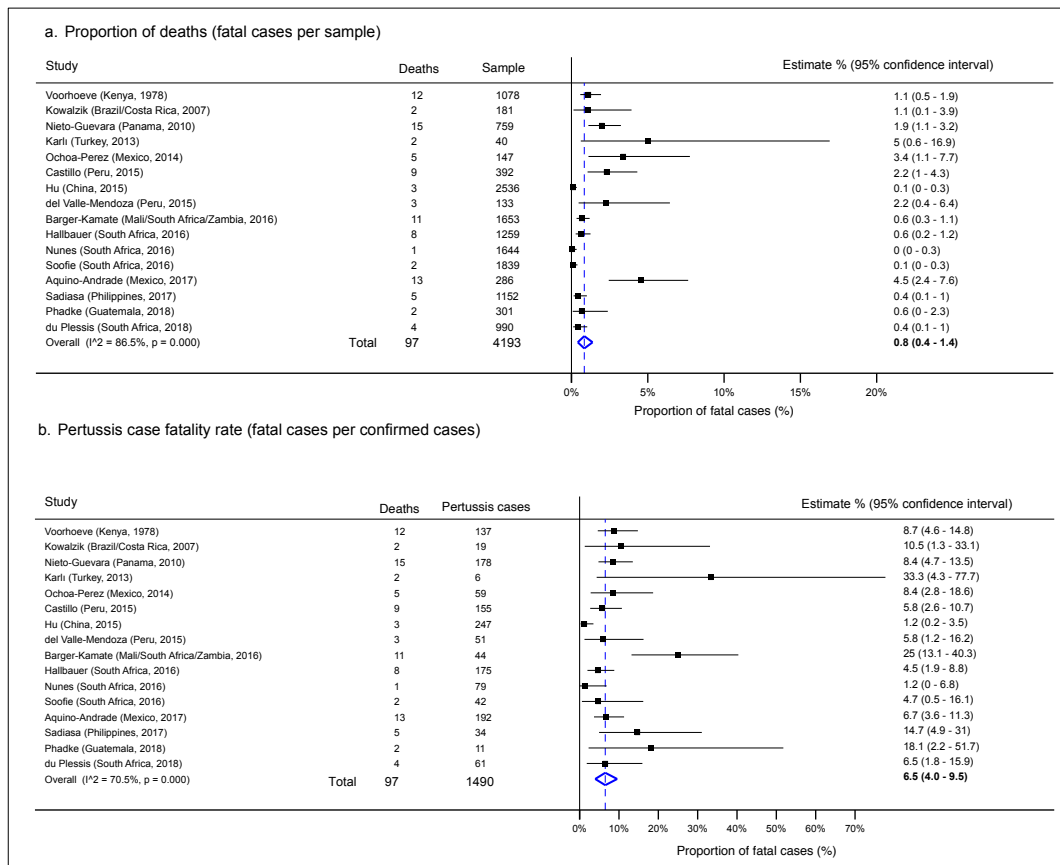


368
 369 **Figure 5. Meta-analysis of the relative risk of pertussis comparing HIV unexposed uninfected**
 370 **(HUU) to HIV exposed uninfected (HEU) (a) and HIV infected (b)**
 371

372 **Deaths and case fatality rate of pertussis**

373 A total of 97 pertussis related deaths out of 1490 confirmed cases were reported in 16
 374 studies (n=14390) representing 13 countries. All deaths were associated with *Bordetella*
 375 *pertussis* with none attributed to *Bordetella parapertussis*. From the 16 studies, the overall
 376 proportion of deaths was 0.8 % (95% CI, 0.4-1.4%) with a pertussis case fatality rate of
 377 6.5% (95% CI, 4.0-9.5%). Figure 6. When only infants were considered (13 studies), the
 378 case fatality rate was 7.2% (95% CI, 3.6-11.8 %) in the studies reporting deaths.

379



380

381 **Figure 6. Mortality and case fatality rate of confirmed pertussis**

382

383 All children who died were younger than five years and the majority were younger than
 384 six months of age. Almost all deaths occurred under one year of age with only one study
 385 (Voorhoeve *et al*, 1978) reporting pertussis deaths after the second year of life (n=5).[23]

386

387 **Quality of the included studies**

388 Using the modified tool published with the protocol for this systematic review, we found
 389 all the included studies to be of high quality.[14] This is because components of the
 390 quality index score, such as laboratory confirmation and availability of raw denominator
 391 and numerator data formed part of the inclusion criteria; which automatically excluded
 392 poor quality status. Similarly, studies had moderate to low risk of attrition and selection
 393 bias.

394

395 **Discussion**

396 This study comprehensively reports on the burden of confirmed pertussis over a 45-year
397 period (1974-2018) in LMICs. This period starts in 1974 with the inception of EPI.
398 Prevalence of confirmed pertussis disease differed in the same study depending on the
399 method of laboratory confirmation with PCR showing the greatest diagnostic sensitivity as
400 expected.[4] Most cases were due to *Bordetella pertussis*. *Bordetella parapertussis* was
401 less common and did not have any reported fatalities associated with it. The study
402 indicates that pertussis deaths are significantly high in LMICs with a disproportionate case
403 fatality rate in young infants. Secondly, the metanalysis shows that HIV has a significant
404 impact in the burden of pertussis in settings where the burden of HIV is high.

405

406 Not surprisingly, the findings from our study agree with those from some HICs: the
407 highest incidence of pertussis was in infants and the greatest pertussis-specific mortality in
408 children younger than 6 months.[105-107] Moreover, we also noted the increase in the
409 prevalence of pertussis in adolescents similarly described in highly vaccinated cohorts
410 from HICs.[108-110] The noted decline in adulthood may indicate protection following
411 the natural boosting in adolescence. Worryingly, the pooled case fatality rate of nearly 6%
412 exceeds the less than 4% estimated by WHO for developing countries.[2] As noted this is
413 even higher when only infants are considered.

414

415 Prevalence data presented in this systematic review suggests that LMICs may also be
416 experiencing a resurgence of pertussis as noted in HICs. Both the GPI and WHO advocate
417 for the strengthening of surveillance systems as a key component in the control of
418 pertussis.[108, 111] Currently, surveillance of pertussis in LMICs is suboptimal. As a

419 result, there are many gaps in accurate pertussis epidemiological data which we observed
420 in this study. The review indicates that the choice of laboratory case-confirmation
421 influences the quantification of pertussis disease burden within the same setting. This is an
422 important finding that suggests that use of PCR to confirm pertussis should be prioritized
423 in LMICs. The higher sensitivity of PCR is more likely to capture the true burden of
424 pertussis and give a better understanding of the global epidemiological pattern of the
425 disease across different settings than any other method. In contrast, culture, recognized as
426 the diagnostic gold standard missed on average 85% of cases identified by PCR in the
427 studies that used both methods in this systematic review.

428

429 A number of authors reported incidences although population denominators could not be
430 independently verified as these went largely unreported. The rates were quite high
431 compared to HICs and showed no pattern of decline over the period in which the
432 incidences were reported.

433

434 An unexpected finding was the significant overlap in the prevalence of pertussis noted
435 with different vaccines in use. Most LMICs use wP vaccines in contrast with the
436 predominant use of aP vaccines in HICs. In general wP vaccines are regarded as offering
437 better protection against pertussis.[112] Despite the predominant use of wP in the
438 reviewed studies, we noted a steady increase in confirmed pertussis since in studies
439 reporting after 2000. This suggests that the observed resurgence of pertussis noted in HIC
440 may only be partly explained by the change of vaccine from wP to aP in these countries.
441 Another possible explanation for the increase may be the increase in the use of PCR for
442 case confirmation – all the included studies conducted after 2010 used PCR as the primary

443 method of confirming cases. The increase in the observed prevalence coincides with the
444 use of these molecular techniques.

445

446 Although data are limited, there is strong evidence from the metanalysis showing that the
447 risk of pertussis is increased in HIV+ and HEU individuals. The risk of pertussis was
448 increased by 50% and 40% respectively in the two groups compared to HUU. With the
449 exception of the study by du Plessis *et al.*, all studies showed increased risk of pertussis
450 incidence or prevalence associated with HIV infection or exposure.[38] In addition, there
451 was a higher risk of hospitalisation and deaths related to pertussis in HIV exposed or
452 infected infants.[32, 35, 36] In considering their pertussis control strategies, LMICs,
453 which have the biggest burden of HIV, need to take into account this increased risk
454 associated with HIV exposure and infection.[7]

455

456 Our study is largely limited by paucity of data, especially longitudinal data for each
457 included country as well as vaccine coverage in the specific population studied. In
458 addition, case detection may have been affected by different selection criteria used as well
459 as the variability of PCR assays used to confirm cases. Regardless, our results will
460 encourage generation of more epidemiological studies on pertussis in LMICs, while in the
461 meantime, assisting policy makers in disease control planning.

462 **CONCLUSIONS**

463 This study indicates an urgent need to review the existing pertussis control programs in
464 LMICs to target children, adolescents, and HIV exposed and infected groups. In addition
465 the study highlights the need to urgently consider measures to reduce the high infant

466 mortality rate, with specific consideration for maternal vaccination that has been shown to
467 be effective in protecting young infants even in an LMIC setting.[113] In their
468 recommendation, the GPI in addition to prioritizing surveillance, made this an urgent area
469 of action for LMICs.[5] Encouragingly, we noted a substantial increase in the number of
470 studies published in the last eight years of the period under review (2010-2018), possibly
471 reflecting recent increase in interest and funding for pertussis research in LMICs.

472 **LIST OF ABBREVIATIONS**

473 aP - acellular vaccine
474 CIs - confidence intervals
475 EPI - Expanded Programme on Immunisation
476 GPI - Global Pertussis Initiative
477 HEU - HIV-exposed uninfected
478 HICs - High Income Countries
479 HIV+ - HIV infected
480 HUU - HIV-unexposed uninfected
481 IQR - interquartile range
482 LMICs - Low and Middle-Income Countries
483 PCR - polymerase chain reaction
484 WHO - World Health Organization
485 wP - whole cell vaccine

486

487 **DECLARATIONS**

488

489 **Ethics approval and consent to participate**

490 The study did not recruit participants. As the study employed secondary analysis of data
491 already in the public domain, no Institutional Review Board or Ethics Committee was
492 approached to give approval for this study.

493

494

495 **Consent for publication**

496 No consent for publication was required. All authors read and approved the final
497 manuscript.

498

499 **Availability of data and materials**

500 All data generated or analysed during this study are included in this published article and
501 in the reference list provided, all of which are in the public domain.

502

503 **Competing interests**

504 The authors declare that they have no competing interests.

505

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507 No funding was received for the submitted work

508

509 **Authors' contributions**

510 RM conceived the research project, extracted the data, designed and executed the
511 analyses, interpreted the findings, wrote the first draft, and revised drafts of the
512 manuscript. BK contributed to search strategy, study selection, and revision of manuscript.
513 ME revised drafts of the report and contributed to the interpretation of the findings. GH
514 contributed to the interpretation of the findings and supervised the research project. All
515 authors reviewed the final manuscript.

516

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519 translation of non-English full texts.

520

521 **TITLES OF INCLUDED ADDITIONAL FILES**

522 **Additional file 1:** Country and year of included studies with confirmed pertussis shown
523 by World Health Organization region

524 **Additional file 2:** Distribution of point prevalence of confirmed pertussis by World
525 Health Organization region and confirmation method [PCR = polymerase chain reaction]

526 **Additional file 3:** Prevalence of paired serology confirmed *Bordetella pertussis*. Dotted
527 lines show subgroup and whole group average estimates

528 **Additional file 4:** Prevalence of polymerase chain reaction and culture confirmed
529 *Bordetella parapertussis*. Dotted line shows group average estimate [# Culture confirmed]

530 **Additional file 5:** Meta-analysis of relative detection rates of polymerase chain reaction
531 (PCR) and culture in confirming *Bordetella pertussis* infection

532

533

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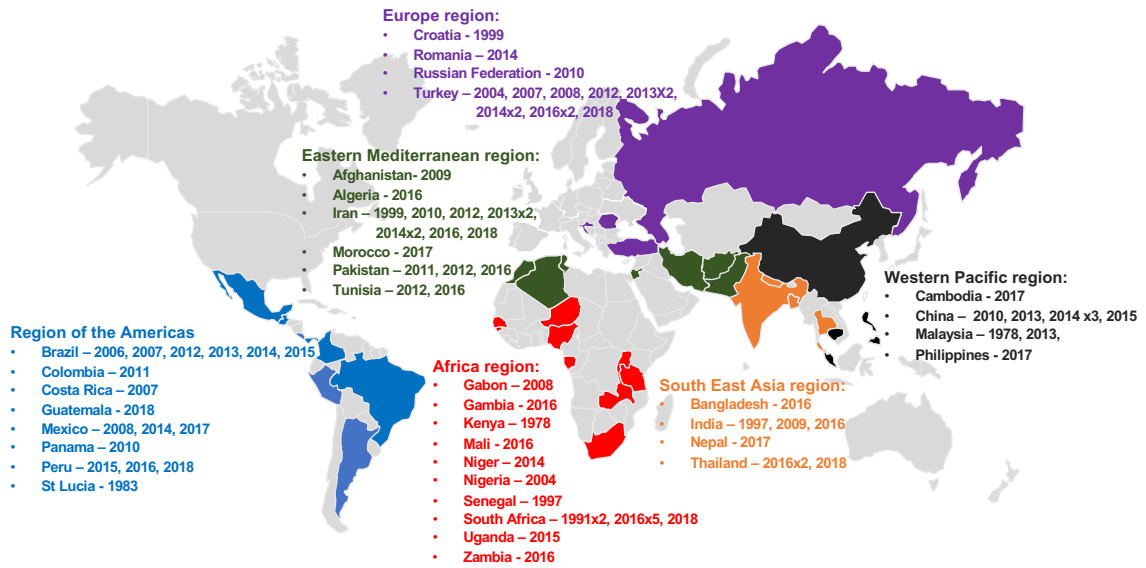
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1 **Additional Files**

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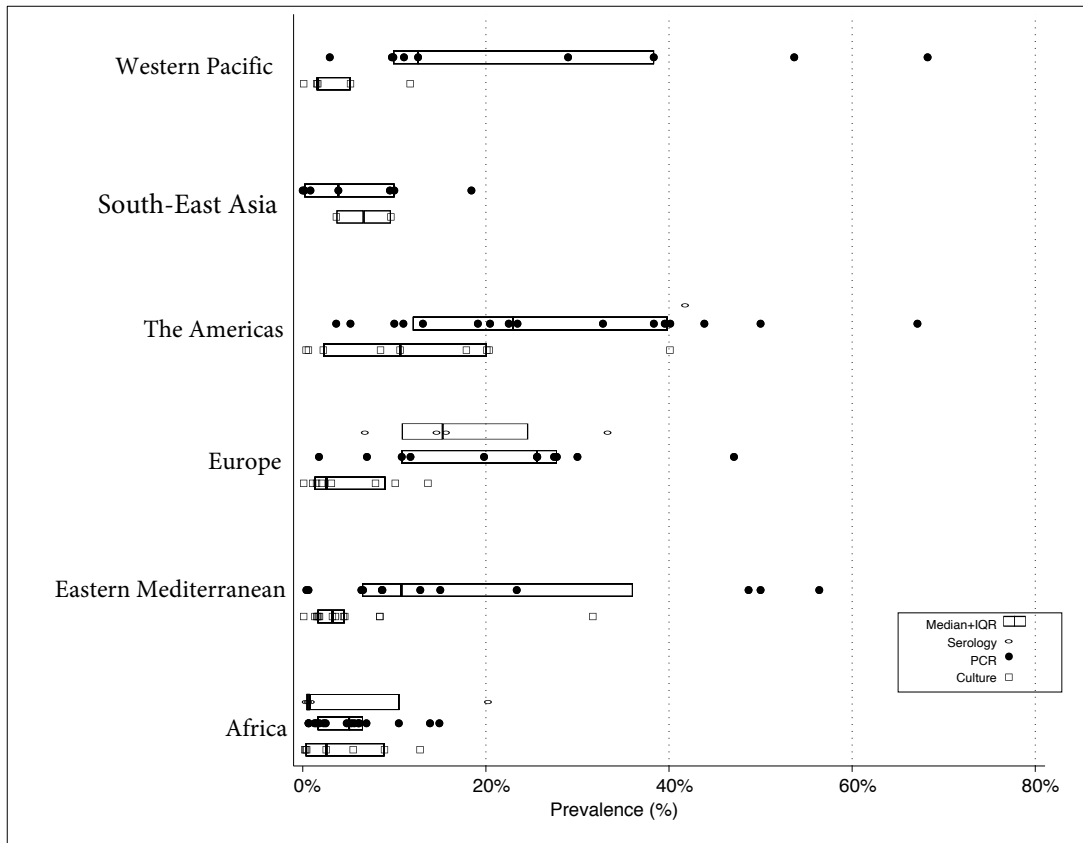


3

4 **Additional file 1:** Country and year of included studies with confirmed pertussis shown
 5 by World Health Organization region

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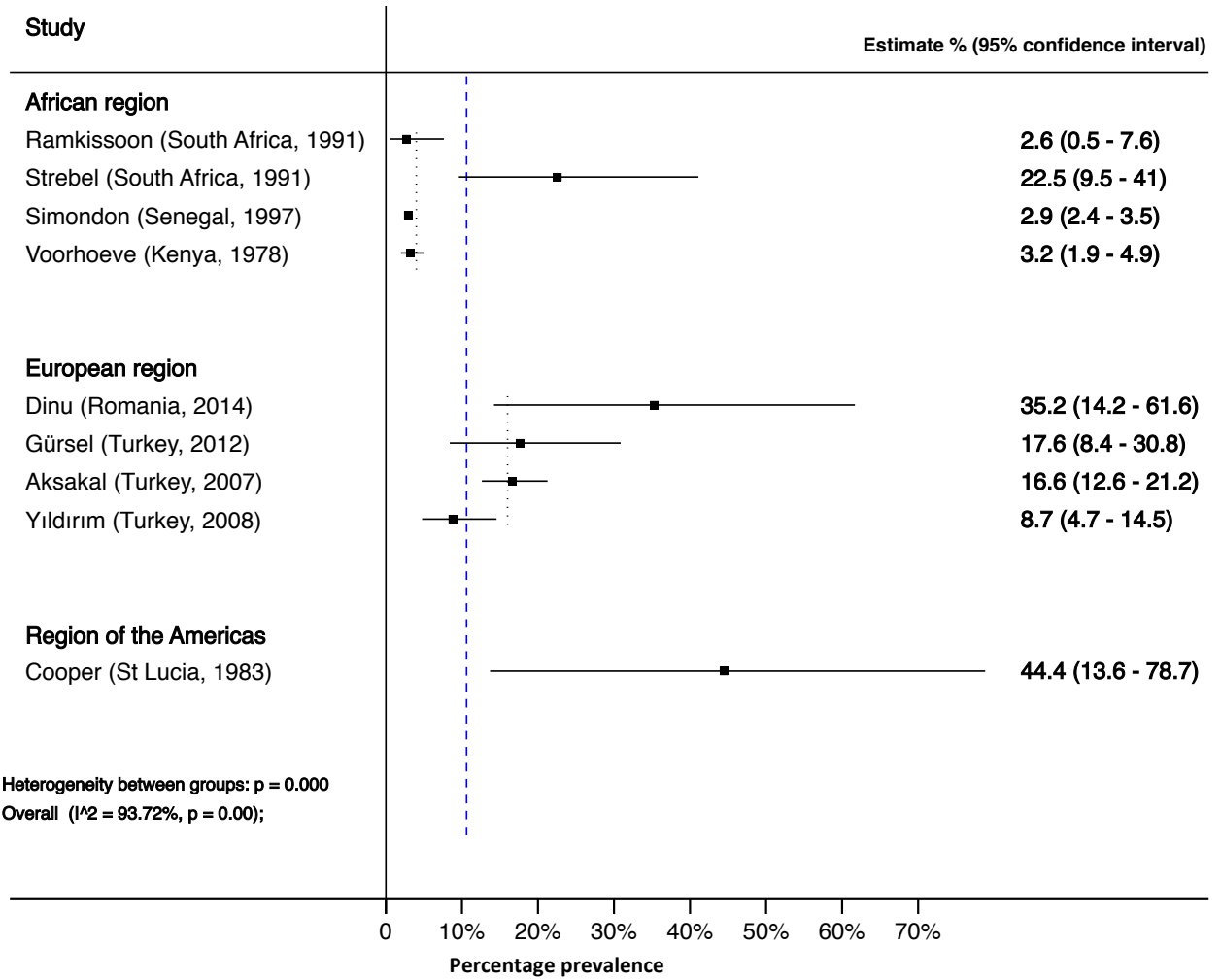
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9 **Additional file 2:** Distribution of point prevalence of confirmed pertussis by World
 10 Health Organization region and confirmation method [PCR = polymerase chain reaction]

