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.

Neither the substance nor any part of the above thesis has been submitted in the past, or is being, or is to be submitted for a degree at this University or at any other university.

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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

Introduction

Epidemiology of pertussis

According to the World Health Organization (WHO), there are more that 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.

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

Pathogenesis

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

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

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

Diagnosis

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

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

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

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

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

PCR has greatly improved the ability to confirm pertussis cases and is increasingly used for diagnosis on clinical specimens. There are many advantages associated with the use of PCR: results are rapid, less dependent on delays in transport and even non-viable organisms may be detected. PCR targets include IS*481,* the common Bordetella target that includes *Bordetella pertussis* and IS1001 for *Bordetella parapertussis*. The qualitative PCR using these gene targets can be performed on a nasopharyngeal swab (Dacron not calcium alginate swabs as the latter may inhibit PCR) and/or aspirate specimens. The

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

In a prior unpublished study[18] to compare a standard nested PCR and a LightCycler-based real-time assay with culture for confirming the diagnosis of pertussis, 48 children presenting to Red Cross War Memorial Children's Hospital with clinical features of pertussis were sequentially enrolled, nasopharyngeal aspirates were collected, and inoculated onto charcoal agar for isolation of *Bordetella pertussis*. The identity of colonies morphologically resembling *B. pertussis* was confirmed by amplification of the pertussis toxin promoter gene (*ptxA-Pr*). A nested PCR assay targeting the *IS*481 sequence was performed directly on the NPA samples. In addition, a real-time PCR assay for detection of the pertussis toxin promoter gene was performed on all samples. *Bordetella pertussis* was cultured from five (10%) patients. However, the nested PCR assay for *IS*481 was positive in 31 (65%) patients, and the toxin promoter gene was present in 17 (55%) of these 31. No patient with a negative *IS*481 assay was culture positive or PCR positive for the toxin promoter. These initial results suggest that PCR is a more sensitive method of detecting *Bordetella pertussis* than culture. But it also shows that ptxA-Pr, even though

more specific for *Bordetella pertussis,* may lack the requisite sensitivity for case confirmation. In the absence of double assays, PCR for the *IS*481 target, even though less specific, may offer the best diagnostic confirmation support for clinically suspected cases of pertussis.

Serological diagnosis using ELISA is available, quick and easy to perform in the laboratory. As the measured response is uses antibodies against common vaccine components, in patients recently vaccinated, it is not easy to distinguish between acute infection and recent infection on a single serum sample.[19] Acute and convalescent paired sera can be taken and the change in titres used for diagnostic purposes. Serology suffers a number of drawbacks: only anti-PT (pertussis toxin) serology has been well-standardized and validated, and only anti-PT IgG ELISA testing is recommended for use in the diagnosis of pertussis. Appropriate cut-offs have not yet been determined in many instances, making interpretation, especially for unpaired testing, difficult. IgG titres need to be interpreted in the context of a diagnostic cut-off determined by local sero-epidemiological surveys. A recommendation for considering anti-PT IgG titres between 50—120 international units per millilitre (IU/ml) as highly suggestive of recent pertussis has been proposed by a collaboration of European laboratories, but this is based on sero-epidemiological surveys in Western Europe.[20] No sero-epidemiological data exists for SA or other countries in Sub-Saharan Africa from which we could infer appropriate cut-off levels; this limits the usefulness of such tests in our setting at present.
Direct fluorescent antibody tests for direct antigen detection have poor sensitivity and specificity and should not be relied on.[21]

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

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

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

The advantages and disadvantages of the available diagnostic methods are summarised in Table 1 [22].

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 pro- vided 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 bed- side.
Molecular techniques (PCR)	Highly sensitive. Can detect *B. pertussis* 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 singletarget PCR is used e.g. IS481. Requires molecularexpertiseand equipment.Relatively expensive.
Serology	Relatively cheap and rapid test.	Onlyusefulifastandardizedanti-PTIgGELISAtestisused(other antibodies lack sensitivity and specificity);eventhen, localcut- offs have not beendetermined. 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.

Accurate diagnosis of pertussis is important for the timely institution of optimal treatment (a macrolide antibiotic) and infection control measures, especially in-hospital, and for appropriate treatment of household contacts.