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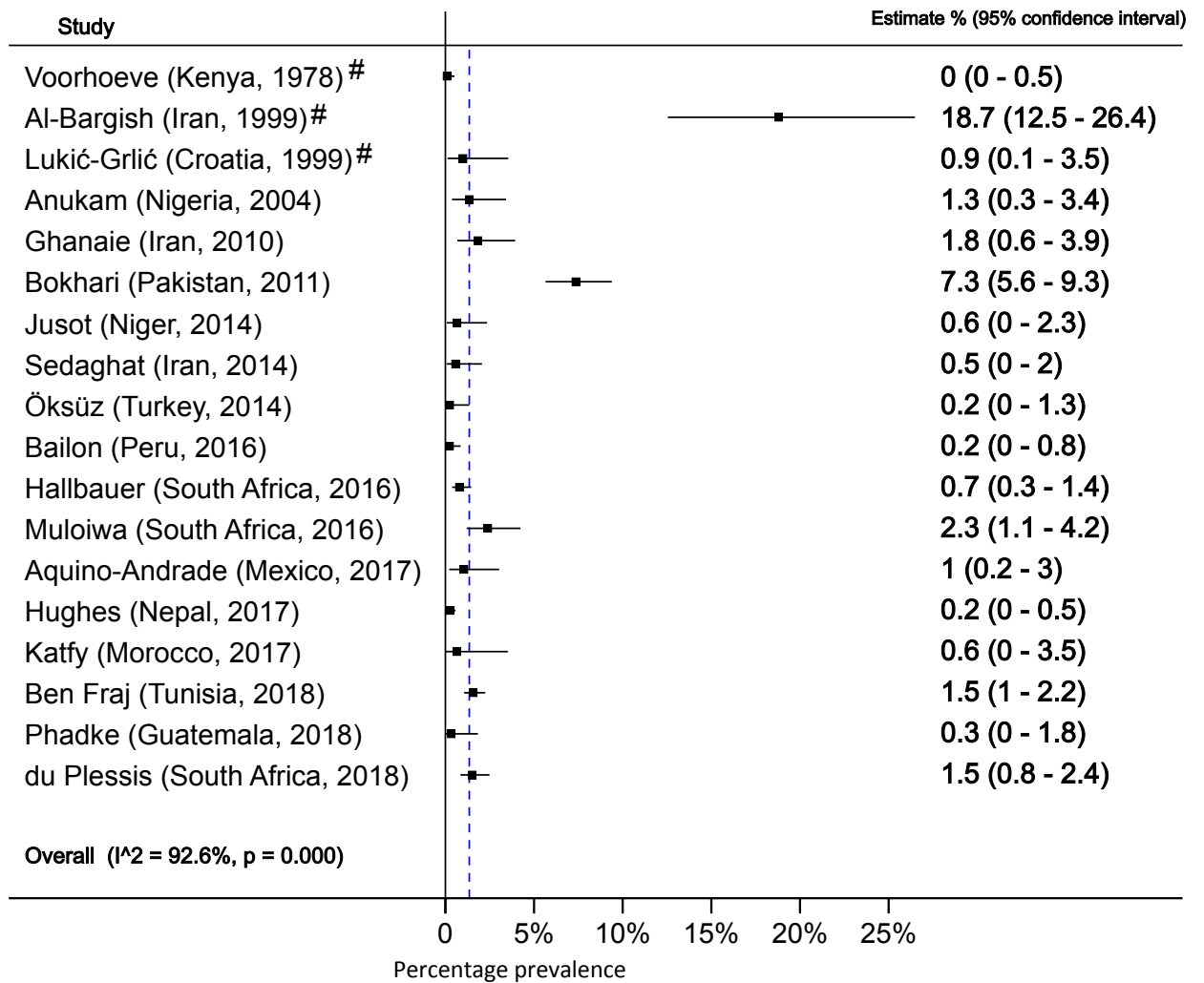
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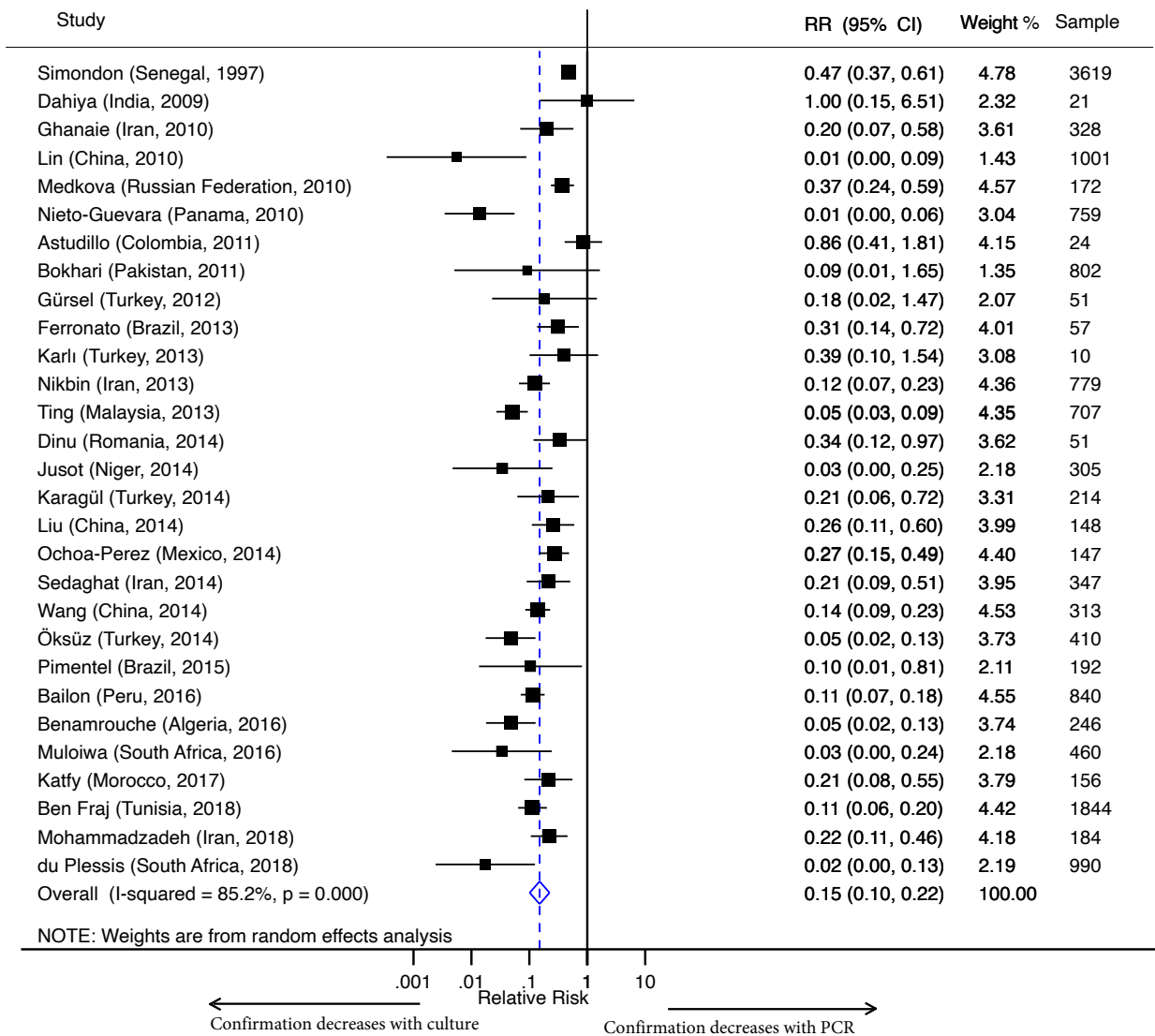
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Additional file 3: Prevalence of paired serology confirmed *Bordetella pertussis*. Dotted lines show subgroup and whole group average estimates



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Additional file 4: Prevalence of polymerase chain reaction and culture confirmed *Bordetella parapertussis*. Dotted line shows group average estimate [# Culture confirmed]



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Additional file 5: Meta-analysis of relative detection rates of polymerase chain reaction (PCR) and culture in confirming Bordetella pertussis infection

Chapter 3

1 **Incidence and diagnosis of pertussis in South African children hospitalised with** 2 **lower respiratory tract infection**

3

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21

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34 Pertussis in hospitalised South African children

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36 Pertussis in hospitalised South African children

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53 **Abstract**

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55 **Background:** The incidence of pertussis in children in low and middle-income
56 countries is poorly described. This study aimed to prospectively investigate the incidence
57 of pertussis in South African children hospitalised with lower respiratory tract infection
58 (LRTI).

59

60 **Methods:** Children hospitalised with LRTI in Cape Town, South Africa were enrolled
61 over one year. Clinical data were collected. A nasopharyngeal swab (NP) and induced
62 sputum (IS) were taken and PCR specific for *Bordetella pertussis* (IS481+/hIS1001-)
63 and *Bordetella parapertussis* (IS1001+) was performed.

64

65 **Results:** 460 children with median age 8 (IQR 4-18) months were studied. *Bordetella*
66 *pertussis* was detected in 17 (3.7%) while total *Bordetella spp.* were identified on 23
67 (5.0 %) of 460 NP. Adding IS testing increased the identification of *B. pertussis* to
68 32/460 cases (7.0%; 95% CI 4.8-9.7%); p=0.028 and total *Bordetella* to 41/460 (8.9%;
69 95% CI 4-10%); p=0.020. Shorter duration of symptoms [median 2 (IQR 2-3) days
70 versus 5 (IQR 3-7) days; p=0.0008] was associated with detection of *B. pertussis* on IS
71 versus NP.

72

73 *B. pertussis* was detected in 15.8% (n=3/19) of HIV infected children, 10.9%
74 (n=10/92) of HIV exposed uninfected and 5.4% (n=19/349) of HIV unexposed
75 uninfected children. Risk of *B. pertussis* decreased with each additional dose of DTaP
76 vaccine [0 doses = 17.9%; 1 dose =7.0%; 2 doses =6.9%; >3 doses =6.2%].

77

78 **Conclusions:** Pertussis is common in South African children hospitalised with LRTI
79 particularly if HIV exposed or infected but decreases sequentially with vaccination
80 doses. PCR on IS specimen provides confirmation earlier than NP while increasing overall
81 diagnostic yield.

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84

85 **Introduction**

86

87 Pertussis is an important cause of severe respiratory disease in children globally. Most
88 cases are due to *Bordetella pertussis* infection with a small percentage attributed to
89 *Bordetella parapertussis*. [1] The World Health Organization (WHO) estimates that
90 between 20 and 40 million cases of pertussis and 300 000 pertussis-related deaths occur
91 around the world each year, 90% of which occur in children in low and middle-income
92 countries (LMICs). [2, 3] Data are largely unavailable in LMICs, making it difficult to
93 assess the true burden of disease and the impact of vaccination. [4, 5]

94

95 Pertussis can be difficult to diagnose clinically, especially in infants. [6] Polymerase
96 chain reaction (PCR), commonly performed on a nasopharyngeal specimen obtained by
97 swab or aspiration, has greatly improved the ability to confirm cases. [7, 8] [9, 10] Due
98 to lack of resources for laboratory confirmation in most LMICs, diagnosis is largely
99 made on clinical grounds utilizing clinical case definitions such as the one
100 recommended by WHO. [5, 11, 12]

101

102 Since 2009, South Africa (SA) has introduced an acellular vaccine in a combination
103 formulation with four other vaccines (DTaP-IPV/HIB; Pentaxim®, Sanofi
104 Pasteur). [13]. Available data indicates that coverage for the pertussis vaccine in the
105 Western Cape Province, where this study was done, declines from six weeks with each
106 extra dose in the schedule. [14]

107 This study aimed to prospectively investigate the incidence and risk factors for pertussis
108 in South African children hospitalised with lower respiratory tract infection (LRTI).

109

110

111 **Materials and Methods**

112

113 A prospective study of the incidence and risk factors for pertussis was performed in
114 children hospitalised with LRTI.

115

116 Participants

117 Sequential children admitted to Red Cross War Memorial Children's Hospital (RCH),
118 Cape Town, South Africa, from 07 September 2012 to 06 September 2013 for LRTI
119 were eligible for enrolment. To be included, the children had to be less than 13 years of
120 age and have WHO-defined age-specific tachypnoea or lower chest indrawing or apnoea.
121 In addition, the children could only be enrolled after informed consent was obtained from
122 the parents for the children to be included. Participants were excluded if they had a
123 previous admission to a health care facility in the preceding two weeks or if they had
124 already been in hospital longer than 48 hours during the current admission. A maximum
125 of four children were enrolled per working day to ensure distribution of enrolment
126 throughout the year. The attending doctors at RCH provided clinical care. Children
127 were followed up until they were discharged from hospital.

128

129 A detailed history and clinical examination was done, especially noting the presence of
130 cough, apnoea, duration of symptoms and use of antibiotics prior to admission. History of
131 HIV exposure, infection and where relevant, antiretroviral treatment (ART) were
132 recorded. Maternal HIV status at the time of pregnancy with the child was abstracted as
133 recorded on the Road to Health Card (RTHC). In addition, caregivers who did not know
134 their current HIV status were counselled and offered HIV testing. All children were
135 screened for HIV infection during the study. Information on immunisation was abstracted
136 from the RTHC, a standardised national record for each child, and the date and type of

137 each vaccine recorded. Vaccination status was regarded as a completed primary
138 schedule if three or more doses were received. The status was classified unknown
139 where RTHC was missing and up to date for age if an appropriate number of doses for
140 the child's age were received.

141

142 HIV screening was done using an ELISA test (Architect HIV Ag/Ab Combo, Abbott
143 Diagnostics, Wiesbaden). Children younger than 18 months who were ELISA positive
144 had their status confirmed with an HIV PCR test (COBAS AmpliPrep/COBAS Taqman
145 HIV-1, Roche Molecular Diagnostics, Pleasanton, CA) while those older than 18 months
146 had a second ELISA test (Enzygnost Anti-HIV 1/2 Plus, Siemens/Dade Behring,
147 Erlangen). HIV infection was defined as a positive PCR in children less than 18 months
148 of age or two positive ELISA tests in older children. Children younger than 18 months
149 who were ELISA positive, but PCR negative were classified as HIV exposed
150 uninfected; older children were classified as HIV exposed uninfected if the mother was
151 HIV infected at the time of the pregnancy but the child tested HIV negative.

152

153 Two nasopharyngeal (NP) swabs followed by an induced sputum (IS) specimen were
154 collected from each child. The first NP specimen was taken using a cotton wool tipped
155 swab and immediately put into a gel Amies transport medium and sent to the laboratory
156 for culture. The second NP specimen was taken with a nylon flocculated swab
157 (FLOQSwabsTM, Copan Diagnostics, Murrieta, CA) and immediately transferred into
158 a nucleic acid preservation medium (PrimeStore®, Longhorn Vaccines & Diagnostics,
159 San Antonio, TX). The IS specimen was collected after nebulization with a
160 bronchodilator and hypertonic saline as previously described.[15] Briefly, children were
161 fasted for 2–3 hours before sputum induction. Salbutamol nebulisation was given via a
162 metered dose inhaler to reduce the risk of bronchospasm, after which 5 ml of 5% sterile

163 saline was given via an oxygen jet nebuliser at a rate of 5 l/min. After 15 min, the children
164 were either suctioned or encouraged to cough, with suctioning performed through the
165 nasopharynx with a sterile mucus extractor. Both specimens were frozen at minus 80°C
166 until thawed for batched molecular diagnostic testing.

167 The Amies swab was inoculated onto a charcoal-enriched culture medium (Greenpoint
168 Media Laboratory, National Health Laboratory Service, Cape Town, South Africa) and
169 incubated for a minimum of seven days. If growth was detected, the bacterial colonies
170 were tested by PCR as described below.

171

172 PCR specific for insertion sequences IS481 for *Bordetella* spp. and IS1001 for *B.*
173 *parapertussis* was done with a validated commercial kit (LightMix® Kit *Bordetella*
174 *pertussis* and *Bordetella parapertussis*, TIB MolBiol, Berlin, Germany) using
175 previously published primers.[16] To exclude *Bordetella holmesii* (IS481 +, IS1001 -,
176 hIS1001 +) before the diagnosis of *B. pertussis* infection was made, all specimens
177 testing positive for IS481 were tested for the presence of insertion site hIS1001.[17]

178

179 Controls

180 Controls were children without symptoms or signs of respiratory illness presenting for
181 other reasons to the same hospital or a nearby primary health care facility. Controls
182 were matched to within 12 weeks of birth and 12 weeks of specimen collection of the
183 cases testing positive for *Bordetella* spp. on the NP specimen. An average of three
184 controls were matched to each case. One nylon flocked NP specimen was collected from
185 each control and processed as described above.

186

187

188

189 Ethics

190 Approval for the study was granted by the Human Research Ethics Committee of the
191 Faculty of Health Sciences, University of Cape Town, South Africa (Reference:
192 371/2011). Written informed consent was obtained from a parent or legal guardian in
193 their preferred language.

194

195 Sample size and statistical analysis

196

197 We calculated that a sample size of between 400 and 500 participants would give
198 95% confidence intervals (CI) of 3% above and below our estimated prevalence of
199 5%. Percentages with 95% CI were used to depict proportions of categorical variables
200 while medians with interquartile ranges (IQR) were used to summarize all continuous
201 variables.

202

203 Cumulative frequencies of confirmed *B. pertussis* were described and stratified by age
204 category, HIV status and vaccination status.

205

206 A χ^2 or Fisher's exact test was used to assess the strength of association between two
207 categorical variables as appropriate. The Mann-Whitney test was used to test the
208 hypothesis of similarity between two groups with respect to a continuous variable. A two-
209 tailed cut-off level of significance at $p < 0.05$ was used in all hypothesis testing. All
210 analyses were done using *Stata Statistical Software Release 13* (StataCorp LP,
211 College Station, TX).

212

213

214

215 **Results**

216 Study participants

217 Over the study period, 7 792 children were hospitalised of whom 987 children had
 218 respiratory illness. Of these, 460 (46.6%) eligible participants were enrolled. The median
 219 age was 8 (IQR 4-18) months and 258 (56.1%) were male. Most children were HIV
 220 unexposed uninfected (n=349; 75.9%) while 92 (20.0%) were HIV exposed uninfected
 221 and 19 (4.1%) were HIV infected, table 1. Among HIV infected children, one was WHO
 222 HIV stage 1 and one HIV stage 2, while 10 were stage 3 and seven were stage 4. Nine
 223 (47.4%) HIV infected children were on ART of whom four had attained viral
 224 suppression.

Table 1: Baseline characteristics of the study participants, *Bordetella* cases and age matched NP controls

Baseline character	Study sample (N=460)	Positive NP cases (n=23)	Matched Controls (N=70)
Age			
Median (IQR) months	8 (4-18) months	8 (2.5 - 14) months	8 (5-16) months
Gender			
	n (%)	n (%)	n (%)
Female	202 (43.9)	13 (56.5)	33 (47.1)
Male	258 (56.1)	10 (43.5)	37 (52.9)
Pertussis doses received			
0	28 (6.1)	2 (8.7)	2 (2.9)
1	57 (12.4)	5 (21.7)	1 (1.4)
2	58 (12.6)	4 (17.3)	4 (5.7)
≥ 3	308 (67)	12 (52.2)	49 (70.0)
Unknown	9 (2.0)	0 (0.0)	14 (20.0)
HIV status			
Unexposed Uninfected	349 (75.9)	17 (73.9)	68 (97.1)
Exposed Uninfected	92 (20)	5 (21.7)	0 (0.0)
Exposed Infected	19 (4.1)	1 (4.4)	2 (2.9)
Presenting symptoms			
Cough	456 (99.1)	23 (100)	NA
Apnoea	20 (4.5)	3 (13.0)	NA
Fever	288 (63.7)	10 (45.5)	NA
Pre-hospital antibiotic			
	n=173	n=10	
Penicillin	77 (44.5)	7 (70.0)	NA
Ceftriaxone	99 (57.2)	3 (30.0)	NA
Cotrimoxazole	4 (2.3)	0	NA
Erythromycin	1 (0.4)	0	NA

NP = Nasopharyngeal specimen; NA = Not applicable; NB. Only cases positive on NP shown for comparison with controls.

226 Immunisation status was unknown in nine children. Of 451 (98.0%) with immunisation
227 records, 369 (85.2%) were up to date with pertussis vaccine doses for age while 308
228 (68.3%) had completed the primary schedule. This did not differ by HIV status with
229 237/345 (68.7%) in unexposed uninfected, 59/89(66.3%) in exposed uninfected and
230 12/17 (70.6%) in HIV infected children respectively completing the primary schedule;
231 $p=0.891$.

232 Antibiotics were received by 173 (37.6%) children for a median duration of one day
233 (IQR 1-1 days) prior to admission. Ceftriaxone ($n=99$; 57.2 %) and penicillin ($n=77$; 44.5
234 %) were the commonest antibiotics received. Seven children received both ceftriaxone and
235 penicillin while one child received both ceftriaxone and co-trimoxazole.

236

237 Cough (median duration 3, IQR 2-5 days) and fever were the commonest presenting
238 symptoms, table 1. Apnoea was reported in 20 (4.4%) participants with a median age
239 of 6 (IQR 3-16) months while those without apnoea had median age 8 (IQR 4-18)
240 months; $p=0.26$.

241

242 Description of microbiologic confirmation

243 Both NP and IS specimen collection were well tolerated and obtained in most children
244 with no severe adverse reaction noted in any participant. NP specimens were obtained
245 in all children. In four participants IS could not be obtained as children were transferred
246 or discharged before the specimen could be collected. In all other children ($n=456$; 99.1
247 %), IS was successfully obtained. Two IS specimens were lost due to container leakage.

248

249 PCR for IS481 was positive in 17 of 460 NP specimens (3.7 %; 95% CI 2.2-5.9 %)
250 while seven (1.5%; 95% CI 0.6-3.2%) were positive for IS1001. PCR was positive for
251 both targets in one participant. Therefore 23 (5%; 95% CI 3.2-7.4%) NP specimens

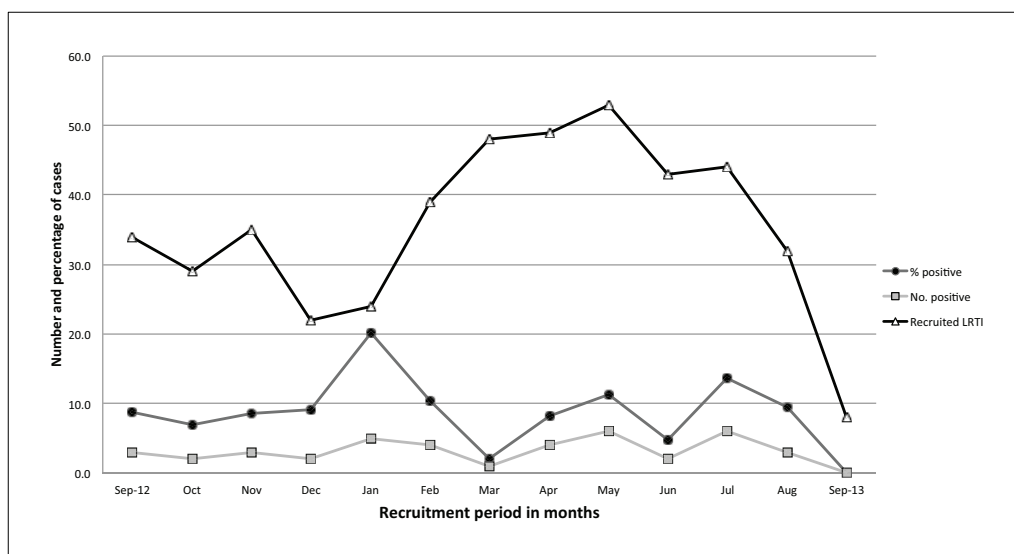
252 were positive for either target. Of the 454 IS specimens processed, 25 (5.5%; 95% CI
 253 3.6-8.0%) were positive for insertion site IS481 and five (1.1%; CI 0.4%-2.5%) positive
 254 for IS1001, giving a total of 30 (6.6%; CI 4.5-9.3%) IS specimens positive for either
 255 *Bordetella* target.

256

257 NP specimens from four participants showed bacterial growth compatible with
 258 *Bordetella* spp. on culture but only one was positive on PCR for the IS481 locus. The
 259 same participant was also positive for IS481 on the flocced-swab NP specimen. NP
 260 specimens from all 70 controls (table 1) were PCR negative for both IS481 and IS1001
 261 insertion sites. As the other three cultures could not be confirmed as *Bordetella pertussis* or
 262 *Bordetella parapertussis*, they were not included with positive cases.

263

264 None of the IS481 positive specimens were positive for the hIS1001 *B. holmesii* locus
 265 and so all were classified as *B. pertussis* positive while all the IS1001 positive specimens
 266 were classified as *B. parapertussis* positive. In total, 32 (7.0%; 95% CI 4.8-9.7%)
 267 participants were *B. pertussis* positive and 11 (2.4%; 95% CI 1.2-4.2%) were *B.*
 268 *parapertussis* positive from NP and IS specimens. Two participants were positive for
 269 both organisms. This gave an incidence of 8.9% (95% CI 6.5-11.9%; n=41/460) for
 270 either organism. The occurrence of *Bordetella* cases did not show any seasonal pattern
 271 although enrolled LRTI cases in general peaked between March and July 2013, figure 1.



272

273 **Figure 1: Recruited lower respiratory tract infection cases showing number and percentage**
 274 **of confirmed *Bordetella* per month**

275 Children with confirmed *B. pertussis* had a median age of 8 months (IQR 2-21), similar
 276 to those without confirmed infection [median of 8 months (IQR 4-18); p=0.43]. A
 277 higher proportion of cases occurred in infants younger than two months of age with
 278 6/41 (14.6%) positive for *B. pertussis* compared to 26/419 (6.2%) in children older
 279 than two months; p=0.043.