Recent evidence has shown that the finding of a pathogen or pathogens in respiratory specimens does not always indicate a causal relationship. Organisms whose presence used to be regarded as being of pathogenic significance are now found in otherwise healthy

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

Pertussis vaccine

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

In the Western Cape Province of South Africa, vaccine coverage in 2005 was found to be 80%, 77% and 48% for vaccines due by 14 weeks, 9 months and 18 months respectively. Thus, a substantial number of children did not receive their early vaccines, while a large 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

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

Rationale for the study

Hypothesis

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

The aims:

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

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

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

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

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

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

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

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

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

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

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)

Outline of the thesis

General statement on the structure of the thesis

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

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

Chapter 1

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

Chapter 2

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

Chapter 3

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

Chapter 4

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

Chapter 5

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

Chapter 6

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

Chapter 7

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

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

Appendix A: Informed consent form

Appendix B: Ethical approval HREC 371/2011

Appendix C: Case Report Form

Author contributions to included manuscripts

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

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

[Under review BMC Medicine]

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

2. **Incidence and Diagnosis of Pertussis in South African Children Hospitalised With Lower Respiratory Tract,** Muloiwa R, Dube FS, Nicol MP, Zar HJ, Hussey GD. *The Pediatric infectious disease journal* 2016; **35**(6): 611-6.

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

3. **Impact of HIV status and maternal carriage on risk of childhood *Bordetella pertussis* disease.** Muloiwa R, Dube FS, Nicol MP, Hussey GD, Zar HJ [Under review Plos One]

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

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

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

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

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

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

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Chapter 2

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

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Abstract

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

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

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

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

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

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

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

KEYWORDS

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

Background

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

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

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

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

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

METHODS

Search strategy and criteria for selecting studies

The protocol for the systematic review was registered with PROSPERO International Prospective Register of systematic reviews (http://www.crd.york.ac.uk/PROSPERO), with registration CRD42015015159. The methods employed in conducting this review have been previously published [14]. The following electronic databases were searched for qualifying literature: MEDLINE, Scopus, Africa-Wide, PDQ-Evidence, WHOLIS, CINAHL , CENTRAL and Web of Science. Search terms used included "pertussis," "*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 Cameroons 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 Timur 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 Malaya 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 Moldovia OR Moldovian 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 Phillippines 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 Tadzhikistan OR Tadjikistan OR Tadzhik 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)

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

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

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

Data extraction

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

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

Incidence data were extracted as reported by the authors.

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

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

Data analysis and reporting

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

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

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

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

Quality of included studies

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

RESULTS

Characteristics of the included studies

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

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

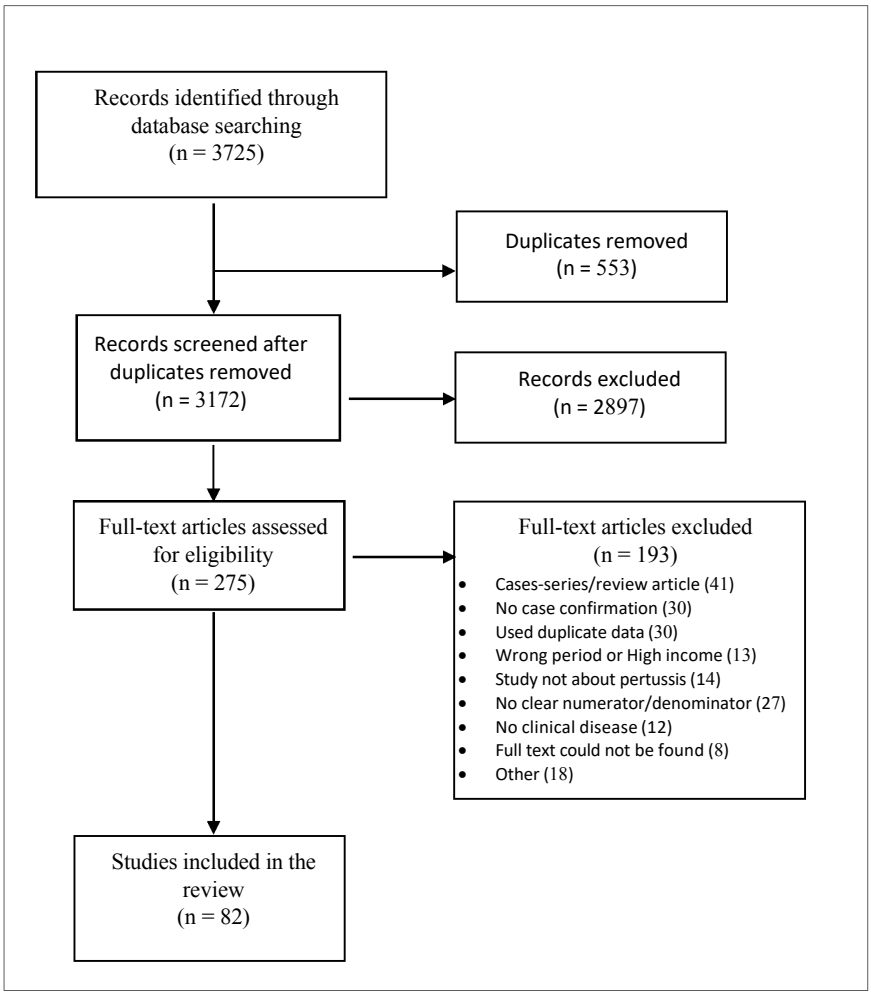

Figure 1: Studies included in the systematic review

Table 2 shows the characteristics of the included studies by WHO region. A large proportion of the studies (n=63; 77%) was published between 2010 and 2018. Sixty-eight studies (83%) involved hospital-based participants. Forty-seven studies (57%) used one laboratory diagnostic confirmatory test while 32 (39%) and three (4%) studies used two 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-Grlić# (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*