280

281 The risk of *B. pertussis* differed by HIV status with HIV unexposed uninfected having the
 282 lowest risk (n=19/349; 5.4%). Exposed uninfected (n=10/92; 10.9%) had an intermediate
 283 risk while HIV infected children (n=3/19; 15.8 %) had the highest risk.

284

285 Risk of *B. pertussis* infection declined with each additional dose of the vaccine
 286 received. The highest risk was seen in children who had not received any vaccine with
 287 5/28 (17.9%) having confirmed *B. pertussis*. The risk was 4/57 (7.0%), 4/58 (6.9%)
 288 and 19/308 (6.2%) for children that received one, two and three or more doses
 289 respectively. Table 2.

Table 2: *Bordetella* incidence stratified by age group, HIV and vaccine doses (N=460)

Stratifying variable	Stratum total (n)	<i>B. pertussis</i>	<i>B. parapertussis</i>	Total <i>Bordetella</i>
		Cases n (%)	Cases n (%)	Cases n* (%)
Crude	460	32 (7.0)	11 (2.4)	41 (8.9)
Age group				
< 2 months	41	6 (14.6)	0 (0.0)	6 (8.4)
≥ 2 months	419	26 (6.2)	11 (2.6)	35 (14.6)
HIV status				
Uninfected unexposed	349	19 (5.4)	10 (2.9)	27 (7.7)
Exposed uninfected	92	10 (10.9)	0 (0.0)	10 (10.9)
Exposed infected	19	3 (15.8)	1 (5.3)	4 (21.1)
Pertussis vaccine doses				
0	28	5 (17.9)	0 (0.0)	5 (17.9)
1	57	4 (7.0)	1 (1.8)	4 (7.0)
2	58	4 (6.9)	3 (5.2)	7 (12.1)
≥ 3	308	19 (6.2)	6 (2.0)	24 (7.8)
Unknown	9	0 (0.0)	1 (11.1)	1 (11.1)

* Two cases were positive for both organisms. NB. Positive cases includes total diagnosed on nasopharyngeal and induced sputum specimens

290

291 Stratified *B. parapertussis* incidences were difficult to interpret because of small number of
292 cases. Table 2.

293

294 Use of IS versus NP specimen for diagnostic confirmation

295 Ten participants had a positive PCR for IS481 on both NP and IS. In one participant
296 PCR for IS1001 was positive on both the NP and IS while in another IS1001 was
297 positive on NP and IS481 positive on IS. The latter was counted as a single case of *B.*
298 *pertussis*. In total 30 out of 41 (73.2%) *Bordetella* confirmed cases were detected on IS
299 while only 23/41 (56.1%) were detected on NP (of which six were not identified on IS).

300

301 Testing of IS was able to identify 25/32 (78.1%) of all confirmed *B. pertussis*
302 infections compared to 17/32 (53.1%) for NP. The use of IS increased the diagnostic
303 yield of *B. pertussis* by 15 (46.9%) from 17/460 (3.7%) when NP was used alone compared
304 to 32/460 (7.0%), when used with IS ($p=0.028$) and that of total *Bordetella spp.* by an
305 additional 18 (43.9%) from 23/460 (5.0%) when NP was used alone compared to 41/460
306 (8.9%) when used with IS ($p=0.020$).

307

308 Participants positive for *B. pertussis* on IS specimens only (IS+/NP-), had a shorter
309 duration of symptoms [median 2 (IQR 2-3) days] compared to those who were *B.*
310 *pertussis* positive on NP specimen (NP+/IS- or NP+/IS+) [median 5 (IQR 3-7) days;
311 $p=0.0008$]. None of the *B. pertussis* cases diagnosed on IS only (IS+/NP-) had a
312 duration of symptoms longer than five days.

313

314 Outcome

315

316 Only 4/41 (9.8%) of confirmed cases were clinically diagnosed as pertussis. The other

317 cases were diagnosed as lobar or bronchopneumonia (n=15; 36.6%), bronchiolitis
318 (n=15; 36.6%) or other LRTI (n=7; 17.1%).

319 All participants were discharged from hospital with no in-hospital deaths occurring.
320 Fourteen (3.0%) children were admitted to a High Dependency or Pediatric Intensive
321 Care Units. Among these, 3/41 (7.3%) were *Bordetella* infected compared to 11/419
322 (2.6%) in the uninfected group; p=0.120).

323

324 The median length of hospitalisation was two days (IQR 1-4); similar in *Bordetella*
325 infected [2 (IQR 1-4.5) days] and uninfected children [2 (IQR 1-5) days; p=0.187]

326

327 **Discussion**

328

329 This study has shown that pertussis is common in South African children hospitalised
330 with LRTI with an estimated 9% of our sample having laboratory confirmed infection. *B.*
331 *pertussis* was responsible for 75% of the cases with *B. parapertussis* contributing the
332 rest. These likely represent true cases of pertussis, as they had both clinical and
333 microbiological evidence of disease while all results obtained from controls were
334 negative. The rate is equivalent to 526 cases of laboratory confirmed pertussis for every
335 100 000 children hospitalised and 4154 of 100 000 children specifically hospitalised
336 with LRTI. The lack of data on the baseline population from which the cases
337 originated made it difficult to estimate the true population incidence. Data from high-
338 income countries report hospitalisation incidences of between 2 and 240 per 100 000
339 population.[18-20] Possible reasons for the high incidence of disease in our study
340 population include incomplete primary vaccination and a high burden of HIV which
341 may reduce protection against pertussis.[21]

342

343 Pertussis cases occurred consistently throughout the year. The proportion of confirmed
344 pertussis cases in our study did not show any seasonal pattern although there was an
345 overall increase in the number of respiratory cases recruited over the winter months.
346 The increase in winter enrolment was an expected finding that reflects a trend we have
347 consistently observed over this time of the year. A study done around the same period
348 in the same province of South Africa reported a similar pattern of increased overall
349 respiratory cases over the winter months.[22] A recent study found the prevalence of
350 pertussis to be lower than 1% in children hospitalised with bronchiolitis over the
351 winter period. Differences in both diagnostic and sampling methodology made it
352 difficult to compare this finding with the current study.[23]

353

354 HIV exposed and infected children showed higher risks for pertussis. This is of
355 particular importance in this region with a high burden of maternal HIV with
356 associated intrauterine exposure and infection.^[24] The observed increase in pertussis
357 cases in these groups may relate to both reduced vaccine effectiveness and passive
358 immunity associated with HIV infection or exposure.[25-28] It is possible that these
359 observations may have been confounded by other factors such as poverty, malnutrition and
360 exposure to biofuels. Consistent with other studies, the risk of *B. pertussis* was higher in
361 younger infants and decreased with each extra dose of vaccine received, with the lowest
362 risk after a completed primary schedule. [18]

363

364 A novel, important finding was the higher sensitivity for diagnosis of *Bordetella* spp. on
365 IS specimens, with more confirmed cases on IS compared to NP specimens. The use of
366 NP specimen on its own would have missed 47% of *B. pertussis* cases compared to
367 22% missed by IS. When used in addition to the traditional NP specimen, IS
368 significantly increased the yield by 47%. IS was successfully obtained in almost all
369 children and was well tolerated even in very young infants. Large studies of children

370 with suspected pulmonary TB have reported excellent safety, tolerability and good
371 diagnostic yield of IS, even in infants.[15, 29] These data add to the increasing evidence
372 of the utility and safety of IS for diagnostic confirmation of the aetiology of LRTI in
373 young children.

374

375 This study also found that testing of IS is more likely to be positive early in the course
376 of disease. Early diagnosis allows for targeted antibiotic therapy, which may reduce
377 severity of illness and allow early prophylaxis for close contacts.[30, 31] The findings of
378 this study suggest that IS should be the recommended specimen for testing children with
379 suspected pertussis either on its own or in addition to NP specimen, rather than the
380 current recommended practice of using NP specimen on its own.[9, 10]

381

382 Confirming prior studies, culture had very low sensitivity, suggesting that although this
383 method of diagnosis offers opportunity for antibiotic sensitivity testing, it is ineffective for
384 confirming a diagnosis.[5]

385

386 Limitations of this study include lack of detection of other pathogens. The finding of
387 *Bordetella* infection does not preclude the possibility of co-infection that may have
388 modified the disease presentation.[32-34] As only hospitalised cases were included in
389 the study, the findings may not be generalizable to children with less severe forms of
390 illness. The small number of confirmed cases limits the interpretation of stratified *B.*
391 *parapertussis* incidence. Further studies to investigate these aspects are needed.

392

393 As our study demonstrates, the difficulty in diagnosing atypical pertussis may substantially
394 underestimate its role in severe LRTI. Accurate diagnosis is important for timely
395 treatment, institution of infection control measures and assessment of vaccine

396 effectiveness.[5] Macrolides may shorten the duration of symptoms and reduce the very
397 high secondary attack rate associated with *B. pertussis*. [30, 31] Although concerns exist of
398 rapid emergence of resistance to macrolide antibiotics, empiric use should be considered in
399 young African infants and HIV exposed or infected children hospitalised with LRTI,
400 particularly where pertussis immunisation is incomplete. [35, 36]

401

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403

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Chapter 4

1 **Risk factors for *Bordetella pertussis* disease in hospitalised children**

2 **Risk factors for pertussis in children**

3
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30 **Abstract**

31 **Background:** Risk factors for pertussis in children in low and middle-income
32 countries are poorly understood, despite a resurgence of disease. This study aimed to
33 investigate risk factors for pertussis disease in African children hospitalised with
34 severe LRTI.

35 **Methods:** A prospective study of children hospitalised with severe LRTI in Cape
36 Town, South Africa was conducted over a one-year period. A nasopharyngeal and
37 induced sputum samples from child and nasopharyngeal sample from caregiver were
38 tested for *Bordetella pertussis* using PCR (IS481+/hIS1001). History and clinical
39 details were documented.

40 **Results:** 460 children with a median age of 8 (IQR 4-18) months were enrolled. *B.*
41 *pertussis* infection was confirmed in 32 (7.0%). The adjusted risk of confirmed
42 pertussis was significantly increased if infants were younger than two months [aRR
43 2.37 (95% CI 1.03-5.42)], HIV exposed but uninfected (aRR 3.53[95% CI 1.04-12.01])
44 or HIV infected (aRR 4.35[95% CI 1.24-15.29]). Mild (aRR 2.27 [95% CI 1.01-5.09])
45 or moderate (aRR 2.70 [95% CI 1.13-6.45]) under-nutrition in the children respectively
46 were also associated with higher risk. The highest adjusted risk occurred in children
47 whose caregivers had *B. pertussis* detected from nasopharyngeal swabs (aRR 13.82
48 [95% CI 7.76-24.62]). Completion of the primary vaccine schedule (three or more
49 doses) was protective (aRR 0.28 [95% CI 0.10-0.75]).

50 **Conclusions:** HIV exposure or infection, undernutrition as well as detection of
51 maternal nasal *B. pertussis* were associated with increased risk of pertussis in African
52 children, especially in young infants. Completed primary vaccination was protective.
53 There is an urgent need to improve primary pertussis vaccine coverage in low and
54 middle-income countries. Pertussis vaccination of pregnant women, especially those
55 with HIV infection should be prioritized.

56 **Introduction**

57

58 The last decade has seen a resurgence of pertussis in high-income countries to levels
59 experienced over half a century ago.[1] Possible reasons for this include waning
60 immunity following acellular vaccination, antigenic divergence of circulating strains
61 from vaccine antigens as well as increased ascertainment due to improved diagnostic
62 tools.[2-5]

63

64 Introduction of the whole cell (wP) pertussis vaccine in the 1940s greatly reduced the
65 incidence of pertussis. wP has since been succeeded by acellular vaccines (aP), mainly in
66 high-income countries. The South Africa National Expanded Programme on
67 Immunisation (EPI) replaced wP with a combination formulation aP (DTaP-IPV/HIB;
68 Pentaxim®, Sanofi Pasteur) in 2009. The primary schedule comprises doses at six, ten
69 and 14 weeks with a booster at 18 months of age.[6]. Current data indicates that vaccine
70 coverage for the Western Cape Province, where this study was done, is 97% at six
71 weeks, 90.8% at 10 weeks and 85.2% at 14 weeks. By 18 months of age coverage had
72 declined to 58.7%.[7]

73

74 Risk factors for pertussis include lack of immunisation or impaired immune
75 responses to vaccination. Laboratory studies suggest that immune responsiveness to
76 pertussis vaccines may be impaired by both infection and intrauterine exposure to HIV
77 even in HIV-uninfected children. [8-11] Another factor associated with reduced immune
78 responses to pertussis vaccine is poor nutritional status, a common problem among children in
79 low and middle-income countries (LMIC).[12] Although household use of biomass fuels,
80 indoor air pollution, and cigarette smoking have been associated with an increased risk of
81 respiratory illness and bacterial carriage in children, it is unclear if these impact on the risk of
82 pertussis.[13]

83

84 With the resurgence of pertussis, adults and adolescents, who tend to exhibit milder
85 and atypical symptoms, are now recognized as important sources of pertussis in
86 infants. In particular, household contacts pose the greatest risk to unvaccinated or
87 partially vaccinated infants.[14-16]

88

89 We aimed to investigate risk factors for *Bordetella pertussis* disease in a cohort of African
90 children less than 13 years of age hospitalised with lower respiratory tract infection (LRTI)
91 in a high HIV prevalence setting.[17]

92

93 **Materials and Methods**

94 Children less than 13 years of age admitted over a one-year period (07 September 2012 to
95 06 September 2013) for LRTI to the Red Cross War Memorial Children's Hospital
96 (RCH), Cape Town, South Africa, were prospectively screened for enrolment. Children
97 with WHO-defined severe pneumonia (age specific tachypnoea or/and lower chest
98 indrawing requiring hospitalisation) or apnoea were eligible to be included. A child
99 was included if the legal guardian gave written consent and the child had not been in
100 contact with a health care facility in the previous 48 hours to two weeks prior to
101 screening for enrolment. Enrolment was limited to the first four qualifying children per
102 working weekday.

103

104 History of symptoms of the presenting illness and information on current socio-
105 demographic factors including type of housing, access to amenities such as tap water,
106 electricity and toilet facilities, was taken from the caregiver. The mother's level of
107 education was recorded. Socio-economic status was categorized into quartiles on the basis
108 of a validated weighted composite score used elsewhere that included asset ownership,

109 employment and education.[18] The use of household biofuels, presence of smokers in the
110 household and the number of people sharing the bedroom with the child were established.
111 Information on day-care attendance was also collected.

112

113 The mother's HIV status (and that of the primary caregiver if this was not the mother) was
114 established. If the mother or caregiver was HIV infected the latest available CD4 count was
115 recorded and used in the staging of HIV disease according to the Centre for Disease
116 Control (CDC) classification.[19] History was taken on the presence and duration of recent
117 primary caregiver respiratory symptoms as well as presence and numbers of other
118 household members with similar symptoms.

119 The vaccination status of each child was verified using the national standardized
120 immunisation handheld record, the Road to Health Card (RTHC); specifically, the date
121 and type of each vaccine was copied from the record.

122

123 The weight of each child, as measured on admission, was used to evaluate nutritional
124 status using WHO weight for age Z scores (WAZ). Mild under-nutrition was defined
125 as ≤ -1 WAZ > -2 , moderate under-nutrition ≤ -2 WAZ > -3 and severe under-
126 nutrition WAZ ≤ -3 .[20]

127

128 Each child was screened for HIV infection using an ELISA test (Architect HIV Ag/Ab
129 Combo, Abbott Diagnostics, Wiesbaden). The diagnosis of HIV infection was made
130 if both the ELISA and an HIV PCR test (COBAS AmpliPrep/COBAS Taqman HIV-1,
131 Roche Molecular Diagnostics, Pleasanton, CA) were positive in children younger than 18
132 months. A positive ELISA was confirmed with a different ELISA test (Enzygnost Anti-
133 HIV 1/2 Plus, Siemens/Dade Behring, Erlangen) in children older than 18 months to
134 diagnose HIV infection. Children younger than 18 months who were ELISA positive,

135 but PCR negative were classified as HIV exposed uninfected while older children were
136 classified as HIV exposed uninfected if the mother was HIV infected at the time of the
137 pregnancy, but the child tested HIV negative. Caregivers who did not know their HIV
138 status were counselled and offered HIV testing. Children or caregivers with HIV who were
139 not accessing appropriate treatment were referred to public health facilities for further
140 follow-up and treatment of HIV.

141

142 Methods employed in the collection of respiratory specimens have been published.[21]
143 Briefly, nasopharyngeal (NP) specimens from caregivers as well as paired NP and
144 induced sputum (IS) specimens from children were tested by PCR for *B. pertussis*. The
145 NP specimen was taken with a flocced nylon swab which was transported in a nucleic
146 acid preservation medium (PrimeStore[®] MTM, Longhorn Vaccines and Diagnostics,
147 San Antonio, TX). The IS specimen was collected after the NP was taken from each
148 child. All specimens were frozen at minus 80°C until they were thawed for batched
149 molecular diagnostic testing.

150

151 A commercially validated duplex real-time PCR assay targeting the insertion sequence
152 IS481 for *Bordetella* and IS1001 for *Bordetella parapertussis* (LightMix[®] *Bordetella*
153 *pertussis* and *parapertussis* Kit, TIB MolBiol, Berlin, Germany) was used for screening
154 all the respiratory specimens.[22, 23] All specimens testing positive for IS481 were
155 further tested for the presence of insertion site hIS1001 in order to exclude *Bordetella*
156 *holmesii* (IS481 +, IS1001-, hIS1001 +) before the diagnosis of *B. pertussis* infection
157 was made.[24]

158

159 **Ethics**

160 The study was approved by the Human Research Ethics Committee of the Faculty of

161 Health Sciences of the University of Cape Town; Reference: 371/2011. Written
162 informed consent was sought and received from the legal guardian for the participation
163 of both the child and the guardian/caregiver in the study.

164

165 **Analysis plan**

166 The study aimed to investigate risk factors for pertussis as a secondary outcome and
167 was thus not specifically powered to achieve this secondary outcome. The study
168 sample was determined to attain a 3% precision above and below a point estimate risk
169 of 5% for the primary outcome (prevalence of pertussis).

170

171 Categorical data are presented as percentages with 95% confidence intervals (CI). All
172 continuous data are summarized as medians with interquartile ranges (IQR). A χ^2 test
173 assessed strength of association between two categorical variables with a two-tailed cut-off
174 significance set at $p < 0.05$.

175

176 A causal model employing the current understanding of respiratory disease processes and
177 pathogenesis of pertussis was constructed using a directed acyclic graph (DAG) to identify
178 variables in the model required for minimal sufficient adjustment sets for estimating total
179 independent effects of each assessed risk factor.[25]

180

181 To adjust for potential confounders for each risk factor as identified by the DAG, a
182 generalized linear Poisson regression model with robust error variance was used to
183 estimate adjusted relative risks (aRR) and their 95% level of confidence in a
184 multivariable analysis. For all analyses, *Stata Statistical Software Release 13*
185 (StataCorp LP, College Station, TX) was employed.

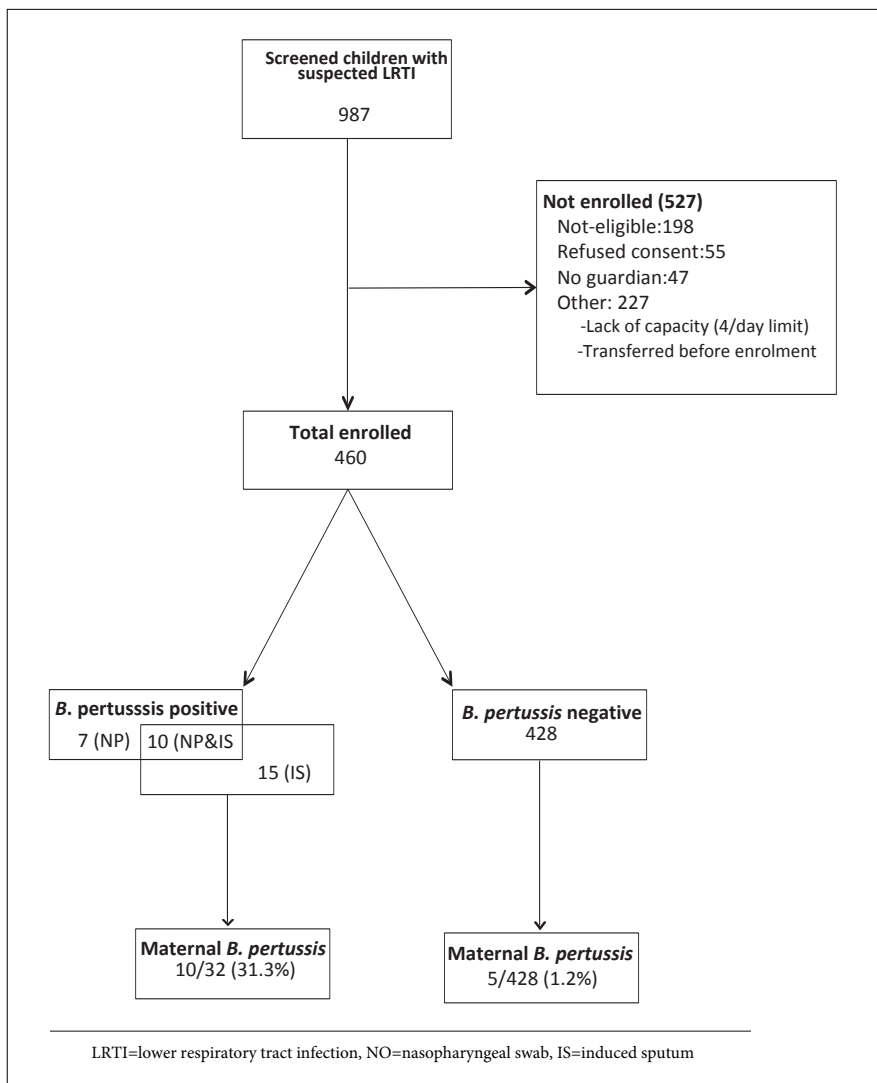
186

187 **Results**

188

189 **Baseline characteristics of children**

190 In total, 987 children hospitalised for acute LRTI were screened; 460 child-caregiver
191 pairs were enrolled; figure 1. The median age of children was 8 (IQR 4-18) months with
192 41 (8.9%) younger than two months of age. The median duration of symptoms was 3
193 days (IQR 2-5 days); 173 (37.6%) received antibiotics prior to admission, Table 1.