Study designs included three clinical trials, four cohort, 62 cross-sectional and 13 surveillance studies. There were 71 studies published in English, four in Mandarin, three each in Spanish and Turkish, and one in Persian. Three studies by Strebel et al, Al-Bargish et al, and Cooper and Fitch, were conducted in outbreak settings [25, 39, 77].

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

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

Prevalence of pertussis

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

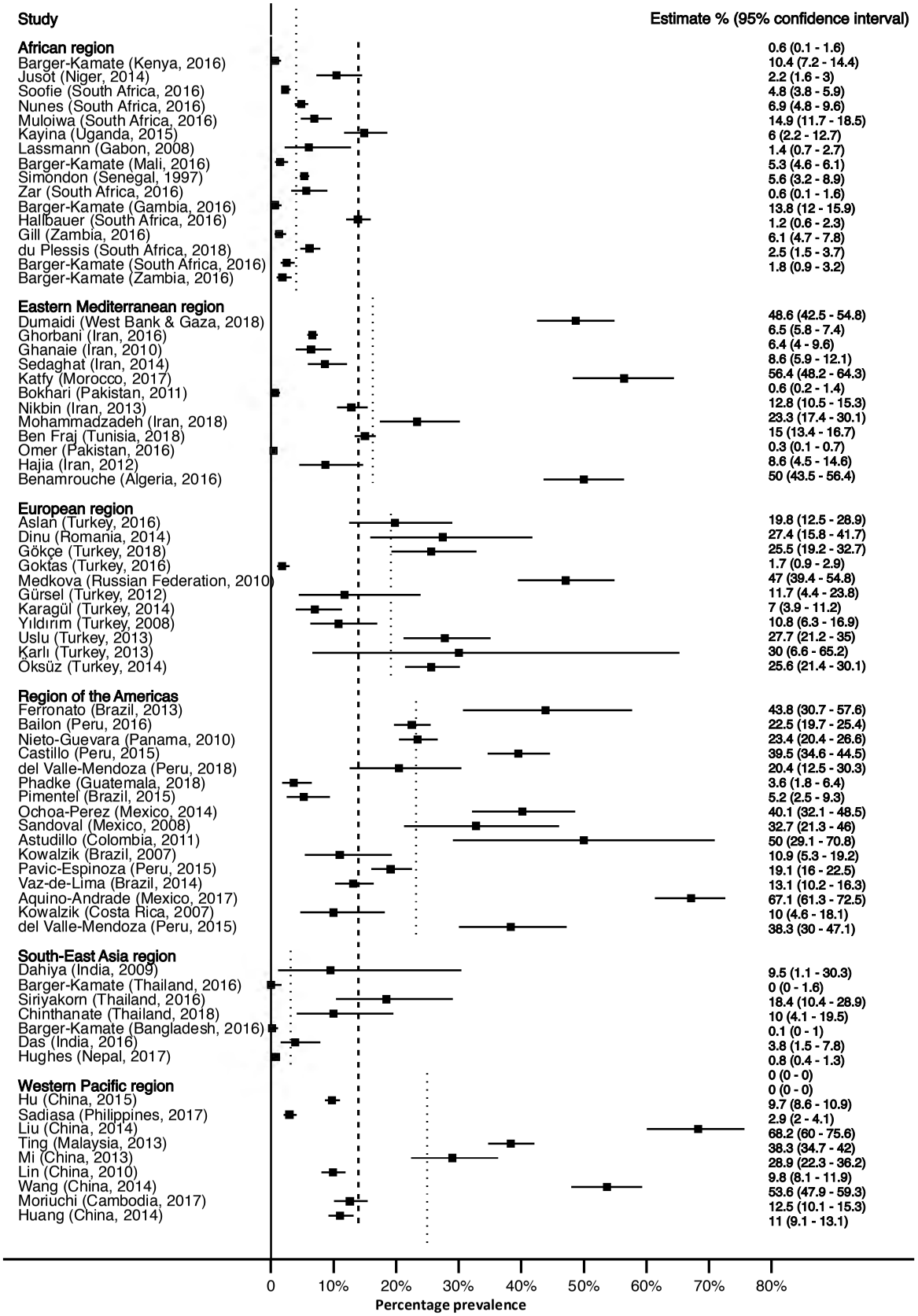

Figure 2. Prevalence (proportion testing positive) **of polymerase chain reaction confirmed *Bordetella pertussis*** Dotted lines show subgroup and whole group average estimates

The median prevalence of culture-confirmed Bordetella *pertussis* was 3% (IQR 1-9%); n=18 868, 44 studies). The point prevalence was similar across WHO regions; P=0.1380. Figure 3 and Additional file 2.

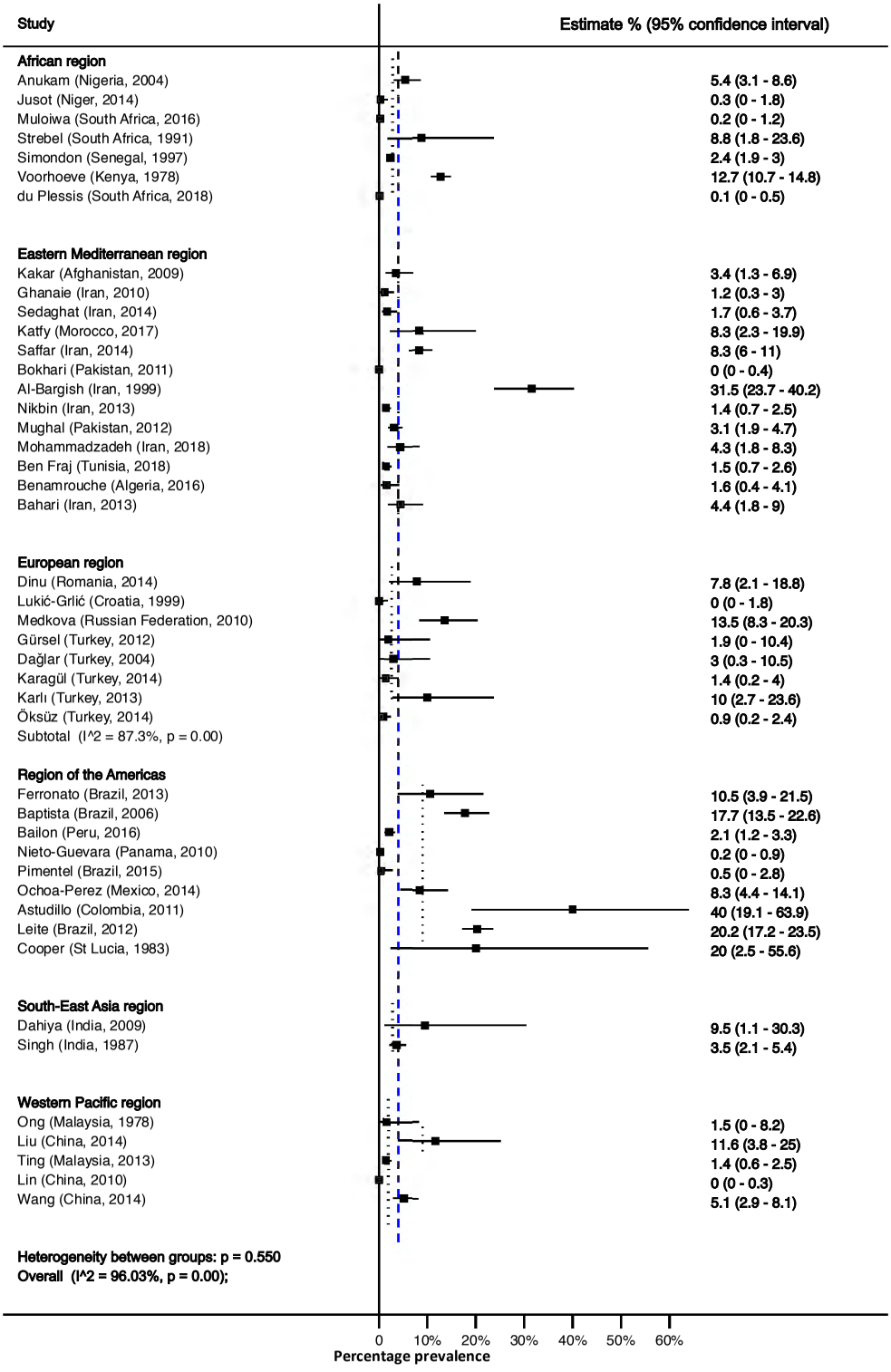

Figure 3. Prevalence (proportion testing positive) **of culture confirmed *Bordetella pertussis*.** Dotted lines show subgroup and whole group average estimates.

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

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

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

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

the highest prevalence of all groups with a median prevalence of 20% (IQR 14 - 32%). Figure 4b.