194

195 **Fig 1. Enrolment flow diagram of study participants showing**
196 **number of *Bordetella pertussis* positive children and caregivers**
197 LRTI=lower respiratory tract infection, NP=nasopharyngeal swab, IS=induced sputum

198
199
200
201

Table 1: Baseline characteristics of enrolled children (N=460)

Baseline character	Frequency n (%)
Age	
< 2 months old	41 (8.9)
≥ 2 months old	419 (91.1)
Gender	
Female	202 (43.9)
Male	258 (56.1)
Pertussis vaccine doses	
0	28 (6.1)
1	57 (12.4)
2	58 (12.6)
≥ 3	308 (67)
Unknown	9 (2)
Nutritional status (WAZ)#	
Normal nutrition	351 (76.3)
Mild under-nutrition	64 (13.9)
Moderate under-nutrition	33 (7.2)
Severe under-nutrition	12(2.6)
HIV status	
Unexposed Uninfected	349 (75.9)
Exposed Uninfected	92 (20)
Exposed Infected	19 (4.1)
Presenting symptoms	
Cough	456 (99.1)
Apnoea	20 (4.5)
Fever	288 (63.7)
Pre-hospital antibiotic (n=173)	
Penicillin	77 (44.5)
Ceftriaxone	99 (57.2)
Cotrimoxazole	4 (2.3)
Erythromycin	1 (0.4)

Normal: WAZ > -1, Mild: ≤ -1 WAZ > -2, Moderate: ≤ -2 WAZ > -3, Severe: WAZ ≤ -3, WAZ = World Health Organization weight for age Z score

202 Ninety-two (20.0 %) children were HIV exposed and uninfected while infection was
 203 confirmed in 19 (4.1%) (table 1) of whom nine were on antiretroviral therapy, four with
 204 viral suppression. Of the 19 children, one was WHO HIV stage 1; another was HIV stage 2
 205 and 10 were HIV stage 3 and 4 respectively.

206

207 Most children 351(76.3%) were adequately nourished with weight for age Z score > -1.
 208 Mild under-nutrition was found in 64 (13.9%), moderate under-nutrition in 33 (7.2%)
 209 and severe under-nutrition in 12 (2.6%) children; table 1. Only 60 (13.0%) of the

210 children had not received any form of breastfeeding, exclusive or otherwise. The majority
211 (n=323, 77.2%) was breast-fed for the first four months of life and 77 (16.7%) for
212 longer than four months.

213

214 Most children (n=451; 98.0%) had their RTHC available and their immunisation status,
215 including the number of vaccine doses could be verified, table 1. Nineteen (4.2%)
216 children were younger than six weeks and had as yet not received the first dose of
217 pertussis vaccine. Of the 432 (95.8%) old enough to receive at least one vaccine dose,
218 369 (85.4%) had received expected doses for age.

219

220 **Confirmed *Bordetella pertussis* in children**

221 NP specimens were obtained from all child participants. Four children were
222 transferred or discharged out of the ward before an IS specimen could be obtained.

223 For the remaining 456 children, IS was successfully obtained with no major adverse
224 events, although two were later lost to container leakage.

225

226 PCR for IS481 was positive in 17 NP specimens and 25 IS specimens. There was an
227 overlap of positive NP and IS specimens in 10 participants giving a total of 32 (7.0%;
228 95% CI 4.8-9.7%) children with confirmed *B. pertussis* infection. *B. parapertussis*

229 (IS1001+) was detected in seven (1.5%; 95% CI 0.6-3.2%) children. *B. holmesii* was

230 excluded in all the positive specimens by the absence of hIS1001.

231 **Caregiver baseline characteristics**

232 All 460 primary caregivers took part in the study of whom 450 (97.8%) were mothers. For
 233 the remaining 10 children, the caregiver was the father in two (0.4%) instances,
 234 grandmother in five (1.1%) and another relative in the other three (0.7%) children. In
 235 451(98.0%) of the recruited pairs, the caregiver slept in the same room as the child. The
 236 median age of the caregivers was 28 (IQR 24-33) years. In the week the child presented to
 237 hospital, 171 (37.2%) of the caregivers had respiratory symptoms. The symptoms,
 238 predominantly of an upper respiratory tract infection, were present in 10 (31.3%) of
 239 caregivers whose children had confirmed pertussis and in 161 (37.7) whose children did
 240 not have pertussis; p=0.466. Baseline characteristics of the caregivers are summarized in
 241 Table 2.

Table 2: Caregiver characteristics by child's *B. pertussis* PCR status

Baseline character	PCR negative (n=428)	PCR positive (n=32)
	n (%)	n (%)
Gender		
Female	426 (99.6)	32 (100.0)
HIV status		
Infected	88 (22.9)	13 (40.6)
Presenting symptoms		
Cough	96 (22.4)	7 (21.9)
Runny nose	107 (25)	6 (18.8)
Wheeze	35 (8.2)	3 (9.4)
Fever	100 (23.4)	6 (18.8)
<i>B. pertussis</i> NP		
PCR positive	5 (1.2)	10 (31.3)

NP = nasopharyngeal swab specimen

243 HIV infection was present in 111 (24.1%) of caregivers whom 55 (49.5%) were on
244 antiretroviral treatment.

245

246 Cigarette smoking was recorded in 162 (35.2%) of the households although only 33 (7.2%)
247 of the caregivers were themselves smokers. The use of biofuels for cooking or heating was
248 uncommon and reported in 18 (3.9%) households.

249

250 **Confirmed *Bordetella pertussis* in caregivers**

251 NP specimens were successfully obtained from all 460 caregivers. IS481 was positive in 15
252 (3.3%; 95% CI 1.8-5.3%) of the caregivers, 10 in mothers of children with confirmed *B.*
253 *pertussis* infection, table 2. All IS481 positive specimens were negative for the hIS1001 *B.*
254 *holmesii* locus.

255

256 Caregivers with detected nasal *B. pertussis* were all mothers of enrolled children and all slept in
257 the same bedroom with the child. No association was noted between presence of maternal
258 symptoms and confirmed *B. pertussis* infection [4/171 (2.3%) symptomatic versus 11/289
259 (3.8%) asymptomatic; $p=0.392$]. There was no difference in the duration of symptoms between
260 caregivers with confirmed pertussis and those without: median 3 (IQR 2.5-5) days and 2 (IQR 2-
261 5) days respectively; $p=0.513$. *B. pertussis* was detected in 6 (5.4%) of HIV infected caregivers
262 compared to 9 (2.6%) of those who were not; $p=0.214$.

263

264 **Effect of risk factors**

265 Unadjusted and adjusted effects of factors on risk of pertussis disease in children are
 266 shown in Table 3.

Table 3: Risk factors for confirmed *Bordetella pertussis* infection in study children

Risk factor	Risk n/N (%)	Relative Risk (95% Confidence Interval)	
		Crude	Adjusted*
Age			
≥ 2 months old	26/419 (6.2)	1	1
< 2 months old	6/41 (14.6)	2.36 (1.03-5.40)	2.37 (1.03-5.42)
Nutritional status			
Normal	19/351 (5.4)	1	1
Mild under-nutrition	8/64 (12.5)	2.31 (1.06-5.05)	2.27 (1.01-5.09)
Moderate under-nutrition	5/33 (15.2)	2.80 (1.12-7.02)	2.70 (1.13-6.45)
Severe under-nutrition	0/12 (0.0)	NA	NA
HIV status			
Unexposed uninfected	19/349 (5.4)	1	1
Exposed uninfected	10/92 (10.9)	2.00 (0.96-4.15)	3.53 (1.04-12.01)
Infected	3/19 (15.8)	2.90 (0.94-8.96)	4.35 (1.24-15.29)
Pertussis vaccine doses			
None	5/28 (17.9)	1	1
One	4/57 (7.0)	0.49 (0.11-1.35)	0.39 (0.11-1.33)
Two	5/58 (6.9)	0.47 (0.14-1.51)	0.33 (0.09-1.19)
Three and more	19/308 (6.2)	0.33 (0.13-0.81)	0.28 (0.10-0.75)
Caregiver <i>B. pertussis</i>			
PCR negative	22/455 (4.9)	1	1
PCR positive	10/15 (66.7)	13.48 (7.84-23.21)	13.82 (7.76-24.62)
Home cigarette smoking			
No home smoker	21/298 (7.0)	1	1
Home smoker	11/162 (6.8)	0.96 (0.48-1.95)	0.98 (0.49-1.98)
Bio-fuel use			
No bio-fuel	29/442 (6.6)	1	1
Use of bio-fuel	3/18 (16.7)	2.54 (0.85-7.57)	2.40 (0.73-7.91)

n/N (%) = stratum specific proportion and percent. * Multivariable models adjusted for age, sex, HIV status, socio-economic status, breast-feeding and number of household members with cough. Risk ratio 95% confidence intervals that do not cross the null value of 1 are shown in **bold typeface**

268 Clinical features of children with and without pertussis were similar except for fever
269 which was present in 274 (64.0%) of children without pertussis compared to 14
270 (43.8%) in children with pertussis; $p=0.022$. LRTI cases with confirmed *B. pertussis*
271 had a median age of 8 months (IQR 2-21), similar to LRTI cases without pertussis [8
272 months (IQR 4-18)]; $p=0.43$). However, the risk of pertussis was significantly
273 increased in young infants less than two months of age; 14.6% versus 6.2%; aRR 2.37
274 (95% CI 1.03-5.42).

275

276 No association was found between household air pollution or smoking and risk of pertussis
277 was identified even after adjusting for potential confounders.

278

279 Both HIV exposure and HIV infection were independently associated with an increased
280 risk of confirmed *B. pertussis* infection with aRR 3.53 (1.04-12.01) and 4.35(1.24-
281 15.29) respectively. The risk of *B. pertussis* declined with each extra dose of pertussis
282 vaccine independent of age, although the reduction only became significant after completion
283 of the 3-dose primary vaccine schedule; aRR 0.28 (95% CI 0.10-0.75).

284

285 Mild and moderate under-nutrition were also associated with an increased risk of pertussis in the
286 adjusted model, however no cases occurred in severely under-nourished children, table 3.

287

288 Detection of maternal nasal *B. pertussis* was most strongly associated with an increased

289 risk of pertussis in the children with aRR 13.82(7.76-24.62). HIV infected caregivers were
290 more likely to have children with confirmed pertussis infection with 13/111 (11.7%) compared
291 to 19/349 (5.4%) in HIV negative caregivers; p=0.024.

292

293 **Discussion**

294

295 This study reports important, novel findings of significant increased risk of pertussis in
296 children exposed to HIV *in utero* and in children with HIV infection as well as in children
297 with poor nutritional status. In addition, the highest risk of pertussis-associated LRTI in
298 hospitalised African children was in those whose mothers had *B. pertussis* detected in
299 nasopharyngeal specimens, with more than 13 fold increased risk. This study also confirms
300 known factors, namely, incomplete primary vaccination and early infancy as important
301 risks for pertussis in an LMIC setting.

302

303 Sub-Saharan Africa, where this study was conducted, carries a high burden of HIV,
304 including a large number of infected or exposed children. In the current study, after
305 adjusting for potential confounding, HIV-infected children had a four-fold increase in
306 the risk of pertussis, possibly due to reduced vaccine effectiveness due to both poor
307 responses to vaccination as well as low persistence of immunoglobulin following
308 vaccination.[8, 9] This increased risk may also reflect increased parental susceptibility. A
309 study Nigerian study showed a 20-fold risk of pertussis in adolescents not yet initiated
310 on anti-retroviral therapy.[26] Other recent studies have reported an increased risk of

311 pertussis in HIV infected individuals.[27-30]The quality and duration of immunity to
312 pertussis in HIV infected children once they are started on antiretroviral therapy is
313 uncertain.[31] The small number of HIV-infected children in our study made it
314 impossible for us to investigate these aspects.

315

316 HIV-exposed, but uninfected, children are increasingly emerging as a group more
317 susceptible to developing disease compared to unexposed children, due to successful
318 implementation of prevention of mother to child transmission strategies with a
319 reduction in vertically transmitted HIV.[32] This study identifies HIV exposure *in*
320 *utero* as a significant important risk factor for pertussis, consistent with other reports
321 that suggested an increased risk in infants, even if the findings were not significant. In
322 our study a quarter of the mothers were HIV infected. The increased risk in HIV
323 exposed uninfected children seems related to reduced immunoglobulin levels
324 passively transmitted from the mother, increased exposure to pertussis in a HIV-
325 household as well as possible impaired responses to vaccination that are not yet
326 clearly understood.[10, 11]

327

328 The high risk of pertussis-associated LRTI in children whose mothers had
329 nasopharyngeal *B. pertussis* is consistent with studies showing that most infants
330 acquire pertussis from an older sibling or parent.[14] Consequently, attempts to protect
331 young infants have advocated cocooning, which involves vaccinating household
332 members, as well as antenatal and postnatal vaccination of mothers of neonates.

333 Whereas cocooning does not seem cost-effective, antenatal vaccination of mothers has
334 shown promising protection for infants with no added risk to either the mother or the
335 pregnancy.[33-37]. In our study, the risk of pertussis may be partially explained by the
336 high proportion of HIV infected caregivers who exhibited a higher risk for
337 nasopharyngeal carriage compared to HIV uninfected caregivers (5.4% vs 2.6%)
338 although these findings were not statistically significant most likely due to small
339 numbers.

340

341 The risk of *B. pertussis* infection independently decreased with each extra dose of
342 vaccine received, but as observed in other studies, statistically significant reduction
343 was only seen with completion of at least three doses.[38, 39] This highlights the great
344 risk pertussis poses to children in LMIC who, according to WHO, largely receive
345 incomplete vaccination.[40, 41] This risk is further increased by the high incidence of
346 endemic childhood malnutrition.[42]

347

348 The study is limited by low frequencies of pertussis in some subgroups. Even when the
349 study possessed sufficient power to demonstrate statistically significant risk, the estimated
350 magnitude had low precision in some instances. A further limitation is that the study was
351 done in children hospitalised with LRTI so the generalizability of the results to children with
352 less severe illness requires further study.

353

354

355 **Conclusions**

356 There is an urgent need for interventions in LMICs to address modifiable risk factors for
357 pertussis. Such interventions should include nutritional support and immunisation.

358 Immunisation programs should be strengthened to ensure high levels of coverage for children
359 with at least three vaccine doses and include catch-up immunisation for missed doses. A key
360 consideration is to prioritise vaccination of pregnant women, particularly those who are
361 HIV infected, as maternal infection is the greatest risk for disease in infants.[43]

362

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367

368

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369

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Chapter 5

1 **Co-detection of *Bordetella pertussis* and other respiratory organisms in children** 2 **hospitalised with lower respiratory tract infection**

3

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20 Keywords: *Bordetella pertussis*, *Bordetella parapertussis*, pertussis

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32 **Abstract**

33

34 Multiple potential pathogens are frequently co-detected among children with lower
35 respiratory tract infection (LRTI). Evidence indicates that *Bordetella pertussis* has an
36 important role in the aetiology of LRTI. We aimed to study the association between *B.*
37 *pertussis* and other respiratory pathogens in children hospitalised with severe LRTI,
38 and to assess clinical relevance of co-detection. Nasopharyngeal (NP) swabs and
39 induced sputa (IS) were tested with a *B. pertussis* specific PCR; additionally, IS was
40 tested for other pathogens using a multiplex PCR. We included 454 children, median
41 age 8 months (IQR 4-18), 31 (7%) of whom tested positive for *B. pertussis*. Children
42 with *B. pertussis* had more bacterial pathogens detected (3 versus 2; $P < 0.001$). While
43 *B. pertussis* showed no association with most pathogens, it was independently
44 associated with *Chlamydia pneumoniae*, *Mycoplasma pneumoniae* and parainfluenza
45 viruses with adjusted risk ratios of 4.01 (1.03-15.64), 4.17 (1.42-12.27) and 2.13 (1.03-
46 4.55), respectively. There was a consistent increased risk of severe disease with *B.*
47 *pertussis*. Patterns indicated even higher risks when *B. pertussis* was co-detected with
48 any of the three organisms although not statistically significant. Improving vaccine
49 coverage against *B. pertussis* would impact not only the incidence of pertussis but also
50 that of severe LRTI generally.

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68 **Introduction**

69

70 Lower respiratory tract infection (LRTI) is responsible for a large burden of morbidity
71 and mortality in children each year. [1] Current understanding is that the aetiology of
72 LRTI may frequently be polymicrobial, with various combinations of viral and
73 bacterial pathogens implicated in the pathogenesis.[2-5] *Bordetella pertussis*, the
74 organism that causes whooping cough, is one of the organisms strongly associated
75 with LRTI in children, however the role of other organisms in the pathogenesis of
76 LRTI is not well-understood. [4]

77

78 Effective vaccines against *B. pertussis* have been available since the 1940's. Initially
79 these were of the whole cell type (wP) but have since the 1990's been superseded by
80 acellular vaccines (aP) mainly in high income countries.[6] The introduction and wide
81 use of vaccines have markedly reduced the burden of pertussis over the last six
82 decades, but there is strong evidence that pertussis has resurged all over the world in
83 recent years, bringing into focus *B. pertussis* as an important respiratory pathogen in
84 the aetiology of respiratory illness, including LRTI.[7, 8]

85

86 Although the presence of *B. pertussis* has been described together with other
87 organisms that are potential co-pathogens in individuals with LRTI, most studies have
88 focused on the role of viruses in respiratory tract co-infections.[9] As a result,
89 potential interactions between *B. pertussis* and other organisms remain poorly
90 understood.[10-12] The organism, *B. pertussis*, produces several toxins that aid the
91 organism in evading the immune system, assisting it to establish infection on the
92 respiratory epithelium.[13, 14]

93 We hypothesise that the conditions produced by *B. pertussis* toxins not only create a
94 conducive environment for *B. pertussis* itself but may facilitate the colonisation or
95 infection of the respiratory tract epithelium by other bacteria or viruses. In this study,
96 we aimed to investigate whether the detection of *B. pertussis* in children hospitalised
97 for LRTI was associated with co-detection of other potential respiratory pathogens.
98 We also explore whether there was an association between co-detection and clinical
99 severity and outcome.

100

101 **Methods**

102

103 **Recruitment and specimen collection**

104

105 Methods for sampling as well as inclusion criteria have been described
106 elsewhere.[15] Briefly, the study recruited inpatient children seen at a referral
107 hospital, Red Cross War Memorial Children's Hospital (RCH) in Cape Town,
108 South Africa over a one-year period (September 2012 to September 2013).
109 Children were recruited if they presented with cough and WHO defined age
110 specific tachypnoea, or apnoea, and were ill enough to warrant admission. Only children
111 whose legal guardians were present to give written consent were enrolment.
112 Being in contact with the health care services in the preceding two weeks was an
113 exclusion criterion.

114

115 To assess the severity of respiratory symptoms, the presence of chest indrawing was
116 noted. In addition, all children had pulse oximetry to assess for oxygen saturation.
117 A cut-off of <94% was used to define hypoxaemia in children at sea level.[16-18]
118 A detailed history of the current illness was collected, and participants underwent

119 testing for HIV infection. The diagnosis of HIV infection was made for children
120 less than 18 months of age if they tested positive for two HIV PCR tests (COBAS
121 AmpliPrep/COBAS Taqman HIV-1, Roche Molecular Diagnostics, Pleasanton,
122 CA). For children above 18 months of age, HIV infection was diagnosed on the
123 basis of two positive ELISA tests using two different assays (Architect HIV
124 Ag/Ab Combo, Abbott Diagnostics, Wiesbaden; and Enzygnost Anti-HIV 1/2
125 Plus, Siemens/Dade Behring, Erlange sequentially).

126

127 All children had anthropometry (weight and height), performed at enrolment by
128 trained study staff. Nutritional status was classified using WHO weight for age z-
129 scores (WAZ). Children were classified as moderate to severely malnourished if
130 their weight for age fell below -2 z-scores.

131

132 The children's vaccination status was sourced from their handheld clinic booklets. The
133 primary schedule according to the South African Expanded Program on
134 Immunisation, in addition to other vaccines, contains an acellular (aP) vaccine against
135 *B. pertussis* combined with that against *Haemophilus influenzae type b* at 6, 10 and 14
136 weeks (with a booster at 18 months), and vaccination with 13-valent pneumococcal
137 conjugate (PCV13) vaccine at 6 and 10 weeks of age (with a booster at 9 months)

138 .[19]

139

140 A nasopharyngeal (NP) swab was collected first after which an induced sputum (IS)
141 specimen was collected on enrolment as previously described.[20] Molecular
142 diagnostic testing was carried out on batched specimens as described below. No
143 blood culture was done as part of the study.

144

145 **Laboratory methods**

146

147 Diagnosis of *Bordetella pertussis*

148 To diagnose *Bordetella pertussis* infection, PCR specific for IS481 for *Bordetella*
149 species was conducted on both NP and IS specimens with a validated commercial kit
150 (Roche LightMix®, Basel) using previously published primers.[21] *Bordetella*
151 *holmesii* (defined as IS481 + and hIS1001 +) infection was excluded by further
152 testing all IS481 positive specimens for the presence of insertion site hIS1001.[22]

153

154 Diagnosis of co-infections

155 The FTDRsp 33 multiplex real-time PCR assay (Fast-Track Diagnostics, Esch-sur-
156 Alzet, Luxembourg) was used to identify presence of a range of viruses and bacteria
157 as well as *Pneumocystis jirovecii* on IS. As the study was designed specifically to
158 study the epidemiology of pertussis in this population, for the analysis, *B. pertussis*
159 results from LightMix® for *B. pertussis* (rather than those from FTDRsp 33)
160 were used as our assessment indicated that the assay had better sensitivity for *B.*
161 *pertussis* than Fast-Track, although both employ the same targets.

162

163

164 **Statistical analysis**

165

166 We used percentages to depict proportions of study participants with organisms
167 detected from respiratory specimens. Continuous data were tested for normality
168 and summarized as medians with interquartile ranges (IQR) or means and
169 standard deviations (SD) as appropriate. The difference in total numbers of organisms
170 detected in participants with and without confirmed *B. pertussis* was compared using

171 Student's t-test. χ^2 or Fisher's exact tests were used to assess the strength of association
172 between infection with *B. pertussis* and each co-pathogen. All associations at a two-tailed
173 $p < 0.1$ were further analysed adjusted for sex, age and HIV status as potential
174 confounders. Generalised linear modelling using Poisson regression with robust
175 error variance was used to estimate adjusted relative risks (aRR) and their 95%
176 confidence intervals in a multivariable analysis. Severity of clinical disease and
177 outcomes were further analysed stratified by a combination pertussis status and organisms
178 showing strong association with pertussis. Continuous data were tested for normality and
179 comparisons between groups were made with the appropriate test for parametric or non-
180 parametric data as indicated. Statistical significance was set at a two-side $P < 0.05$. All
181 analyses were carried out using *Stata Statistical Software Release 13* (StataCorp
182 LP, College Station, TX).

183

184 **Statement on ethics approval**

185 Prior approval for the study was obtained from the Human Research Ethics
186 Committee of the Faculty of Health Sciences of the University of Cape Town;
187 reference: 371/2011. Written informed consent was sought and received from the
188 parent or legal guardian of each child in order for the child to participate in the
189 study. All methods were carried out in accordance with the relevant guidelines
190 and regulations.

191

192

193

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196

197 **Results**

198

199 **Baseline data**

200 Four hundred and sixty children were enrolled. Six children, including four whose IS
201 could not be collected and two whose IS were lost prior to processing during transportation
202 or storage, were excluded, providing 454 participants with sufficient data for analysis, of
203 which 253 (55.7%) were male.

204

205 The median age of the children was 8 (IQR 4 -18) months. HIV infection was
206 confirmed in 19 (4.2%) of the children. Nine children (2.0%) did not have their
207 immunisation records with them. Of the 445 (98.0%) with known vaccination
208 status, 321(72.1%) were up to date with pertussis and *Haemophilus influenzae type*
209 *b* vaccine doses for age, while 427 (96.0%) had received at least one dose of the
210 combination. Similarly, 312 (70.1%) were up to date with PCV13 doses for age
211 with 385 (86.5%) having received at least one dose of the vaccine. Baseline
212 characteristics of the study group are summarized in Table 1.