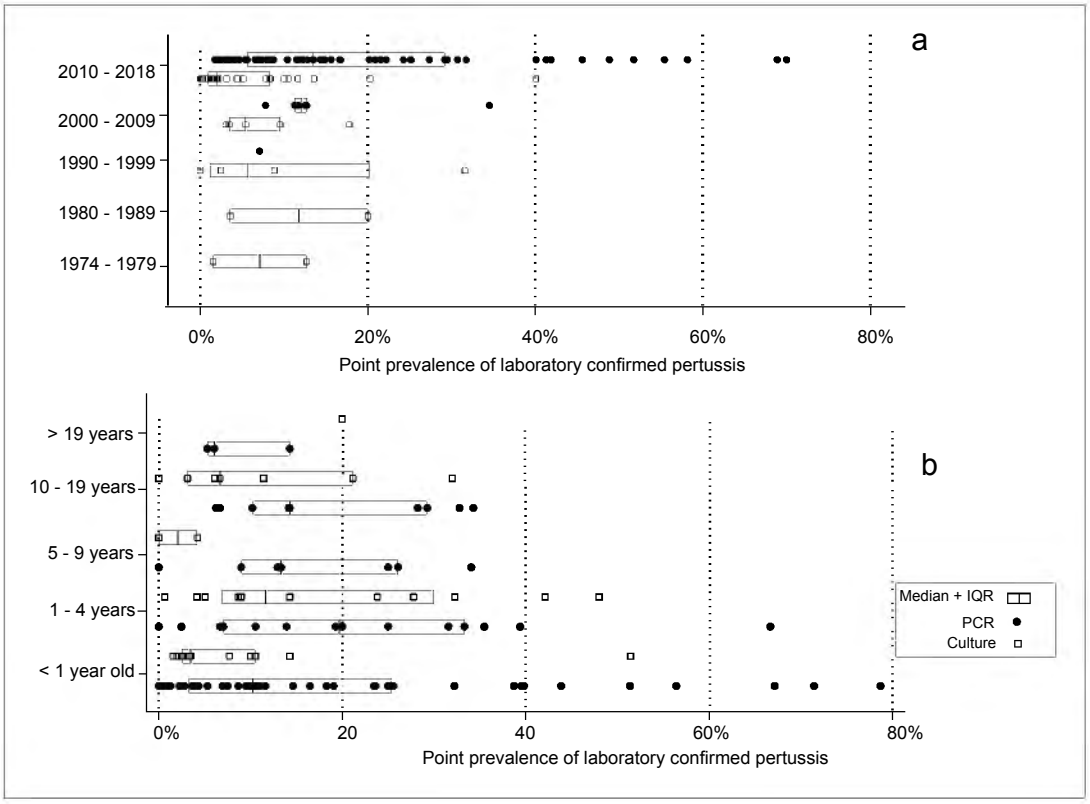

Figure 4. Distribution of point prevalence (proportion testing positive) **of polymerase chain reaction and culture confirmed pertussis by period (a) and age group (b)**

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

Incidence of pertussis

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

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

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

Risk of pertussis in HIV exposed and infected

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

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

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

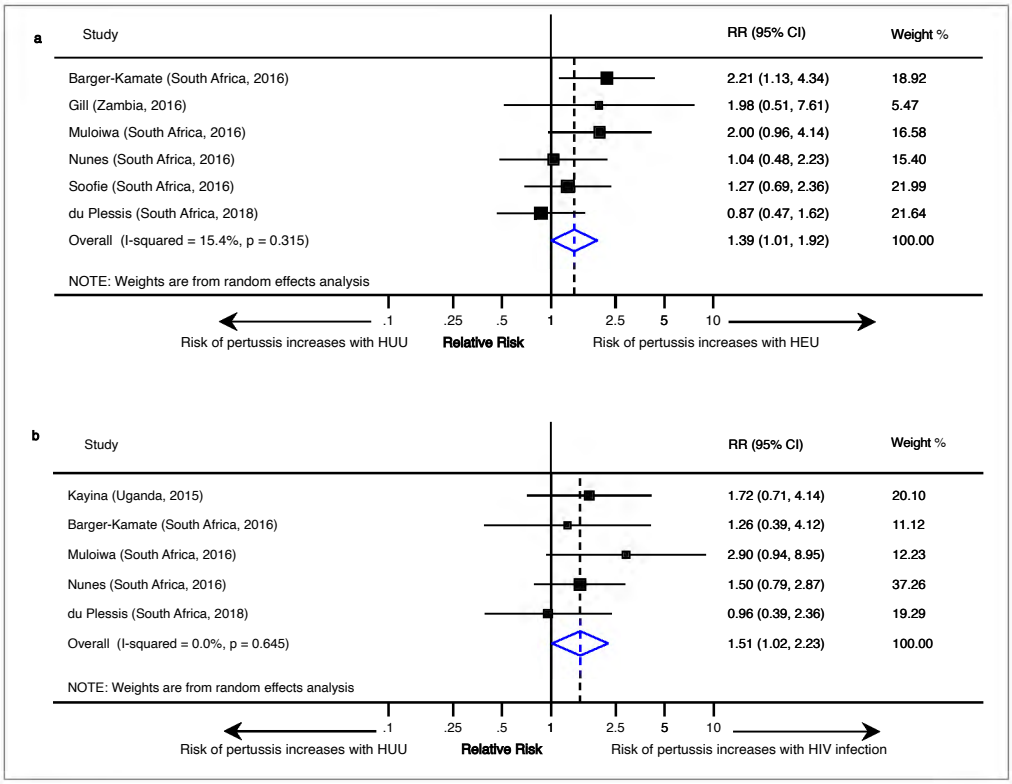

Figure 5. Meta-analysis of the relative risk of pertussis comparing HIV unexposed uninfected (HUU) to HIV exposed uninfected (HEU) (a) and HIV infected (b)

Deaths and case fatality rate of pertussis

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

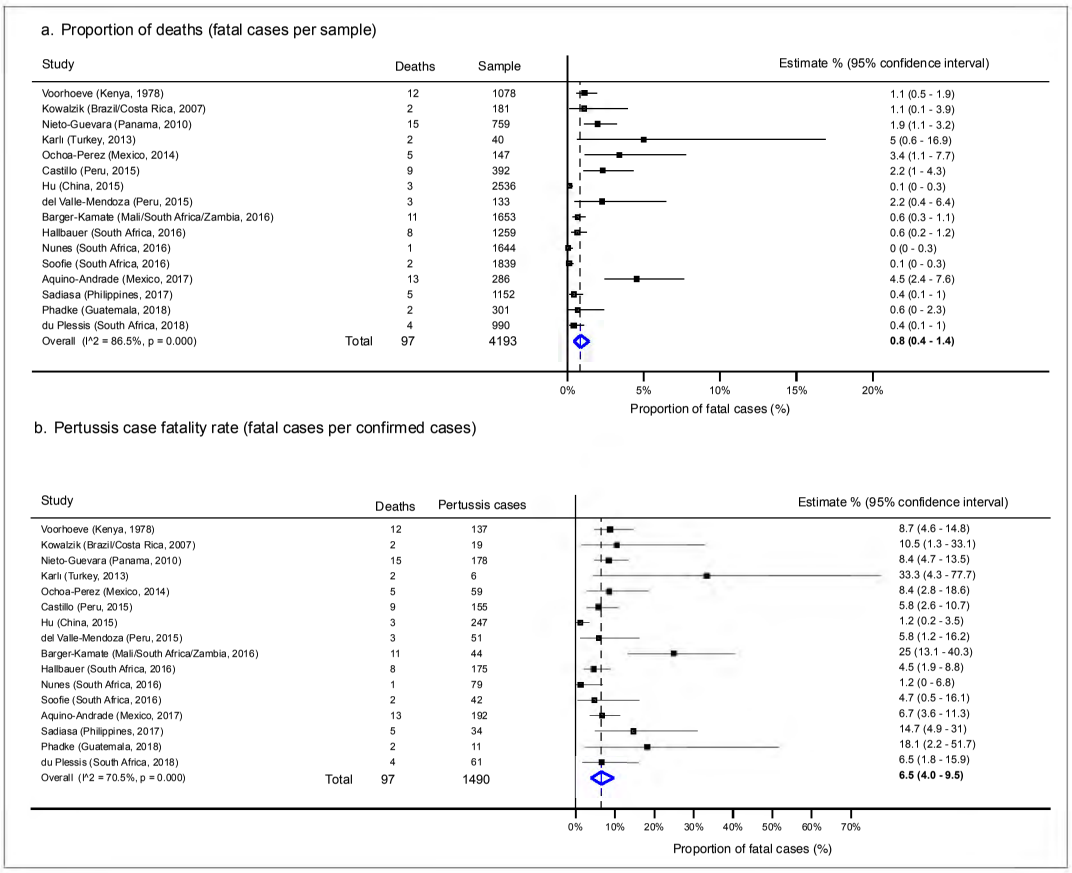

Figure 6. Mortality and case fatality rate of confirmed pertussis

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

Quality of the included studies

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

Discussion

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

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

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

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

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

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

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

LIST OF ABBREVIATIONS

aP - acellular vaccine
CIs - confidence intervals
EPI - Expanded Programme on Immunisation
GPI - Global Pertussis Initiative
HEU - HIV-exposed uninfected
HICs - High Income Countries
HIV+ - HIV infected
HUU - HIV-unexposed uninfected
IQR - interquartile range
LMICs - Low and Middle-Income Countries
PCR - polymerase chain reaction
WHO - World Health Organization
wP - whole cell vaccine