213

214

215

216

217

Table 1: Baseline characteristics of study participants

Baseline character	<i>N</i> =454
Age	
Median (IQR) months	8 (4-18)
Male sex	n (%) 253 (55.7.)
Pertussis/<i>H. influenzae type b</i> vaccines	
0	31 (6.8)
1	59 (13.0)
2	61 (13.4)
≥ 3	294 (64.8)
Unknown	9 (2.0)
PVC13 vaccine doses	
0	60 (13.2)
1	108 (23.8)
2	139 (30.6)
3	138 (30.4)
Unknown	9 (2.0)
HIV infected	19 (4.2)
Nutritional status	
Normal	384 (90.8)
WAZ ≤ -2	39 (9.2)
Pre-hospital antibiotic	
Yes	153 (36.2)
No	270 (63.8)
Oxygen saturation < 94% in room air	70 (15.4)
Chest indrawing	380 (83.7)
Confirmed <i>Bordetella pertussis</i>	31(6.8)

WAZ=World Health Organization weight for age Z-score < -2

218

219 **Confirmed *Bordetella pertussis* infection**

220 PCR for insertion site *IS481* was positive in 16 NP specimens and 25 IS specimens

221 on LightMix®. Ten participants had a positive PCR on both NP and IS specimens,

222 therefore 31 (6.8%; 95% CI 4.7-9.6%) participants were confirmed as having *B.*

223 *pertussis* infection. The *B. holmesii* insertion site *hIS1001*, was not identified in

224 any of the *IS481* positive specimens. Only 10 (2.2%) samples, all also found to be

225 positive on LightMix®, were positive for *B. pertussis* on Fast-Track testing of IS
226 samples.

227

228 The median age of children with confirmed *B. pertussis* was 8 (IQR 2-22) months
229 while those testing negative had a median age of 8 (IQR 4-18) months; P=0.559.

230

231 **General description of PCR detected pathogens**

232 In most participants (n=412; 90.7%) both a viral and bacterial organism were co-
233 detected from the IS specimen. Four hundred and forty-one (97.1%) participants had
234 at least one virus identified from an IS specimen. There were 29 (6.4%) participants in
235 whose specimens only a viral pathogen was detected with no bacterial pathogens detected. In
236 421 (92.7%) at least one bacterial species was identified. There were 9 (2.0%) participants in
237 whose specimens only a bacterial pathogen was detected with no viral pathogens detected.
238 Four (0.9%) of the 454 children did not have any organism (including *B. pertussis*)
239 identified from their IS specimen.

240

241 For all participants with confirmed *B. pertussis* infection, a minimum of two
242 organisms were identified. The average number of viruses detected in children with
243 and without confirmed pertussis were 2.5 (SD 1.4) and 2.3 (SD 1.3) respectively;
244 P=0.665. Children with confirmed pertussis had on average 3.0 (SD 1.6) different
245 bacterial species detected while those without had 2.0 (SD 1.1); P<0.001. When both
246 bacterial and viral organisms were considered, the average number identified in
247 pertussis positive participants was 5.5 (SD 2.0) and 4.4 (SD 1.9) in the pertussis
248 negative group; P=0.009. Figure 1.

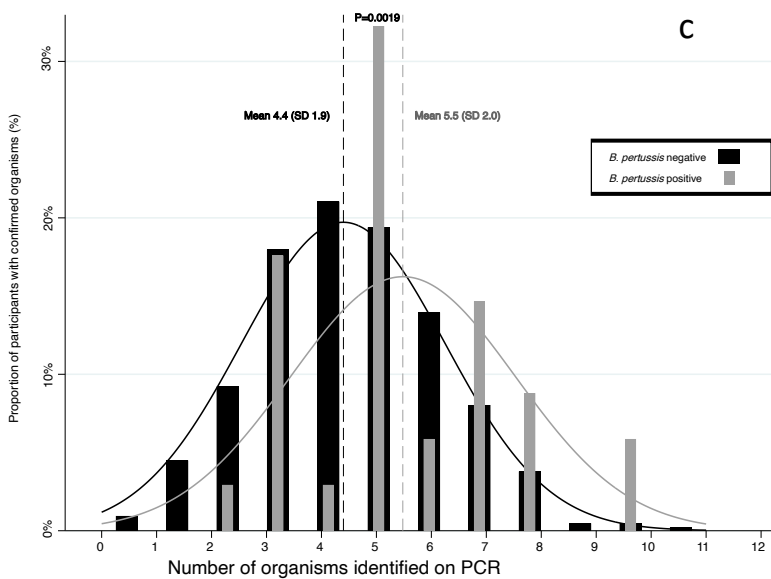
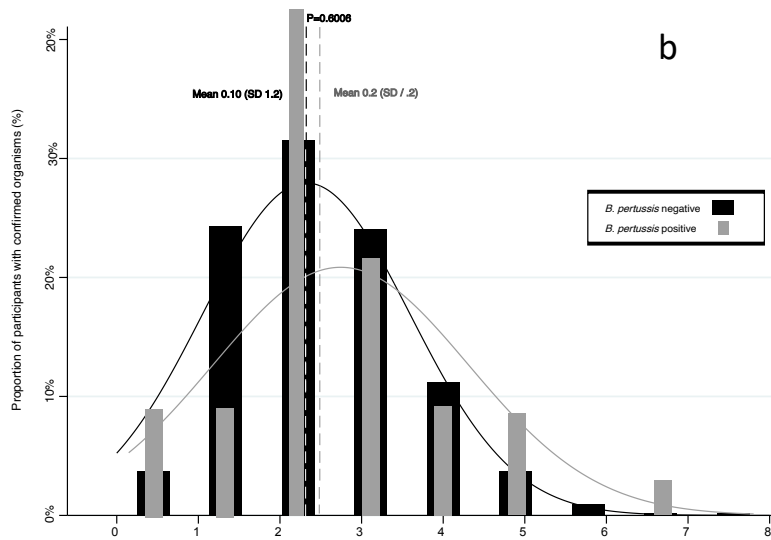
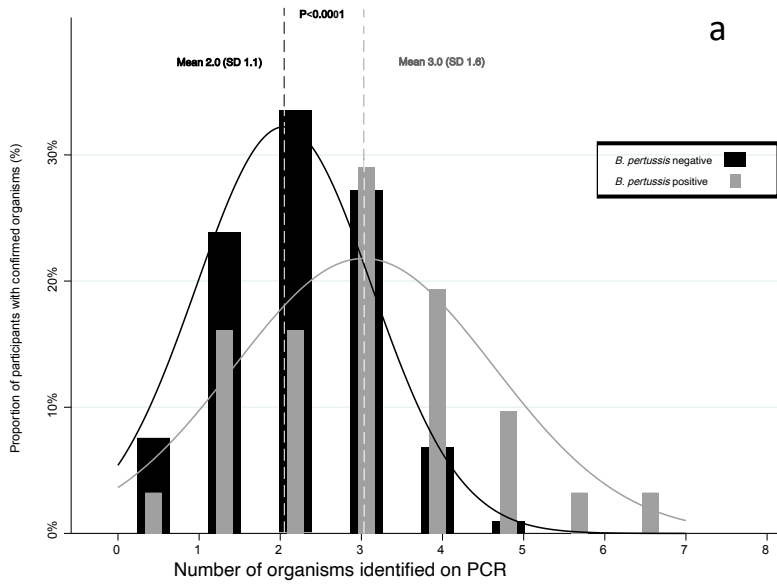


Figure 1. Distribution of number of bacteria (a), viruses (b) and bacteria + viruses (c) identified on polymerase chain reaction (PCR) in participants with and without *Bordetella pertussis*

250 The prevalence of specific organisms identified on IS in participants with and without
 251 confirmed pertussis is shown in descending order of frequency in Table 2.

252

Table 2: Association between *Bordetella pertussis* and other organisms isolated on IS (N=454)

Pathogen [¶]	Total n (%)	<i>Bordetella pertussis</i> PCR n (%)		
		Positive n=31	Negative n=423	P value [#]
Viral organisms				
Cytomegalovirus	253 (55.7)	17 (54.8)	236 (55.8)	0.918
Rhinovirus	222 (48.9)	17 (54.8)	205 (48.5)	0.493
Respiratory Syncytial	135 (30.7)	5 (16.1)	130 (30.7)	0.086
Adenovirus	117 (25.8)	9 (29.0)	108 (25.5)	0.667
Bocavirus	80 (17.6)	8 (25.8)	72 (17.0)	0.215
Enterovirus-parechovirus	80 (17.6)	7 (22.6)	73 (17.3)	0.453
Parainfluenza (1,2,3,4)	75 (16.5)	9 (29.0)	66 (15.6)	0.052
Metapneumovirus A & B	45 (9.9)	1 (3.2)	44 (10.4)	0.345
Coronavirus	35 (7.7)	3 (9.7)	32 (7.6)	0.723
Influenza (A, B, C)	28 (6.2)	0 (0.0)	28 (6.6)	0.244
Bacterial organisms				
<i>Moraxella catarrhalis</i>	295 (65.0)	18 (58.1)	277 (65.5)	0.403
<i>Streptococcus pneumoniae</i>	240 (52.9)	17 (54.8)	223 (52.7)	0.819
<i>Haemophilus influenzae</i>	231 (50.9)	18 (58.1)	213 (50.4)	0.407
<i>Staphylococcus aureus</i>	136 (30.0)	9 (29.0)	127 (30.0)	0.907
<i>Haemophilus influenzae B</i>	16 (3.5)	2 (6.5)	14 (3.3)	0.299
<i>Mycoplasma pneumoniae</i>	10 (2.2)	3 (9.7)	7 (1.7)	0.025
<i>Chlamydia pneumoniae</i>	7 (1.2)	2 (6.5)	5 (1.2)	0.076
Fungal organism				
<i>Pneumocystis jirovecii</i>	98(21.6)	8 (25.8)	90 (21.3)	0.554

IS=induced sputum. # Two-sided Fisher's exact or Chi Square tests P-values; Bold Typeface = P < 0.1

¶ Organisms shown in descending order of total frequency for each pathogen group

253

254 Co-detection of *Bordetella pertussis* with specific organisms

255 Overall, *Moraxella catarrhalis* was the commonest bacterium identified with 295

256 (65.0%) samples testing PCR positive for the organism. *Mycoplasma pneumoniae*

257 showed association with *B. pertussis* infection (P=0.025) while *Chlamydia*

258 *pneumoniae* displayed weak evidence of association with *B. pertussis* (P=0.076). No

259 other bacteria were significantly associated with confirmed pertussis. Table 2.

260 Cytomegalovirus was the commonest virus, identified in 253 (55.7%) of the

261 participants. Parainfluenza viruses (1, 2, 3 &4) were weakly associated (P=0.052)

262 with the presence of *B. pertussis*, while Respiratory Syncytial Virus (RSV) was

263 weakly associated with the absence of *B. pertussis* (p=0.086). The other viruses did
 264 not show any association with *B. pertussis* infection. Table 2. *Pneumocystis jirovecii*
 265 was detected in 8 (25.8%) and 98 (21.6%) of participants with and without confirmed
 266 pertussis, respectively, P=0.554).

267

268 After adjusting for potential confounding, two bacterial pathogens, namely *C.*
 269 *pneumoniae* (aRR 4.01 (95% CI 1.03-15.64) and *M. pneumoniae* (aRR 4.17 (95% CI
 270 1.42-12.27) remained independently associated with confirmed *B. pertussis* infection.
 271 Parainfluenza viruses (aRR 2.13 (95% CI 1.03-4.55) was the one group of viruses that
 272 showed significant independent association with confirmed pertussis. Lack of strong
 273 association between the absence of *B. pertussis* and RSV remained unchanged after
 274 adjusting for potential confounding. Table 3.

275

Table 3. Risk of lower respiratory co-infection in children with confirmed *Bordetella pertussis* infection

Co-infection	<i>Bordetella pertussis</i> PCR n (%)		RR (95% Confidence interval)	
	Positive n=31	Negative n=423	Crude	Adjusted [#]
<i>Chlamydia pneumoniae</i>	2 (6.5)	5 (1.2)	4.40 (1.29-14.98)	4.01 (1.03-15.64)
<i>Mycoplasma pneumoniae</i>	3 (9.7)	7 (1.7)	4.75 (1.73-13.11)	4.17 (1.42-12.27)
Parainfluenza (1,2,3,4)	2 (6.5)	4 (1.0)	2.07 (0.99-4.31)	2.13 (1.03-4.55)
Respiratory syncytial virus	5 (16.1)	130 (30.7)	0.45 (0.18-1.16)	0.45 (0.17-1.20)

RR = Relative risk; [#] Multivariable model adjusted for age in months, sex and HIV status; confidence intervals not overlapping the null value of 1 are shown in bold typeface.

276

277 **Clinical presentation and outcome**

278

279 Only three (9.7%) of the 31 *B. pertussis* PCR positive cases were diagnosed clinically
 280 with pertussis. All 454 participants were discharged from hospital with no in-hospital
 281 deaths occurring in both the *B. pertussis* PCR positive and negative groups. Twelve
 282 (2.4%) children required a High Dependency Unit or Paediatric Intensive Care Unit
 283 admission; slightly higher frequency in children with confirmed *B. pertussis* with

284 two out of 31 (6.5%) and 10 out of 423 (2.4%) in the positive and negative groups
285 respectively; P=0.194. Due to the small numbers, no further analysis was possible.
286 Hypoxaemia as indicated by oxygen saturation of <94% was noted in 70 (15.4%)
287 children while chest indrawing was noted in 380 (83.7%). In general, children with
288 confirmed pertussis showed higher frequencies of chest indrawing with 28 (90.3%)
289 out of 31 compared to 352 (83.2%) of the 423 (P=0.449) without pertussis.
290 Similarly, there were 9 (29.0%) out of 31 compared to 61(14.4%) out of 423
291 (P=0.039) showing hypoxaemia in children with and without *B. pertussis*,
292 respectively.

293

294 The same pattern was noted with the two bacterial organisms whose detection was strongly
295 associated with *B. pertussis*. Figure 2. A higher risk of severe disease was seen when *B.*
296 *pertussis* was detected with each organism than when *B. pertussis* or each of the bacteria
297 was detected on its own. No hypoxaemia was noted in any of the *C. pneumoniae* positive
298 children who did not have *B. pertussis*. With parainfluenza viruses, this pattern was noted
299 only with respect to hypoxaemia but not with chest indrawing. The small sample sizes of
300 each stratum were not sufficient to allow for meaningful formal assessment of strength of
301 association.

302

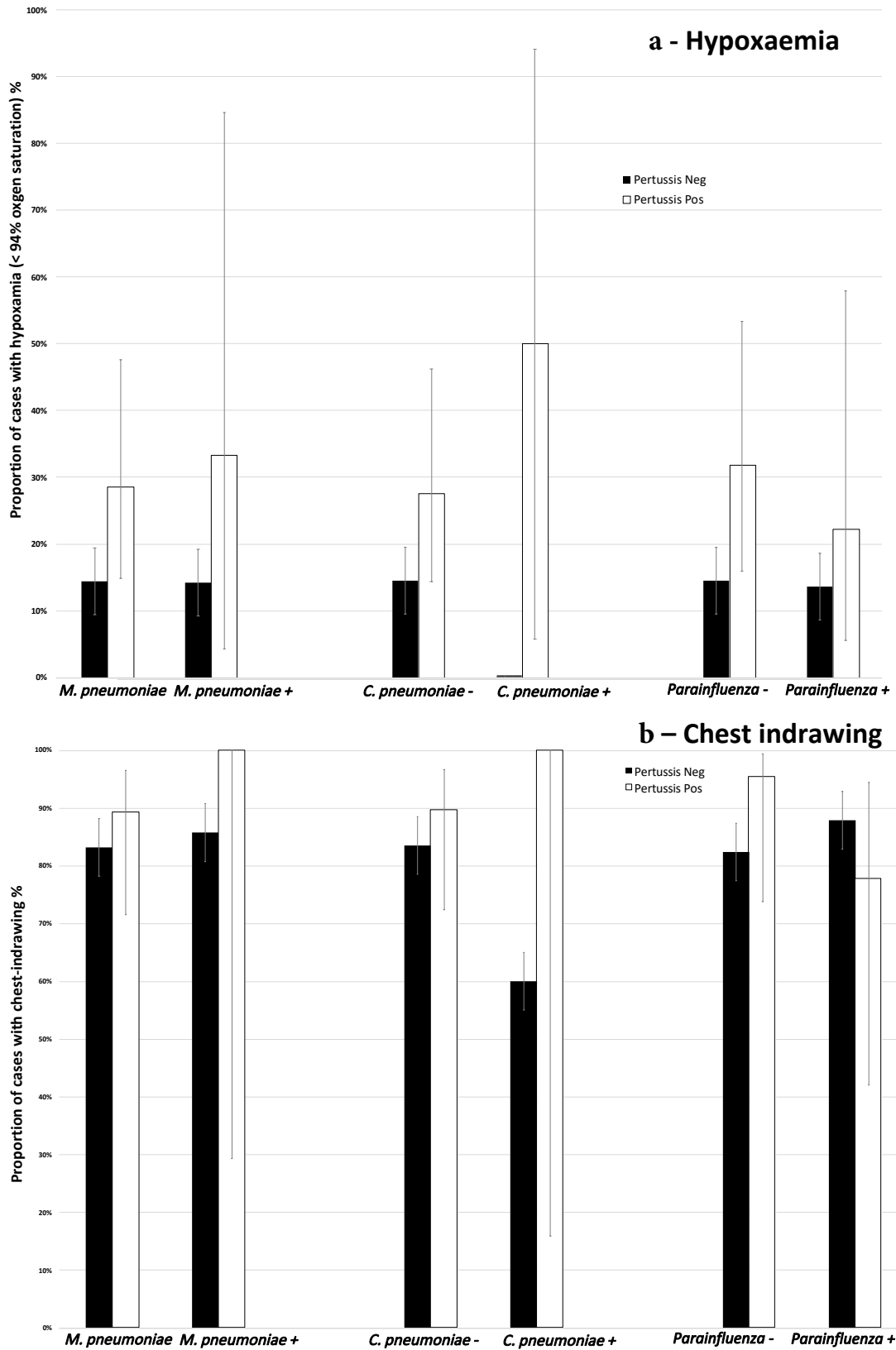


Figure 2. Proportion of children with hypoxaemia and chest indrawing by presence of co-detected organisms that were strongly associated with pertussis

304 The median length of hospital stay was similar in children with confirmed *B.*
305 *pertussis* [2 (IQR 1- 5) days] and children testing negative [2 (IQR 1-4) days];
306 P=0.522. The co-detection of organisms independently associated with pertussis
307 did not have an effect on the length of hospital stay.

308

309 **Discussion**

310 This study demonstrates a high prevalence of bacteria and viruses involved in LRTI in
311 children requiring hospitalisation for severe disease. The study also shows that only a
312 few of these organisms are specifically associated with *B. pertussis* co-detection. The
313 detection of *B. pertussis* was however strongly associated with a higher number of co-
314 detected potential respiratory pathogens, specifically bacterial organisms. Although
315 most of the studied potential respiratory pathogens did not show association with the
316 presence of *B. pertussis*, three, namely *C. pneumoniae*, *M. pneumoniae* and
317 parainfluenza viruses, were independently associated with being co-detected with *B.*
318 *pertussis*. In addition, the study shows some evidence, albeit weak, that the co-
319 detection of *B. pertussis*, together with these three organisms may be a risk for severe
320 illness.

321

322 The finding of other pathogens in a respiratory tract specimen together with *B.*
323 *pertussis* is not by itself remarkable. As noted earlier, several studies have shown that
324 multiple potential pathogens are frequently identified from children with respiratory
325 infections, including pertussis.[4, 23] What is of interest, however, is the finding of
326 higher numbers of potential pathogens, in particular bacterial, significantly associated
327 with the presence of confirmed *B. pertussis*; and the association of specific organisms
328 with confirmed *B. pertussis*.

329

330 Participants with *B. pertussis* had more organisms detected in their sputum samples,
331 compared to those without. This association was significant for bacterial pathogens, a
332 finding which had an impact also on association with overall total number of all
333 detected organisms. In addition, patients with *B. pertussis* had a fourfold increase in
334 the risk for detection of either *C. pneumoniae* or *M. pneumoniae*, as well as twice the
335 risk to detect parainfluenza viruses.

336

337 In this study, although the number of viruses associated with *B. pertussis* was
338 marginally higher, this finding was not of statistical significance. This is in keeping
339 with findings of other studies that show little association between *B. pertussis* and
340 viral infections.[24] Specifically, our study showed a negative correlation between *B.*
341 *pertussis* and *RSV* although this finding was also not of statistical significance. This
342 pattern was however in keeping with published literature where a negative association
343 was noted between *RSV* and pertussis or between pertussis and bronchiolitis, a disease
344 mainly attributable to *RSV*.[25, 26] We did however, find independent association
345 between detection of parainfluenza viruses and the presence of *B. pertussis*.

346

347 Children with pertussis generally showed more severe clinical illness (hypoxaemia,
348 chest-indrawing and need for high dependency care). Although these findings were
349 not all statistically significant due to the small numbers, the pattern was consistent.

350 Children who had both strongly associated organisms and *B. pertussis* detected
351 showed additional risk of severe disease, again without statistical significance. It is of
352 note that the three organisms independently associated with *B. pertussis* are associated
353 with 'atypical' or interstitial pneumonia which commonly presents with impaired
354 oxygenation. There was no difference in mortality (study registered no deaths) and

355 length of hospital stay.

356

357 There is evidence associating *B. pertussis* with pneumonia in children. [4] Although
358 the first vaccine specifically targeting pneumonia was only introduced with the
359 registration of a vaccine against *Haemophilus influenzae b* in 1985, the decline in
360 pneumonia-associated mortality in the United States was noted three decades
361 earlier.[27] This decline can not be fully explained by the improvement in quality of
362 health care alone, and may in part be explained by the rapid decline in reported
363 pertussis cases following the introduction of DPT in the 1940s. The decline in
364 pneumonia-associated mortality mirrors that of pertussis over the period.

365

366 Due to the small sample size, our study was limited both in exploring the effect of
367 other factors such as HIV infection and in its ability to establish strong evidence for
368 some associations, even where patterns suggested a correlation. In some instances, in
369 which strong association was demonstrated, the precision of the estimated risk was
370 low due to the same limited sample size. In addition, the study is limited only to the
371 organisms included in the multiplex PCR, noting also that distinguishing between
372 benign colonisation and pathological infection from PCR detection of an organism in
373 the respiratory tract remains a challenge. [3] As such these findings must be
374 interpreted with caution.

375

376 Further well powered studies or creative metanalytical systematic reviews are
377 required, to study this phenomenon further.

378

379 Partial and waning immunity, especially in individuals vaccinated with acellular

380 pertussis (aP) vaccines, has been shown to lead to pertussis not presenting in a
381 classical manner.[28-30] This atypical nature of presentation may lead to the
382 diagnosis being missed unless a high index of suspicion is maintained. South Africa,
383 where this study was conducted, changed from wP to aP containing vaccines in early
384 2009. [19]

385

386 Less than 10% of children with confirmed *B. pertussis* in this study were clinically
387 suspected of having possible pertussis, which highlights the need for laboratory
388 support in the diagnosis of the disease. Where the diagnosis needs confirmation, the
389 use of an additional IS specimen seems to improve detection rates.[4, 15]

390

391 As we have shown previously, isolation of *B. pertussis* in this cohort of children was
392 strongly associated with incomplete primary vaccination.[15] Improving vaccine
393 coverage for pertussis remains the most affordable and effective tool to decrease the
394 incidence of pertussis, and indirectly that of severe LRTI due to associated pathogens.

395

396

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399 staff for their immense contribution. The authors acknowledge financial support to
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401 South African Medical Research Council.

402 **Author contribution statement**

403 RM conceptualised and designed the study with the input from GDH and HJZ. FSD
404 was responsible for the laboratory aspect of the study under the supervision of MPN.
405 RM did the data analysis. All authors were involved in the overall interpretation of
406 the data. RM drafted the initial manuscript and updated it following inputs from all
407 the authors. All authors reviewed and approved the final manuscript.

408 **Competing interests statement**

409 The authors declare no competing interests.

410

411 **Data availability**

412 All data analysed during the current study have been presented in this manuscript, but

413 the corresponding datasets generated are available from the corresponding authors on

414 reasonable request.

415

416

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418

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- 506

Chapter 6

1 **Diagnostic limitations of clinical case definitions of pertussis in infants and** 2 **children with severe lower respiratory tract infection**

3

4 **Limited diagnostic accuracy of pertussis clinical case definitions**

5

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30

31 **Abstract**

32

33 **Introduction:** Diagnosis of pertussis is challenging especially in infants. Most
34 low and middle-income countries (LMIC) lack resources for laboratory confirmation,
35 relying largely on clinical diagnosis alone for both case management and surveillance.
36 This necessitates robust clinical case definitions.

37 **Objectives:** This study assesses the accuracy of clinical case definitions with and
38 without lymphocytosis in diagnosing pertussis in children with severe lower
39 respiratory tract infection (LRTI) in a LMIC setting.

40 **Methods:** Children hospitalised with severe LRTI in a South African hospital were
41 prospectively enrolled and evaluated for pertussis using PCR on respiratory
42 samples. Clinical signs and differential white cell counts were recorded.
43 Sensitivity and specificity of pertussis clinical diagnosis using WHO and Global
44 Pertussis Initiative (GPI) criteria; and with addition of lymphocytosis were
45 assessed with PCR as the reference standard.

46 **Results:** 458 children <10 years were enrolled. *Bordetella pertussis* infection was
47 confirmed in 32 (7.0%). For WHO criteria, sensitivity was 78.1% (95% CI 60.7–
48 89.2%) and specificity 15.5% (95% CI 12.4 –19.3%); for GPI sensitivity was 34.4%
49 (95% CI 20.1– 52.1) and specificity 64.8% (95% CI 60.1 – 69.2%). Area under the
50 curve (AUC) on receiver operating character (ROC) analysis was 0.58 (95% CI 0.46-
51 0.70 for WHO criteria, and 0.72 (95% CI 0.56-0.88) for GPI with highest likelihood
52 ratios of 5.33 and 4.42 respectively. Diagnostic accuracy was highest between five and
53 seven days of symptoms for both criteria. Lymphocytosis had sensitivity of 31.3%
54 (95% CI 17.5 - 49.3%) and specificity of 70.7% (95% CI 66.1-74.8%) and showed a
55 marginal impact on improving clinical criteria.

56 **Conclusion:** Clinical criteria lack accuracy for diagnosis and surveillance of
57 pertussis. Non-outbreak settings should consider shorter durations in clinical
58 criteria. New recommendations still fall short of what is required for a viable
59 clinical screening test which means the need to improve access to laboratory
60 diagnostic support remains crucial.

61

62 **Introduction**

63

64 Pertussis has resurged globally over the last decade. Waning immunity change in
65 primary schedule to acellular vaccines and improvement in diagnostics have been
66 given as possible explanations. [1, 2] A large proportion of reported cases have not
67 shown the classic presentation of a prolonged spasmodic cough with inspiratory
68 whoop and post tussive vomiting.[3] In response, some high income countries (HICs)
69 have modified their case definitions to suit local diagnostic and surveillance criteria;
70 usually this includes reducing duration of cough symptoms to lower than the two
71 weeks historically recommended as the minimum cut-off time.[4]

72

73 The World Health Organization (WHO) developed criteria for clinically defining
74 cases of pertussis. These include presence of a cough for at least 14 days
75 characterized by one of paroxysms, inspiratory whoop or post-tussive vomiting, table
76 1.[5] Apart from reduction by a week in the duration of cough from more than 21
77 days, very little has changed over the last three decades in the WHO case definition of
78 pertussis.[6]

79

80 Acknowledging shortcomings of WHO clinical case definition, the Global Pertussis

81 Initiative (GPI), following a Roundtable discussion in 2010, recommended changes to
 82 improve diagnostic sensitivity and specificity for pertussis. In addition to clinical
 83 features found in WHO criteria, the GPI suggests adding presence of coryza, apnoea,
 84 seizures or cyanosis as well as absence of fever, and shortens duration of symptoms to
 85 7 days. GPI clinical features vary by age categories: less than four months of age, four
 86 months to nine years and 10 years and above, table 1.[4]

Table 1: Clinical features for diagnosis of pertussis cases

<u>World Health Organization</u>	<u>Global Pertussis Initiative</u>	
<ul style="list-style-type: none"> ▪ A case diagnosed as pertussis by a physician, <u>OR</u> ▪ A person with a cough lasting ≥ 2 weeks with ≥ 1 of the following: symptoms: <ul style="list-style-type: none"> • Paroxysms (i.e., fits) of coughing • Inspiratory “whooping” • Post-tussive vomiting (i.e., vomiting immediately after coughing) without other apparent cause 	▪ Cough or illness in a person without or with only minimal fever <u>AND:</u>	<ul style="list-style-type: none"> ▪ Cough or illness in a person without or with only minimal fever <u>AND:</u>
	<u>0 – 3 months</u>	<u>4 months to 9 years</u>
	Cough and coryza PLUS any of <ul style="list-style-type: none"> • Whoop • Apnoea • Post-tussive emesis • Cyanosis • Seizure • Pneumonia • Close exposure to an adolescent or adult (usually a family member) with a prolonged afebrile cough illness 	Paroxysmal cough PLUS any of <ul style="list-style-type: none"> • Whoop • Apnoea • Post-tussive emesis • Worsening of symptoms at night • Seizure • Pneumonia • Close exposure to an adolescent or adult (usually a family member) with a prolonged afebrile cough illness

Adapted from World Health Organization & Cherry et al (2012)[4, 5] NB. Global Pertussis Initiative criteria for individuals older than 9 years not shown

87

88 Although culture, ELISA and direct fluorescent antigen testing can all be used to
 89 confirm pertussis, polymerase chain reaction (PCR) has gained favour as the most
 90 practical confirmatory method with an acceptable level of sensitivity and
 91 specificity.[7, 8] In contrast to HICs which have access to laboratory resources to
 92 validate clinical suspicion, most low and middle-income countries (LMICs) lack
 93 resources for laboratory confirmation, relying largely on clinical diagnosis alone.[9] It
 94 is therefore imperative to have a robust clinical definition in these settings.

95

96 Pertussis is commonly associated with leukocytosis, with an increase in lymphocytes.
 97 [10] In the absence of resources for confirming pertussis, clinicians have also used

98 lymphocytosis to support a clinical diagnosis.[11]

99

100 Here we investigate the sensitivity and specificity of clinical criteria recommended by
101 WHO and GPI to diagnose pertussis with PCR as the diagnostic reference standard, in
102 a LMIC. The analysis includes assessment of the impact of duration of symptoms on
103 sensitivity and specificity for both sets of diagnostic criteria. Secondly, we assess
104 the impact of adding lymphocytosis to clinical criteria to improve diagnostic
105 accuracy. WHO criteria were selected as they represent the reference criteria used by
106 most countries and they also form the basis for pertussis clinical criteria adopted with
107 some amendments by most national health administrations. The GPI was used as a
108 comparator as it represents the most radical proposal to changes to the WHO criteria
109 independent of national context.

110

111 **Materials and Methods**

112

113 A prospective study was conducted from September 2012 to September 2013, to
114 investigate the incidence of pertussis in children hospitalised for severe lower
115 respiratory tract infection (LRTI), or with apnoea, at a tertiary hospital in South
116 Africa. Children were included if they presented with WHO defined severe LRTI or
117 apnoea and had not been in touch with the health care services in the preceding two
118 weeks after written informed consent was received from the parent or legal guardian.
119 A nasopharyngeal swab and an induced sputum specimen were taken from each child
120 and sent to the laboratory for culture and pertussis PCR.

121

122 The duration and character of cough, especially presence of paroxysms, inspiratory

123 whoop, or post-tussive vomiting; as well as presence of apnoea, cyanosis, seizures
124 and fever were recorded. In addition, any prior exposure to antibiotics including
125 macrolides and cotrimoxazole were noted.

126

127 A blood cell count was done including white cell count with a differential. An
128 absolute count of more than 9000 cells/ μ L or 7000 cells/ μ L was used to define
129 lymphocytosis in infancy or in children 12 months and older respectively, according
130 to local laboratory guidelines.

131

132 **Pertussis PCR**

133 PCR targeting the IS481 common *Bordetella* insertion site and IS1001 for *Bordetella*
134 *parapertussis* was done after which specimens testing positive for the former were
135 further tested with PCR for hIS1001 to exclude *Bordetella holmesii* which shares the
136 same IS481 insertion site. IS481+/hIS1001- samples were classified as confirmed
137 *Bordetella pertussis* infection. The methods and epidemiological findings of this
138 study have been published elsewhere.[12] Only assessment of *Bordetella pertussis*
139 was included in the current analysis to allow for comparison with other studies.

140

141 **Analysis of data**

142

143 Data were analysed using STATA statistical package version 14 (StataCorp, College
144 Street, Texas).

145

146 Proportions were summarized as percentages with a 95% interval of confidence, as
147 indicated. Age in months and duration of symptoms in days were summarized using

148 medians and interquartile ranges.

149

150 The Wilcoxon rank sum test was used to test the difference between continuous
151 variables while the χ^2 or Fisher's exact tests were used to compare proportions of
152 categorical variables as appropriate. Where hypothesis testing was undertaken the P-
153 value was set at a two-tailed $p < 0.05$ as a cut-off point of statistical significance
154 Proportions of cases conforming to positive and negative clinical case definitions
155 were compared with PCR, the reference standard, to respectively estimate sensitivity
156 and specificity of using clinical signs in the diagnosis of pertussis. Clinical features,
157 chosen to conform to WHO and GPI recommendations (Table 1), were assessed with
158 and without taking duration of symptoms into consideration in this analysis.

159

160 As our sample did not include older individuals, the analysis was restricted to children
161 less than 10 years of age who presented with respiratory illness and underwent
162 confirmatory diagnostic testing for pertussis.

163

164 Although GPI criteria do not include duration, for comparison, both WHO and GPI
165 criteria were additionally assessed for sensitivity and specificity at different durations
166 of symptoms. A receiver operating character (ROC) analysis was done to determine
167 area under the curve (AUC) with PCR as diagnostic reference standard. Finally, the
168 utility of adding lymphocytosis to WHO and GPI criteria was analysed.

169

170 Our data allowed us to stratify analysis to reflect only two of the GPI suggested age
171 groups: below four months of age and four months to nine years of age.

172

173 **Results**

174

175 Four hundred and fifty-eight children were enrolled with a median age of 8 (IQR 4-
 176 17) months; 132 (28.8%) were younger than 4 months of age. HIV infection was
 177 confirmed in 19 (4.2%) while 92 (20.1%) were exposed to HIV in utero but tested
 178 negative for HIV infection. Forty-five children (9.8%) were classified as moderately
 179 to severely malnourished using WHO criteria. Table 2.

Table 2: Baseline characteristics of study participants (N=458)

	n (%)
PCR confirmed cases	32 (7.0)
Lymphocytosis	135 (29.5)
Age group	
0 - 3 months	132 (28.8)
4 months – 9 years	372 (71.2)
Sex	
Female	200 (43.7)
Pertussis vaccine doses	
0	28 (6.1)
1	57 (12.4)
2	58 (12.6)
≥ 3	308 (67.2)
Unknown	7 (1.5)
HIV status	
Infected	19 (4.1)
Exposed uninfected	92 (20.1)
Nutritional status	
Moderate-severe malnutrition#	45 (9.8)
Macrolide/cotrimoxazole in preceding week	5 (1.1)

PCR= polymerase chain reaction, # As per World Health Organization criteria of weight for age Z score less than -2

180

181 A total of 32 (7.0%, 95% CI 4.8 – 9.7%) cases were confirmed to have *Bordetella*
 182 *pertussis* on PCR, including 13 (9.9%) of the 132 infants younger than four months
 183 and 19 (5.8%) of 326 in the older age group. One of the PCR confirmed cases was
 184 also positive on culture. Lymphocytosis was found in 135 (29.5%) of which the two

185 age groups had 33/132 (25.0%) and 102/326 (31.3%) respectively. Clinical features of
 186 PCR positive and negative cases were similar across age groups, table 2. Only 3 (9%)
 187 of the 32 PCR positive participants were suspected to have pertussis by the attending
 188 clinicians while 12 (2%) of the PCR negative participants were diagnosed with
 189 pertussis; P=0.079.

190

191 The median duration of symptoms was 3 (IQR 2-5) days. Paroxysmal cough was
 192 reported as a presenting feature in 398 (69.9%) children; similar in confirmed
 193 pertussis cases and those with negative PCR under four months of age with 8/13 (61.5
 194 %) and 79/119 (66.4%) respectively; p=0.726. Similarly, paroxysmal cough was
 195 found in 15/19 (79.0%) participants four months to nine years old with confirmed
 196 pertussis compared to 218/326 (71.0%) with negative PCR; P=0.837. The other
 197 presenting clinical features are shown in Table 3.

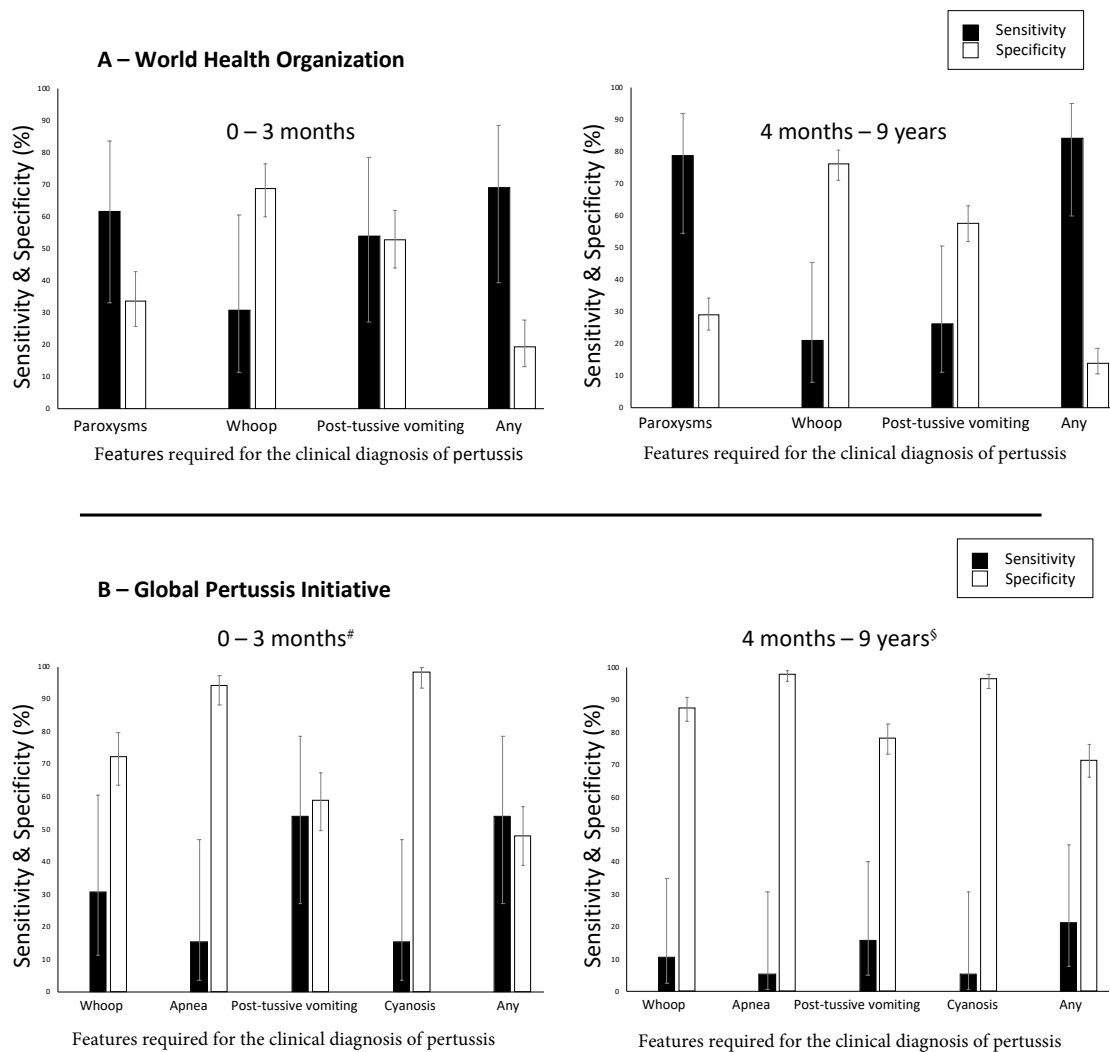
198

Table 3: Clinical presentation of children by *Bordetella pertussis* PCR status N=458

Clinical Feature		PCR+[n (%)]	PCR- [n (%)]	P Value
0 - 3 months		n=13	n=119	
	Paroxysmal cough	8(61.5)	79(66.4)	0.726
	Whoop	4(30.8)	37(31.1)	1.000
	Apnoea	2(15.4)	7(5.9)	0.217
	Post-tussive emesis	7(53.9)	56(47.1)	0.642
	Cyanosis	2(15.4)	3(2.5)	0.076
	Seizure	0(0.0)	1(0.8)	1.000
	Pneumonia	5(38.5)	35(29.4)	0.532
Absence of fever	3(23.1)	33(27.7)	1.000	
4 months - 9 years		n=19	n=307	
	Paroxysmal cough	15(79.0)	218(71.0)	0.457
	Whoop	4(21.1)	73(23.8)	1.000
	Apnoea	1(5.3)	10(3.3)	0.489
	Post-tussive emesis	5(26.3)	130(43.4)	0.169
	Seizure	2(10.5)	6(2.0)	0.073
	Night cough	15(79.0)	239(78.0)	1.000
	Pneumonia	6(31.6)	109(35.5)	0.728
Absence of fever	5(26.3)	142(46.3)	0.090	

PCR= polymerase chain reaction

199 Of the clinical features recommended by WHO for diagnosing pertussis, presence of
 200 paroxysms had the highest sensitivity (61.5% 0 to 3 months and 78.9% 4 months to 9
 201 years, respectively) while inspiratory whoop gave the highest specificity (68.9% 0
 202 to 3 months and 76.2% 4 months to 9 years, respectively) of any single feature on its
 203 own, figure 1. Overall, when the whole group was considered, the use of any feature
 204 suggested by WHO had sensitivity of 78.1% (95% CI 60.7– 89.2%) while GPI
 205 suggested clinical features had sensitivity of 34.4% (95% CI 20.1– 52.1%).
 206 Specificity was 15.5% (95% CI 12.4 –19.3%) and 64.8% (95% CI 60.1 – 69.2%) for
 207 WHO and GPI, respectively. Figure 1.

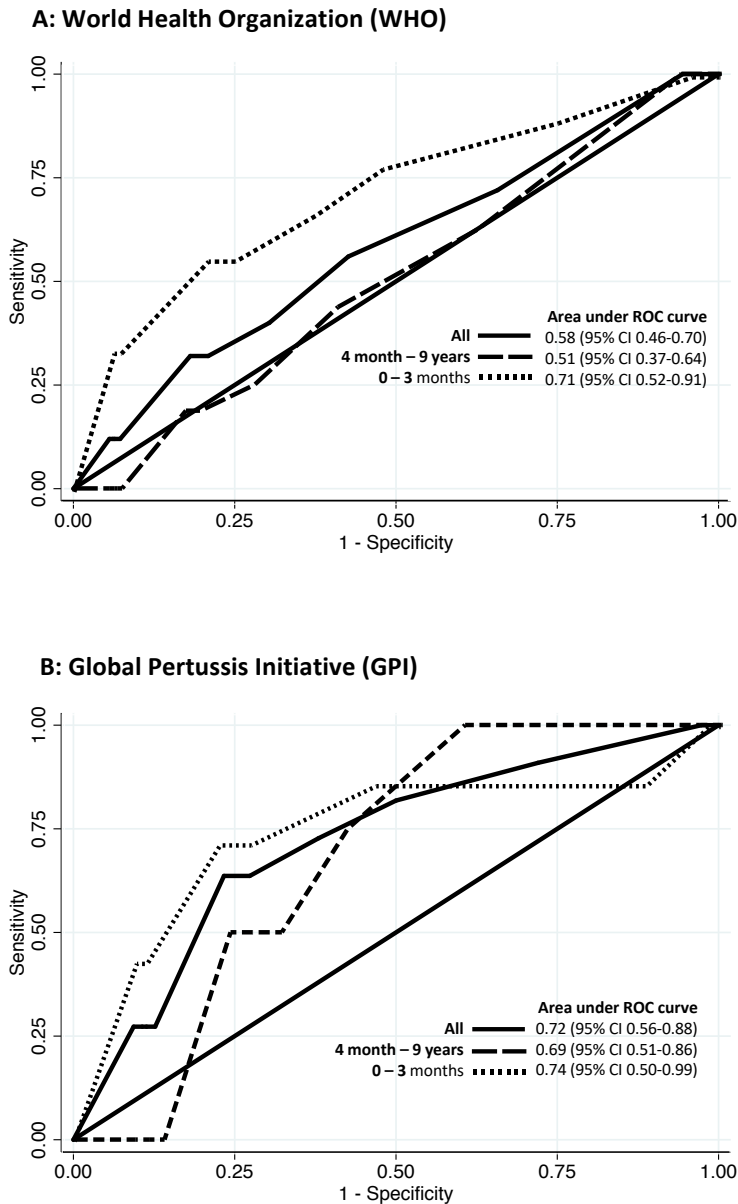


Clinical features must occur in the presence of coryza; § Clinical features must occur in the presence of paroxysms

208

209 **Figure 1. Sensitivity and specificity of clinical features in the diagnosis of pertussis**

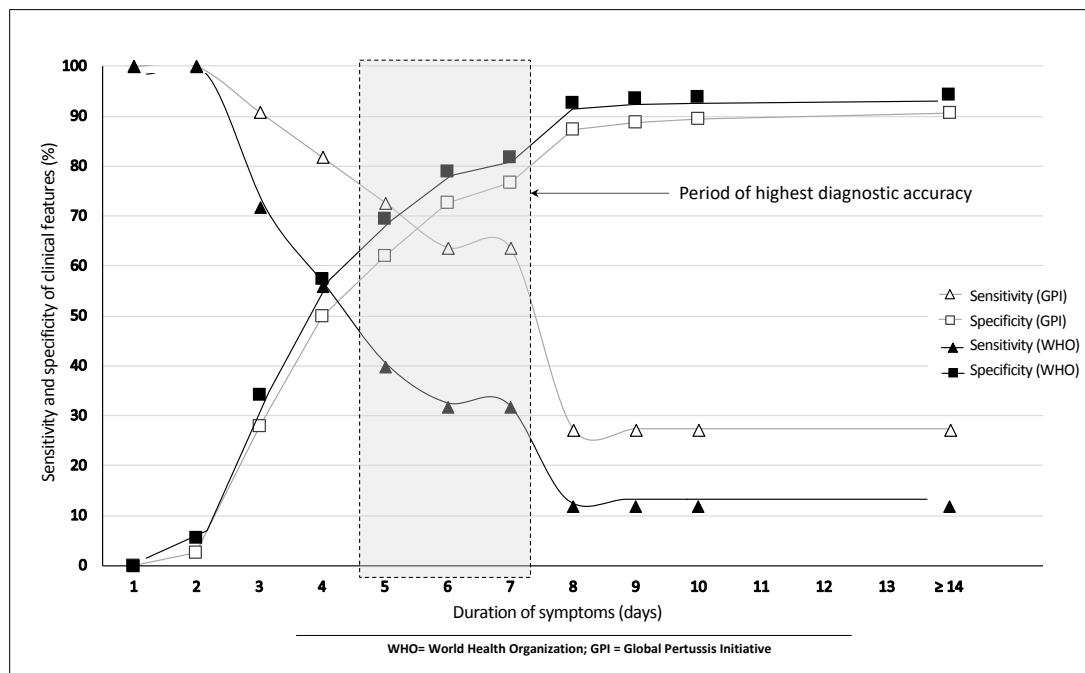
210 When ROC analysis was undertaken on duration of symptoms, GPI recommended
 211 features had AUC of 0.72 (95% CI 0.56-0.88) while those suggested by WHO had
 212 AUC of 0.58 (95% CI 0.46-0.70). The AUC was greater in younger infants than in the
 213 older age group for both WHO and GPI criteria. Figure 2.



214
 215 **Figure 2. Receiver operating characteristics (ROC) curves for duration of symptoms**
 216
 217

218 Specificity increased for both WHO and GPI criteria as duration of symptoms
 219 increased while the opposite was seen with sensitivity declining with duration. The

220 decline in sensitivity occurred earlier and steeper when using WHO clinical criteria
 221 than when GPI criteria were utilized. Sensitivity was 12.0% and 27.3% at ≥ 14 -day
 222 duration for WHO and GPI criteria respectively, while specificity was 94.0% and
 223 90.7% respectively for WHO and GPI criteria at a similar duration cut-off.
 224 In general, sensitivity declined, and specificity increased with increase in duration of
 225 symptoms. When any symptom was considered, the highest combination of sensitivity
 226 and specificity with the use of GPI clinical features was seen between five and seven
 227 days with sensitivity ranging between 63.6% and 72.7% and specificity ranging
 228 between 62.0% and 76.7%. Criteria recommended by WHO showed their highest
 229 combination of sensitivity and specificity between three and five days duration of
 230 symptoms with sensitivity ranging between 40.0% and 72.0%, and specificity ranging
 231 between 34.3% and 69.6%. Figure 3. The highest likelihood ratio for sensitivity was
 232 seen in the young infant group. This ranged between 1.00 and 5.33 using WHO based
 233 criteria and between 0.96 and 4.42 when GPI based criteria were applied.

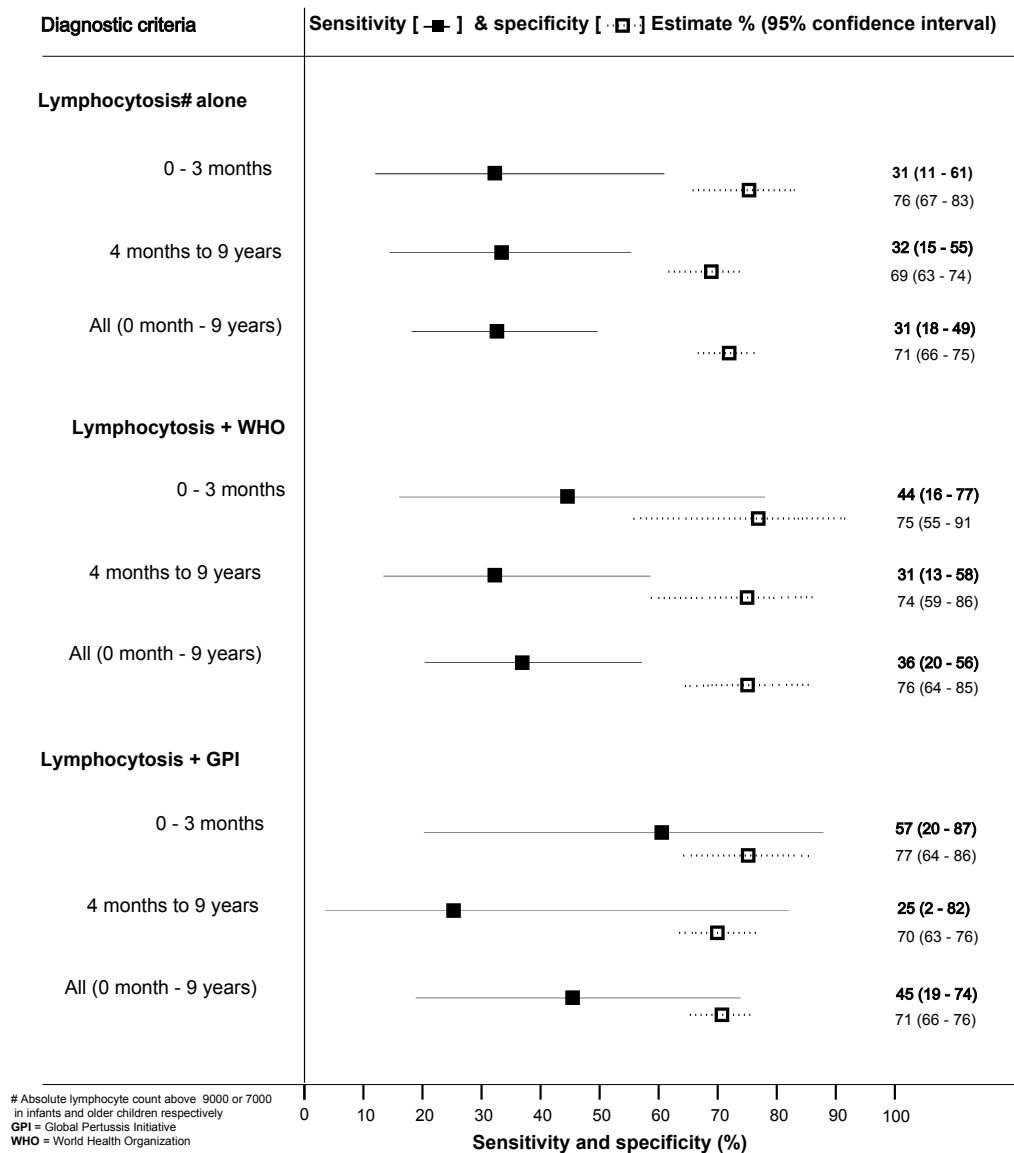


234

235 **Figure 3. Sensitivity and specificity of clinical features in the diagnosis of pertussis with**
 236 **changing duration**

237

238 Lymphocytosis when used alone had a sensitivity of 31.3% (95% CI 17.5 - 49.3%)
 239 and a specificity of 70.7% (95% CI .66.1-74.8%) compared to PCR. The sensitivity
 240 and specificity when lymphocytosis was combined with WHO clinical criteria were
 241 36.0% (95% CI 19.6 - 56.4%) and 75.6% (95% CI 63.7- 84.8%) for the whole group,
 242 respectively; while combining GPI criteria with lymphocytosis gave sensitivity and
 243 specificity of 45.5% (95% CI 19.2 - 74.5%) and 71.4% (95% CI 65.7- 76.4%),
 244 respectively. Age stratified sensitivity and specificity of lymphocytosis when
 245 combined with WHO and GPI criteria is shown in Figure 4.



246

247 **Figure 4. Sensitivity and specificity of lymphocytosis in the diagnosis of pertussis**

248 **Discussion**

249

250 Pertussis remains difficult to diagnose clinically with certainty as demonstrated by
251 low sensitivity and specificity using current clinical case definitions. Adding
252 lymphocytosis had a marginal impact in improving the diagnosis of pertussis in cases
253 preselected with the use of WHO and GPI clinical criteria. In addition, the low
254 sensitivity was across age groups.

255

256 The overall sensitivity and specificity of clinical features were generally low
257 irrespective of whether WHO or GPI diagnostic criteria were used. Criteria
258 recommended by WHO showed better sensitivity than those suggested by the GPI
259 when duration of symptoms was not considered, but the latter showed better
260 specificity. Paroxysmal cough and post-tussive vomiting as standalone clinical
261 features, gave the best sensitivity for both sets of criteria. The highest likelihood ratios
262 of just above five and four for WHO and GPI based criteria, respectively, are too low
263 to make for a functional diagnostic tool for clinical practice.

264

265 A systematic review conducted on the use of symptoms in the diagnosis of pertussis
266 concluded that the presence of whooping or post-tussive vomiting could be used to
267 make a possible diagnosis of pertussis in adults while the absence of paroxysmal
268 cough would possibly exclude it.[13] This study concluded that post-tussive vomiting
269 was less helpful as a clinical diagnostic test in children. However, when Cornia *et al*
270 analysed the likelihood ratios of clinical features used in the same systematic review,
271 they found all positive likelihood ratios to be less than 2, significantly lower than a
272 threshold regarded robust enough for clinical use.[14] A similar likelihood was
273 observed when data from other systematic reviews on sensitivity of clinical signs in

274 the diagnosis of pertussis were analysed.[15, 16]

275

276 In our study, when duration was added to clinical features, GPI criteria had overall
277 better diagnostic accuracy compared to WHO as indicated by the higher AUC (0.72
278 versus 0.58). For both criteria, duration based diagnostic accuracy was better in young
279 infants than in older infants and children (AUC 0.71 vs. 0.51 and AUC 0.74 vs. 0.68
280 for WHO and GPI, respectively). Increase in the duration of symptoms reduced the
281 sensitivity of clinical diagnosis in both sets of criteria, while the opposite was noted
282 with respect to specificity. The best combinations of sensitivity and specificity (60%
283 to 70% for both sets of criteria) were seen between five- and seven-days duration of
284 symptoms. This suggests that suspected cases of pertussis are more likely to be
285 correctly classified as positive or negative in children with this symptom duration.
286 However, early diagnosis is desirable for effective treatment and to prevent
287 transmission

288

289 An Iranian study reported 95% sensitivity and 15% specificity using a 14 day cut-off
290 with at least one WHO clinical criterion in a cohort of children and adolescents 6 to
291 14 years of age.[17] Duration of symptoms equal to or longer than 14 days gave a
292 specificity of 63% and sensitivity of 93% in outbreak settings in one American
293 study.[18] A recently published Serbian study testing diagnostic of GPI criteria in
294 individuals older than 3 months reported sensitivity ranging between 5% and 76% in
295 the 4 months to 9 year old group and between 2% and 73% in the older group; while
296 for both groups specificity ranged between 50% and 100%.[19] For each clinical
297 feature evaluated, there was an inverse relationship between sensitivity and
298 specificity. All the studies mentioned here seem to have selected participants on the

299 bases of clinical criteria some of which were later tested for their sensitivity, which
300 may explain the observed high sensitivity in some.

301

302 Adding lymphocytosis to our analysis marginally improved the diagnostic sensitivity
303 of suspected cases screened with either criteria but showed a specificity higher than
304 70% for both. The increase in sensitivity was higher in younger infants than in older
305 children for both WHO and GPI when lymphocytosis was added to the criteria. This
306 suggests that although lymphocytosis may be less useful in confirming the diagnosis
307 of pertussis, when used age stratification should be considered. Very high lymphocyte
308 counts have been shown to predict the severity and likelihood of dying in infants with
309 pertussis; and therefore, should best be utilized for this role in the management of
310 pertussis rather than diagnosis.[10, 20]

311

312 The difficulty involved in the diagnosis of pertussis has major implications for clinical
313 management, infection control, surveillance and the conduct of pertussis vaccine
314 trials. Missed diagnoses of pertussis due to low sensitivity affects both appropriate
315 clinical intervention in the index patient and contacts as well as appropriate reporting
316 and surveillance. In addition, low sensitivity undermines optimizing of preventive
317 measures including immunisation. On the other hand, over diagnosis of pertussis may
318 lead to overtreatment and inappropriate use of resources. Overall, diagnostic
319 shortcomings have serious economic implications, particularly in poor resourced
320 settings. Clinical and surveillance practice favours higher sensitivity at the expense of
321 specificity, but low specificity has a huge impact on the estimated efficacy of
322 pertussis vaccines.[6] There may be a need to develop context specific definitions of
323 pertussis rather than attempting to come with one for all settings.

324 Our study was limited by a small sample size as well as the use of a single laboratory
325 confirmation method performed at a single point in the course of the illness. As the
326 diagnostic sensitivity of PCR is not constant throughout the course of illness, a
327 negative result at a single point does not necessarily exclude infection with *B.*
328 *pertussis*. [9, 21] We were also unable to assess the impact of HIV on clinical criteria
329 as the sample size was not sufficient to make such an analysis. In addition, as our
330 study population consisted of children with severe disease requiring hospitalisation,
331 these findings may not be generalisable to children with less severe disease.
332

333 While acknowledging the observed poor diagnostic accuracy of clinical criteria, the
334 slight improvement observed with use of GPI criteria should encourage development
335 of screening criteria. Screening with a high sensitivity (but acceptably low specificity)
336 triage algorithm before laboratory testing would potentially reduce the waste of
337 testing patients that are likely to return a negative result, while increasing the
338 sensitivity of confirmatory PCR. Finding such screening criteria will require well-
339 designed, prospective studies specifically investigating clinical diagnosis of pertussis.
340 Such studies should ideally employ different laboratory diagnostic methods with
341 testing at multiple periods in the course of the illness and include appropriate controls
342 to allow estimation of specificity. A majority of published studies exploring
343 sensitivity and specificity of pertussis clinical diagnosis are done in participants
344 included on clinical suspicion of pertussis. Involving only suspected cases of pertussis
345 to assess diagnostic accuracy of the same features on which the sample was selected
346 has the potential of exaggerating the diagnostic accuracy of clinical criteria. [22]
347
348

349 **Conclusions**

350 Ultimately, in the absence of laboratory confirmation, confident diagnosis of
351 pertussis on clinical grounds is difficult even for experienced physicians,
352 especially when cases do not have the classical presentation. In our study, only 9%
353 of confirmed cases were suspected to have pertussis, substantially underestimating
354 pertussis as a possible cause of severe respiratory illness in our cohort. There is no
355 obvious substitute for pertussis laboratory confirmation, and more effort is needed
356 to increase this resource in LMIC settings where most pertussis is speculated to
357 occur.[3, 23] In the current presence of limited resources, it would seem prudent to
358 consider using modified case definitions, such as the one suggested by the GPI.[24] It
359 is clear that longer durations of symptoms in a number of criteria used all over the
360 world - including the ones suggested by WHO and the Centre for Disease control -
361 greatly undermine sensitivity.[5, 25] Due consideration must be given to abandon these
362 in favour of shorter durations of five to seven days used in conjunction with age-
363 stratified criteria, even if this is at the expense of over-diagnosing pertussis.

364

365 **Acknowledgements**

366 We would like to acknowledge the participants, laboratory personnel and study
367 staff for their immense contribution.

368

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370

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Chapter 7

1 **Highlights and Conclusions**

2

3 The thesis aimed to explore the full spectrum of pertussis in children who require
4 hospitalisation for lower respiratory tract infection (LRTI). This included descriptions
5 of prevalence, risk factors, clinical presentation, and to describe other organism
6 associated with pertussis. I have used primary data supported by a comprehensive
7 literature review - both structured as part of each chapter and formally as a systematic
8 review - to come to some unambiguous conclusions about the burden of pertussis as
9 seen in this group of children with severe illness.

10 The systematic review highlights the high mortality and case fatality rate in infants
11 secondary to persistently high prevalence of pertussis in low and middle-income
12 countries (LMIC). In addition, the systematic review is able, for the first time to
13 provide consolidated evidence showing that both HIV exposed but uninfected
14 children and HIV infected individuals are at a higher risk of acquiring pertussis and
15 having worse outcomes. This is an important finding especially for countries in Sub-
16 Saharan Africa, that carry the greatest burden of HIV.

17 Data from the systematic review is supported by the primary data that show that
18 pertussis is quite common in South African children hospitalised with LRTI.
19 Almost 10% of children had laboratory confirmed pertussis. The data suggests that
20 one of the reasons for severity of disease, such that hospitalisation is required,
21 may very well be the significant association between pertussis and bacteria and
22 viruses that tend to cause interstitial pneumonia - a condition that is known to
23 typically lead to hypoxaemia.

24 A worrying finding was that more than 90% of children with confirmed pertussis were

25 not clinically recognised by the attending clinicians. This should not be too surprising
26 as this thesis shows the futility of using clinical features to confidently diagnose
27 pertussis. Part of the aim of this study was to try find features that would improve
28 clinical diagnosis of pertussis. It becomes however, the conclusion of this thesis,
29 that there is currently no obvious substitute for pertussis laboratory confirmation in
30 non-outbreak settings.

31

32 This highlights the need for laboratory support for confirming pertussis. The findings
33 in this thesis show that laboratory confirmation can be further enhanced by testing an
34 induced sputum in addition to a nasopharyngeal specimen; a novel finding of the
35 thesis. Both the primary study and the systematic review concur that the only
36 useful test in this instance is polymerase chain reaction (PCR) as culture, the
37 supposed diagnostic gold standard, and serology, do not have the requisite
38 sensitivity and convenience to be of practical value.

39 In addition to confirming the increased risk of pertussis in children exposed to HIV *in*
40 *utero* and in children with HIV infection – a finding that was novel at the time – the
41 primary data of the thesis also shows poor nutritional status as a major risk. Detection
42 of *Bordetella pertussis* in nasopharyngeal specimens of mothers, was independently
43 associated with a more than 10-fold increased risk of pertussis in their children. The
44 thesis also confirms incomplete primary vaccination and early infancy as important
45 risk factors for pertussis.

46

47 Both the primary study and the systematic review indicate an urgent need to
48 strengthen immunisation programs to prioritise vaccination of pregnant women,
49 particularly those who are HIV infected.

50 In conclusion, I would like to list the recommendations of the Global Pertussis
51 Initiative (GPI) for the African continent.[1] I got involved with the GPI as a result
52 of this doctoral project. These recommendations mirror the conclusions and
53 recommendations of this thesis. Table 1.
54

Table 1. Summary of GPI recommendations for African countries

-
- Public health and laboratory-confirmed pertussis surveillance should be improved both at regional and national levels to better understand the pertussis burden and to help healthcare authorities and policy makers make informed decisions.
 - More research on pertussis aetiology, disease pattern and vaccine development is needed to prevent pertussis.
 - Pertussis vaccination coverage should be improved through education and better outreach programs in rural areas.
 - Based on the available resources, infants and toddlers should be prioritized for vaccination followed by pregnant women and other risk groups.
 - Better disease and treatment awareness should be advocated to help prevent pertussis.
-

From Muloiwa et al. *Pertussis in Africa: Findings and recommendations of the Global Pertussis Initiative (GPI)*[1]

55
56 Seventy-five years after the introduction of effective vaccination against pertussis
57 as a public health strategy, pertussis is still with us and causing severe disease.
58 This thesis does more than just highlight the problem, pointing also to some
59 potential targets for public health policy to prioritise in order to reduce the disease
60 burden of pertussis.

61

62

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63

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68



Informed Consent form for parents and guardians

Pertussis in children hospitalised with Lower Respiratory Tract Infection

You and your child are invited to participate in a study on an illness called pertussis (whooping cough).

Whooping cough is common in children but may also occur in adults. It is caused by a germ. This infection may cause the child to have a severe cough. A vaccine (immunization) is available to prevent this infection, and your child may have been given some or all of the doses of the vaccine at the clinic,

The aim of this study is to understand why some children get pertussis and to develop better ways of diagnosing this infection.

All children admitted into our ward (S11) with a chest infection can participate in this study. You and your child may choose to participate or not. This will not affect the care that you or your child receives. Your child will have all the usual tests for a chest infection including, a chest x-ray, blood culture, HIV test (if the result is not yet known), and sputum tests for tuberculosis (TB) if they are needed, Your child will receive usual care whether or not you choose to participate in this study.

Study investigations

You and your child will have extra tests done to look for the cause of the chest infection. These are:

- Two samples of mucus from the nose will be taken as well as a mucus sample from the chest. These will be sent to the laboratory to be tested for pertussis as well as for other infections that may cause cough and chest infection.
- A small amount of blood (about a teaspoon full) will be taken from your child to store for new tests in the future to diagnose this infection.
- You as the caregiver will have a sample of mucus taken from your nose. This will be sent to the laboratory and stored to test for pertussis, (if your child's specimen is found to have pertussis).
- After two weeks, you will be contacted over the phone to see how your child and other household members are doing.

Side Effects and Risks

The cotton wool swabbing is a bit uncomfortable but it is not dangerous.

Benefits

If you and your child participate in this research, your child will have the benefit of extra tests to look for the cause of cough and chest infection. This may improve treatment as it may indicate a specific treatment to be used. Should your child have pertussis other family members in the family will be offered treatment to protect them against this infection.

Reimbursements

The research will not cost you anything. You will not be given any money or gifts to take part in this research.

Confidentiality

The information that we collect from this research project will be kept confidential. Any study information about you and your child will have a number on it instead of a name. Only the researchers will know what you and your child's number is.

Who to Contact

If you have any questions you may ask them now or later, even after the study has started. If you wish to ask questions later, you may contact any of the study staff or

Dr Rudzani Muloiwa
Red Cross War Memorial Children's Hospital
Klipfontein Road
Rondebosch, 7700
Tel: 021 658 5111/5445E-mail: Rudzani.muloiwa@uct.ac.za

This proposal has been reviewed and approved by the Human Research Ethics Committee of the University of Cape Town, which is a committee whose task it is to make sure that research participants are protected from harm. If you wish to find about more about the ethics committee, contact

Mrs Lamees Emjedi

Research Ethics Committee
E 52 Room 24, Old Main Building, Groote Schuur Hospital, Observatory
Telephone: +27 21 406 6338
Fax: 27 21 406 6411
Email: nosi.tsama@uct.ac.za and shuretta.thomas@uct.ac.za

PART II: Certificate of Consent

Storage of samples

If any of the blood or mucus samples my child has provided for this research project is unused or leftover when the project is completed

- I give my permission for my child's samples to be stored and used in future research of any type which has been properly approved
- I give permission for my child's samples to be stored and used in future research but only for research on Pertussis.
- I give permission for my child's samples to be stored and used in future research except for research about _____.

OR I wish my child's samples to be destroyed immediately.

AND

- I want my child's identity to be removed from my child's samples.
- I want my child's identity to be kept with my child's samples.

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily for my child and myself to participate in this research.

Print Name of Parent/Guardian _____

Signature of Parent/Guardian _____

Date _____

Day/month/year

If illiterate

A literate witness must sign (if possible, this person should be selected by the Parent/Guardian and should have no connection to the research team). Parents/Guardians who are illiterate should include their thumb-print as well.

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness _____ AND Thumbprint of Parent/Guardian

Signature of witness _____

Date _____

Day/month/year



Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands that the following will be done:

1. Nasopharyngeal swabs will be done on the child and on the caregiver.
2. An induced sputum will be collected from the child.
3. Specimens will be analysed for Bordetella pertussis and other respiratory tract infections.
4. Samples may be stored for possible future analysis.

I confirm that the Parent/Guardian was given an opportunity to ask questions about the study, and all the questions asked by the Parent/Guardian have been answered correctly and to the best of my ability. I confirm that the Parent/Guardian has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this information and consent form has been provided to the Parent/Guardian.

Name of Researcher/person taking the consent _____

Signature of Researcher/person taking the consent _____

Date _____
Day/month/year



UNIVERSITY OF CAPE TOWN

Health Sciences Faculty
Faculty of Health Sciences Human Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6338 • Facsimile [021] 406 6411
e-mail: sumayah.ariefdien@uct.ac.za

25 August 2011

HREC REF: 371/2011

Dr R Muloiwa
Paediatrics
5th Floor ICH Building
Red Cross War Memorial Children's Hospital
Rondebosch

Dear Dr Muloiwa

PROJECT TITLE: PERTUSSIS IN CHILDREN HOSPITALISED WITH LOWER RESPIRATORY TRACT INFECTIONS.

Thank you for submitting your study to the HREC for review.

It is a pleasure to inform you that the Human Research Ethics Committee has **formally approved** the above-mentioned study.

Approval is granted for one year till the 28 August 2012.

Please submit an annual progress report if the research continues beyond the expiry date. Please submit a brief summary of findings if you complete the study within the approval period so that we can close our file.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the REC. REF in all your correspondence.

Yours sincerely

Signature removed to avoid exposure online

PROFESSOR M BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

sAriefdien

This serves to confirm that the University of Cape Town Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP) and Declaration of Helsinki guidelines.

The Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

STUDY TITLE	
Pertussis in children hospitalised with Lower Respiratory Tract infection (LRTI)	
Study reference number:	371/2011

CLINICAL TRIAL SITE/UNIT:	Red Cross War Memorial Children’s Hospital Department of Paediatrics University of Cape Town Klipfontein Road Rondebosch, 7700
PRINCIPAL INVESTIGATOR:	Rudzani Muloiwa

Patient’s Name Hospital sticker
--

Subject Study Number:	<table border="1"><tr><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td></tr></table>								

<i>I am confident that the information supplied in this case record form is complete and accurate data. I confirm that the study was conducted in accordance with the protocol and any protocol amendments and that written informed consent was obtained prior to the study.</i>																	
Investigator’s Signature:																
Date of signature:	<table border="1"><tr><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td></tr><tr><td style="text-align: center;">d</td><td style="text-align: center;">d</td><td style="text-align: center;">m</td><td style="text-align: center;">m</td><td style="text-align: center;">y</td><td style="text-align: center;">y</td><td style="text-align: center;">y</td><td style="text-align: center;">y</td></tr></table>									d	d	m	m	y	y	y	y
d	d	m	m	y	y	y	y										

Parent contact details& Inclusion criteria

Care giver's First and Middle Names: _____

Care giver's Last Name: _____

Home Address (If different from child's address)

Home Telephone Number: _____

Work Telephone Number: _____

Cellular Telephone Number: _____

Alternate contact details

1. Name _____ Relationship _____

Home Telephone Number: _____

Work Telephone Number: _____

Cellular Telephone Number: _____

Inclusion Criteria		Yes	No*
1	Is the subject a child aged 13 years or younger?	<input type="checkbox"/>	<input type="checkbox"/>
2	Has the subject's parent/legal guardian willingly given written informed consent?	<input type="checkbox"/>	<input type="checkbox"/>
3	Has the subject been admitted for LRTI or apnoea?	<input type="checkbox"/>	<input type="checkbox"/>
<i>*If any inclusion criteria are ticked 'No' then the patient is not eligible for the study.</i>			
Exclusion Criteria		Yes*	No
1	Is child too ill to be enrolled?	<input type="checkbox"/>	<input type="checkbox"/>
2	Has the child been admitted for more than 72 hours?	<input type="checkbox"/>	<input type="checkbox"/>
<i>*If any exclusion criteria are ticked 'Yes' then the patient is not eligible for the study.</i>			

1. Parent Interview Form

Pertussis study number: _____: _____

A. GENERAL: Complete all questions

1.	Are you the primary caregiver for this child?	<input type="checkbox"/> Yes	<input type="checkbox"/> No
2.	What is your relationship to him or her?	<input type="checkbox"/> Mother <input type="checkbox"/> Grandparent <input type="checkbox"/> Caregiver	<input type="checkbox"/> Father <input type="checkbox"/> Other relative <input type="checkbox"/> Other: _____
3.	During the first 4 months of life, how was your child fed?	<input type="checkbox"/> Breast milk <input type="checkbox"/> Formula (never breastfed)	<input type="checkbox"/> Breast milk and formula <input type="checkbox"/> Unknown
4.	If older than 4 months: how is your child being fed at present? (<i>In addition to solids</i>)	<input type="checkbox"/> Breast milk <input type="checkbox"/> Formula <input type="checkbox"/> Unknown	<input type="checkbox"/> Breast milk and formula <input type="checkbox"/> Other: _____ <input type="checkbox"/> Not applicable
5.	What is your house made of?	<input type="checkbox"/> Bricks <input type="checkbox"/> Tin/iron sheeting <input type="checkbox"/> Unknown	<input type="checkbox"/> Mud/traditional <input type="checkbox"/> Other: _____
6.	How many people sleep in the same room as the child (<i>not counting the child</i>)?	_____ people	<input type="checkbox"/> Unknown
7.	Do you use any of the following for heating and/or cooking in the household? (<i>check all that apply</i>)	<input type="checkbox"/> Electricity <input type="checkbox"/> Coal <input type="checkbox"/> Wood <input type="checkbox"/> Unknown	<input type="checkbox"/> Gas <input type="checkbox"/> Paraffin <input type="checkbox"/> Other: _____
8.	What is the main source of water in the household?	<input type="checkbox"/> In-door tap water <input type="checkbox"/> River water <input type="checkbox"/> Other: _____	<input type="checkbox"/> Borehole <input type="checkbox"/> Outdoor/communal tap <input type="checkbox"/> Unknown
9.	What type of toilet do you have at the house?	<input type="checkbox"/> Flush toilet <input type="checkbox"/> Bucket system <input type="checkbox"/> Other: _____	<input type="checkbox"/> Pit latrine <input type="checkbox"/> None/ Outdoors <input type="checkbox"/> Unknown
10.	Does the person who usually cares for the child smoke inside the house?	<input type="checkbox"/> Yes	<input type="checkbox"/> No <input type="checkbox"/> Unknown
11.	Does anyone else smoke inside the house?	<input type="checkbox"/> Yes	<input type="checkbox"/> No <input type="checkbox"/> Unknown
12.	Does your child attend a nursery or crèche? (<i>at least 2 other children for at least 4 hours per day, 3 days per week</i>)	<input type="checkbox"/> Yes	<input type="checkbox"/> No <input type="checkbox"/> Unknown
13.	What is the highest level of education the mother has completed?	<input type="checkbox"/> No School <input type="checkbox"/> Higher education <input type="checkbox"/> Unknown	<input type="checkbox"/> Schooling: highest grade completed _____

B. MEDICAL HISTORY

14.	Did your child have any of the following symptoms during the current illness?		
14.1	Fever	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	<i>(skip to 14.2 if No or Unknown)</i>
14.1.1	<i>If yes:</i> Duration _____ days	<input type="checkbox"/> Unknown	
14.2	Cough	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	<i>(skip to 14.3 if No or Unknown)</i>
14.2.1	<i>If yes:</i> Duration _____ days	<input type="checkbox"/> Unknown	
14.2.2	Night time cough	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	
14.2.3	Paroxysms of cough	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	
14.2.4	Whooping cough	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	
14.2.5	Vomiting after cough	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	
14.2.6	Facial cyanosis	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	
14.2.7	Barking cough	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	
14.2.8	Normal state between cough	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	
14.3	Apnoea (<i>stopping breathing</i>)	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	
14.4	Loss of weight	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	
14.5	Fits (seizures)	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	
14.6	Presence of any of these symptoms	<input type="checkbox"/> Runny nose <input type="checkbox"/> Sneezing	<input type="checkbox"/> Wheeze <input type="checkbox"/> None <input type="checkbox"/> Unknown <input type="checkbox"/> Fast breathing
15.	Did you seek health care prior to this hospitalization?	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	<i>(skip to 16 if No or Unknown)</i>

Staff initials: _____

Date completed: ____ / ____ / 20 ____

1. Parent Interview Form – (continue)

15.1	If Yes, did you go to:	<input type="checkbox"/> Clinic <input type="checkbox"/> General practitioner	<input type="checkbox"/> Traditional healer <input type="checkbox"/> Other: _____
16.	Did the child receive an antibiotic before coming to the hospital? If Yes, name of antibiotic	<input type="checkbox"/> Yes, oral antibiotic <input type="checkbox"/> Yes, injection	<input type="checkbox"/> Unknown <input type="checkbox"/> No <input type="checkbox"/> Unknown
16.1	If Yes, number of days on antibiotics?	_____ days	<input type="checkbox"/> Unknown
17.	Does your child take cotrimoxazole (bactrim)?	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	(skip to 18 if No or Unknown)
17.1	If Yes, for how long?	_____ months or _____ weeks	<input type="checkbox"/> Unknown
18.	Has any one at home been coughing for more than two weeks? If yes, how many people? Relationship with coughing person/s	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown _____	
19.	Has your child been in contact with an adult known to have TB in the past 6 months?	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	(skip to 20 if No or Unknown)
19.1	If Yes, is the person with TB currently on treatment?	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	
20	Is your child currently receiving INH prophylaxis	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	(skip to 21 if No or Unknown)
20.1	If Yes, about how long has your child been taking it?	_____ months or _____ weeks <input type="checkbox"/> Unknown	
21	Has the child ever been admitted to hospital? (Before this admission)	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	(skip to 22 if No or Unknown)
21.1	If Yes,		
21.2	How long ago was the last admission?	_____ months _____ weeks _____ days <input type="checkbox"/> Unknown	
21.3	Number of previous hospitalizations (not including current illness):	<input type="checkbox"/> 1-2 <input type="checkbox"/> 3-5 <input type="checkbox"/> >5 <input type="checkbox"/> Unknown	
21.4	Were any of these previous admissions for apnoea?	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	
21.5	Were any of these previous admissions for a chest infection?	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	
22	Has your child ever been diagnosed with one of the following chronic medical conditions? (before this admission)	<input type="checkbox"/> TB Disease: If yes: <input type="checkbox"/> On treatment <input type="checkbox"/> Asthma <input type="checkbox"/> Completed treatment <input type="checkbox"/> Heart problem <input type="checkbox"/> Defaulted <input type="checkbox"/> Pertussis/Whooping cough <input type="checkbox"/> Other lung problems Specify _____ <input type="checkbox"/> Cerebral palsy <input type="checkbox"/> Malnutrition <input type="checkbox"/> Kidney problem Specify _____ <input type="checkbox"/> Other Specify _____ <input type="checkbox"/> None known	
23	Did the child's mother have an HIV test during pregnancy?	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	(skip to 24 if No or Unknown)
23.1	If Yes, what was the latest result?	<input type="checkbox"/> Positive <input type="checkbox"/> Negative <input type="checkbox"/> Unknown	
24	Has the child ever been tested for HIV?	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	(skip to end if No / Unknown)
24.1	If Yes, What was the result?	<input type="checkbox"/> Positive <input type="checkbox"/> Negative <input type="checkbox"/> Unknown	(skip to end if Neg / Unknown)
25	Is the child currently receiving Antiretroviral Therapy (ART)?	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	(skip to end if No / Unknown)
25.1	For how long has the child taken ART?	_____ months <input type="checkbox"/> Unknown	

2. RTHC / Vaccination History

Pertussis study number: _____ : _____

1.	Does the child have a road to health card available?	<input type="checkbox"/> Yes (<i>Skip to 2</i>)	<input type="checkbox"/> No
1.1	If No, Has the child ever received any vaccines other than the vaccines <i>he or she</i> received at birth?	<input type="checkbox"/> Yes	<input type="checkbox"/> No <input type="checkbox"/> Unknown

Copy the following information from the card, if no RTHC available, ask person completing interview

2.	Date of Birth (dd/mm/yy)	___ / ___ / 20__	<input type="checkbox"/> Unknown
2.1	If DOB unknown, enter age in months:	_____	
3.	Is the child failing to thrive/crossing centiles	<input type="checkbox"/> Yes	<input type="checkbox"/> No <input type="checkbox"/> Unsure
4.	Birth weight	___ . ___ kg	<input type="checkbox"/> Unknown
5.	Gestational age	___ weeks	<input type="checkbox"/> Unknown
5.1	If gestational age unknown	<input type="checkbox"/> Term <input type="checkbox"/> Premature (<37 weeks)	<input type="checkbox"/> Unknown

If the RTHC is available, please complete

No RTHC available

6.	Vaccine	Date given (dd/mm/yyyy)	
6.1	BCG	___ / ___ / 20__	<input type="checkbox"/> Not given
6.2	OPV	0 ___ / ___ / 20__	<input type="checkbox"/> Not given
		1 ___ / ___ / 20__	<input type="checkbox"/> Not given
		2 ___ / ___ / 20__	<input type="checkbox"/> Not given
		3 ___ / ___ / 20__	<input type="checkbox"/> Not given
		4 ___ / ___ / 20__	<input type="checkbox"/> Not given
6.3	PENTAXIM LOCATION GIVEN -	<i>(i.e. Health facility where the child received vaccine)</i>	
6.3	OR	1 ___ / ___ / 20__	<input type="checkbox"/> Not given
		2 ___ / ___ / 20__	<input type="checkbox"/> Not given
		3 ___ / ___ / 20__	<input type="checkbox"/> Not given
		4 ___ / ___ / 20__	<input type="checkbox"/> Not given
	DTP/HIB	1 ___ / ___ / 20__	<input type="checkbox"/> Not given
		2 ___ / ___ / 20__	<input type="checkbox"/> Not given
		3 ___ / ___ / 20__	<input type="checkbox"/> Not given
		4 ___ / ___ / 20__	<input type="checkbox"/> Not given
6.4	PCV <input type="checkbox"/> 7 <input type="checkbox"/> 13 <input type="checkbox"/> Unknown	1 ___ / ___ / 20__	<input type="checkbox"/> Not given
		2 ___ / ___ / 20__	<input type="checkbox"/> Not given
		3 ___ / ___ / 20__	<input type="checkbox"/> Not given
6.5	ROTAVIRUS	1 ___ / ___ / 20__	<input type="checkbox"/> Not given
		2 ___ / ___ / 20__	<input type="checkbox"/> Not given
6.6	MEASLES	1 ___ / ___ / 20__	<input type="checkbox"/> Not given
		2 ___ / ___ / 20__	<input type="checkbox"/> Not given
7.	INFLUENZA	1 ___ / ___ / 20__	<input type="checkbox"/> Not given
		2 ___ / ___ / 20__	<input type="checkbox"/> Not given
8.	OTHER: _____	___ / ___ / 20__	
9.	Did the child have any of the following within 6 weeks before or after any of the vaccines in 6.3? If yes, which vaccines? Enter 1- 4	<input type="checkbox"/> Steroid use	<input type="checkbox"/> Measles
		<input type="checkbox"/> Immunoglobulin therapy	<input type="checkbox"/> None
		<input type="checkbox"/> Other: _____	<input type="checkbox"/> Unknown
10.	Has the child received Vitamin A in the previous six months?	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Unknown

Staff initials: _____

Date completed: ___ / ___ / 20__

3. Medical Records

The following questions relate to the current admission. Information should be obtained from clinical records or measured (e.g. weight and temperature) if not available in records. Temperature, heart rate and respiratory rate should be the maximum recorded within 24 hours of admission

Pertussis study number: _____: _____

1.	Sex	<input type="checkbox"/> Male <input type="checkbox"/> Female
2.	Date of admission	____ / ____ / 20 ____ (dd/mm/yyyy)
3.	Time of admission	____ : ____ <input type="checkbox"/> Am <input type="checkbox"/> Pm <input type="checkbox"/> Unknown
4.	Race	<input type="checkbox"/> Asian/Indian <input type="checkbox"/> Black <input type="checkbox"/> White <input type="checkbox"/> Coloured
5.	Admission height/length	____ , ____ cm
6.	Admission weight	____ . ____ kg
7.	Mid upper arm circumference (MUAC)	____ , ____ cm
8.	Head Circumference	____ , ____ cm
9.	Temperature	____ . ____ °C
10.	Heart rate	____ beats / min <input type="checkbox"/> Not recorded
11.	Respiratory rate	____ breaths / min <input type="checkbox"/> Not recorded
12.	Oxygen saturation	____ % <input type="checkbox"/> Room air <input type="checkbox"/> On oxygen <input type="checkbox"/> Not recorded
13.	Presence of oedema	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Not recorded
14.	Did the child receive any of: Bronchodilators Antibiotics If Yes, name(s) of antibiotic(s)	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> No _____
15.	HIV Stage of the child	<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3 <input type="checkbox"/> 4 <input type="checkbox"/> Not Applicable

16.	Lower chest wall indrawing:	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Not recorded
17.	Crackles/crepitations	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Not recorded
18.	Wheezing	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Not recorded
19.	Clubbing	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Not recorded

Outcome Summary – to be obtained on all participants when discharged.

20.	Did child receive supplemental oxygen	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Not recorded
21.	Admission to a High Care unit or ICU If admitted to above, number of days	<input type="checkbox"/> ICU <input type="checkbox"/> High Care <input type="checkbox"/> None <input type="checkbox"/> Unknown ICU days: ____ High Care days: ____ <input type="checkbox"/> Unknown
22.	Assisted ventilation If Yes, Duration Type of support Number of days	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Not recorded ____ days <input type="checkbox"/> Not recorded <input type="checkbox"/> CPAP <input type="checkbox"/> IPPV ____ days ____ days <input type="checkbox"/> Not recorded
23.	Outcome of child	<input type="checkbox"/> Discharged <input type="checkbox"/> Refused Hospital Treatment (RHT) <input type="checkbox"/> Died <input type="checkbox"/> Transferred to another hospital
24.	Date of discharge/ death/ transfer/RHT	____ / ____ / 20 ____ (dd/mm/yyyy)
25.	If discharged, on what treatment was child discharged? (mark all that apply)	<input type="checkbox"/> Antibiotics, specify: _____ <input type="checkbox"/> Bronchodilators <input type="checkbox"/> Inhaled steroids <input type="checkbox"/> Anti TB treatment <input type="checkbox"/> Other, specify _____
26.	Discharge diagnosis (mark all that apply)	<input type="checkbox"/> Bronchopneumonia <input type="checkbox"/> Bronchiolitis <input type="checkbox"/> Lobar Pneumonia <input type="checkbox"/> Tuberculosis <input type="checkbox"/> Pertussis <input type="checkbox"/> Pneumocystis pneumonia <input type="checkbox"/> Chronic lung disease <input type="checkbox"/> Sepsis <input type="checkbox"/> Underweight/Kwashiorkor/Marasmus <input type="checkbox"/> Not recorded <input type="checkbox"/> Other, specify _____

Staff initials: _____

Date completed: ____ / ____ / 20 ____

5. Investigations & Results Form

Pertussis study number: _____ : _____

Questions 1 and 2 to be completed for all participants

1. HIV testing <input type="checkbox"/> Not done (skip to 2)			
	<u>Test</u>	<u>Date of test</u>	<u>Result</u>
1.1	<input type="checkbox"/> ELISA – child	___ / ___ / 20 ___	<input type="checkbox"/> Reactive <input type="checkbox"/> Non reactive
1.2	<input type="checkbox"/> ELISA - mother	___ / ___ / 20 ___	<input type="checkbox"/> Reactive <input type="checkbox"/> Non reactive
1.3	<input type="checkbox"/> PCR - child	___ / ___ / 20 ___	<input type="checkbox"/> Positive <input type="checkbox"/> Negative
1.4	<input type="checkbox"/> Most recent CD4 count - child	___ / ___ / 20 ___	_____ Absolute CD4 _____ % of lymphocytes
1.5	<input type="checkbox"/> Most recent viral load - child:	___ / ___ / 20 ___	_____ copies/ml <input type="checkbox"/> >750 000
2. Micro/Virology results - Obtained in the first 48 hours of admission <input type="checkbox"/> Not done (skip to 3)			
	<u>Lab number</u>	<u>Date of test</u>	<u>Site</u> <u>Organism</u>
2.1		___ / ___ / 20 ___	
2.2		___ / ___ / 20 ___	
2.3		___ / ___ / 20 ___	
2.4		___ / ___ / 20 ___	

3. CRP (C-reactive protein) (<72 hrs of admission) <input type="checkbox"/> Not done (skip to 4)			
	Date ___ / ___ / 20 ___	Time ___ : ___ <input type="checkbox"/> Am <input type="checkbox"/> Pm	Result _____ mg/l
	<input type="checkbox"/> Not recorded		
3.1	Haematology	Result	
3.1	Haemoglobin	_____	
3.2	Platelets	_____	
3.3	White cell count	_____ (Total)	
4.	Differential count	Lymphocytes	Absolute _____ Percentage _____
4.1		Neutrophils	Absolute _____ Percentage _____
4.2		Monocytes	Absolute _____ Percentage _____
4.3		Bands	Absolute _____ Percentage _____
4.4		Other	Absolute _____ Percentage _____
5.	STUDY SPECIMENS TAKEN	Date ___ / ___ / 20 ___	
		<input type="checkbox"/> NP Swab for culture	<input type="checkbox"/> NP Swab for PCR (Child)
		<input type="checkbox"/> Induced sputum	<input type="checkbox"/> NP Swab for PCR (Care-Giver)
6.	TB Results <input type="checkbox"/> Not done (skip to 6)	Date	Specimen
6.1	___ / ___ / 20 ___	Specimen	Result –AFB smear Culture requested
		<input type="checkbox"/> Induced sputum	<input type="checkbox"/> Positive <input type="checkbox"/> Yes
		<input type="checkbox"/> Gastric Washings	<input type="checkbox"/> Negative <input type="checkbox"/> No
		<input type="checkbox"/> Not recorded	
7.	Other (skip to 8 if not done)		
	<u>Test</u>	<u>Date of test</u>	<u>Result</u>
7.1	<input type="checkbox"/> Albumin	___ / ___ / 20 ___	_____ . _____ g/dL
7.2	<input type="checkbox"/> Total protein	___ / ___ / 20 ___	_____ . _____ g/dL
7.3	<input type="checkbox"/> Chemistry	___ / ___ / 20 ___	Na _____ K _____ Urea _____ Creatine _____
8.	Radiology		
8.1	Date of chest x-ray	___ / ___ / 20 ___	
8.2	X-ray findings	<input type="checkbox"/> Normal	<input type="checkbox"/> Interstitial infiltrate
		<input type="checkbox"/> Pleural effusion	<input type="checkbox"/> Consolidation or air bronchograms
		<input type="checkbox"/> Cavitations	<input type="checkbox"/> Lobar consolidation
		<input type="checkbox"/> Sub-optimal quality	<input type="checkbox"/> Other _____

Staff initials: _____

Date completed: ___ / ___ / 20 ___