DECLARATIONS

Ethics approval and consent to participate

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

Consent for publication

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

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

Funding

No funding was received for the submitted work

Authors' contributions

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

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TITLES OF INCLUDED ADDITIONAL FILES

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

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

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

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

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

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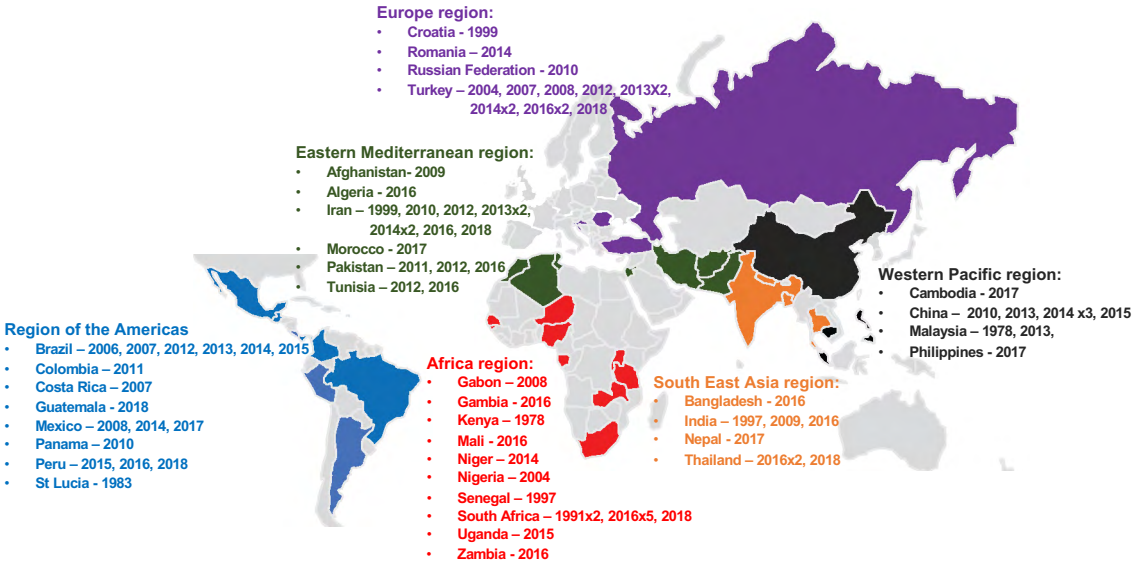
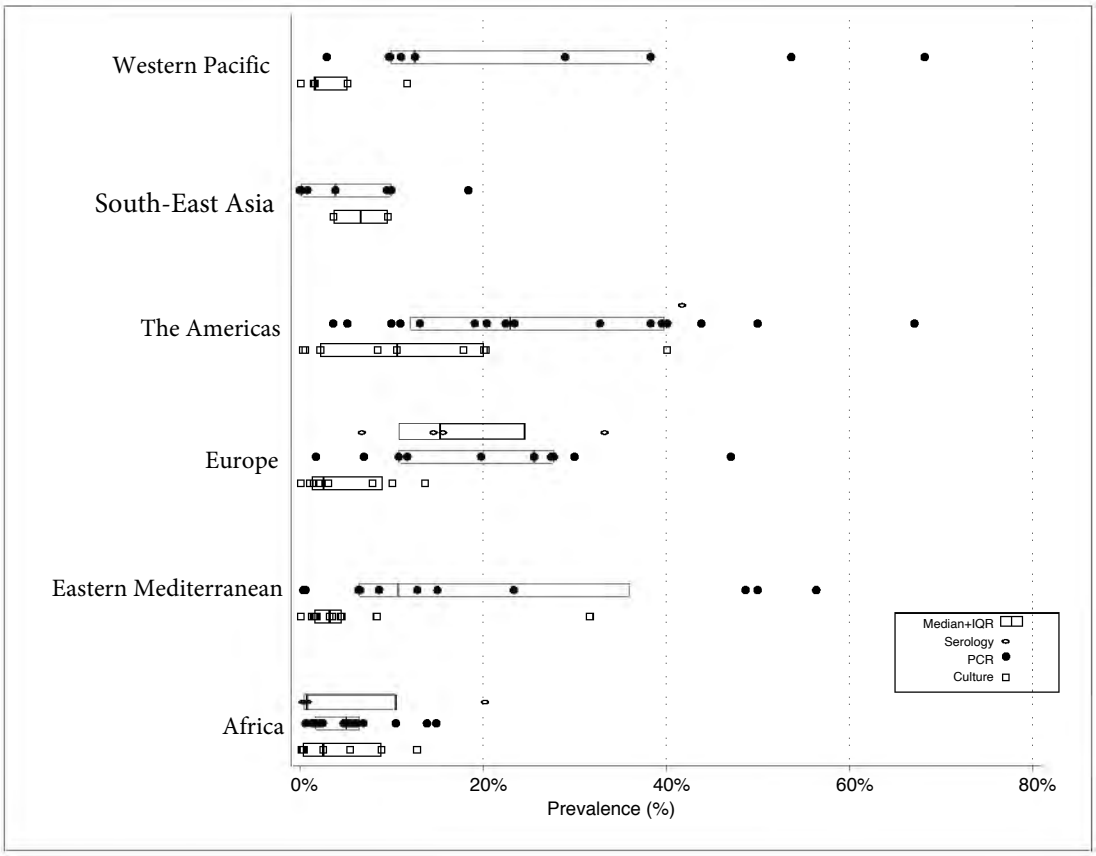
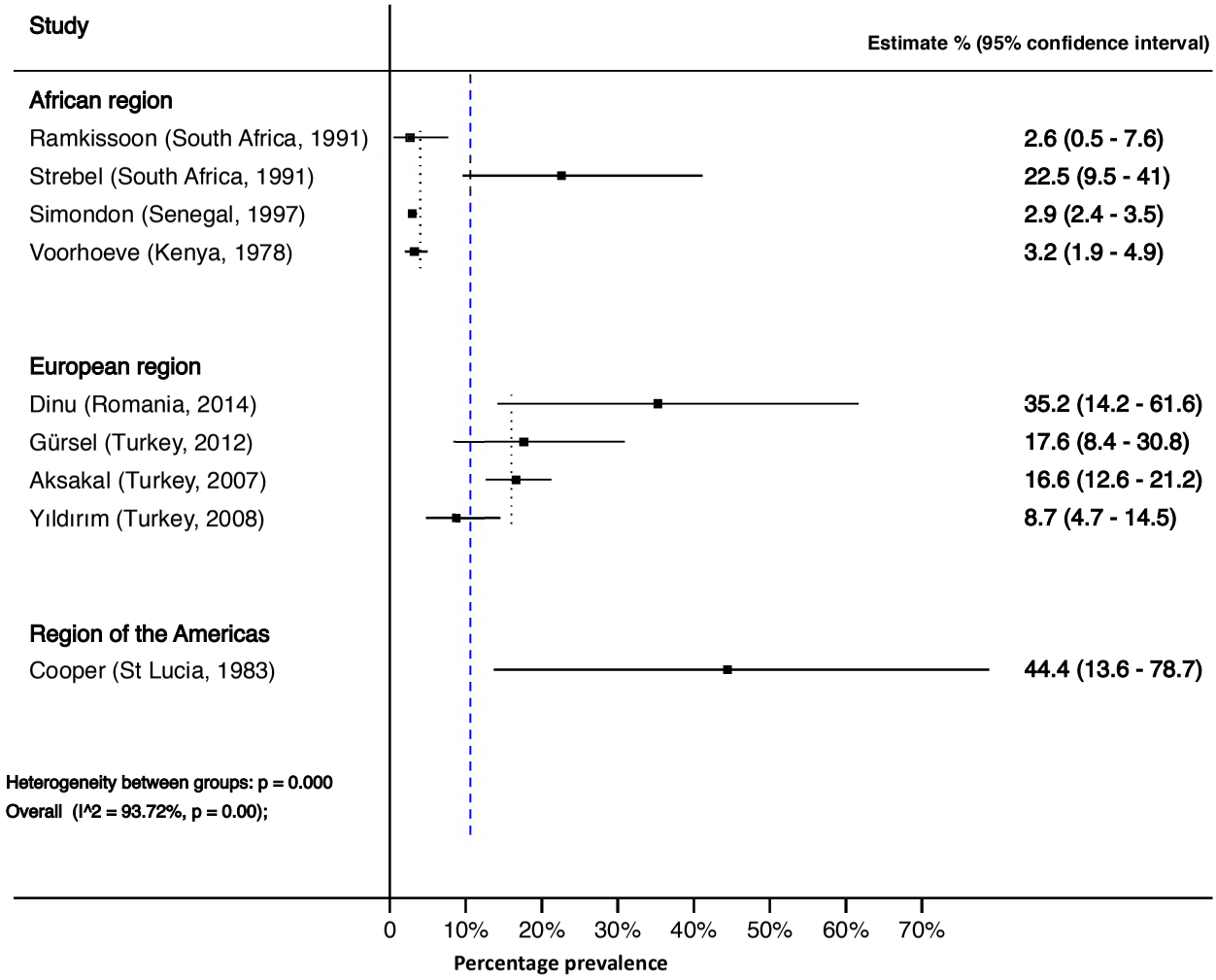
Additional Files



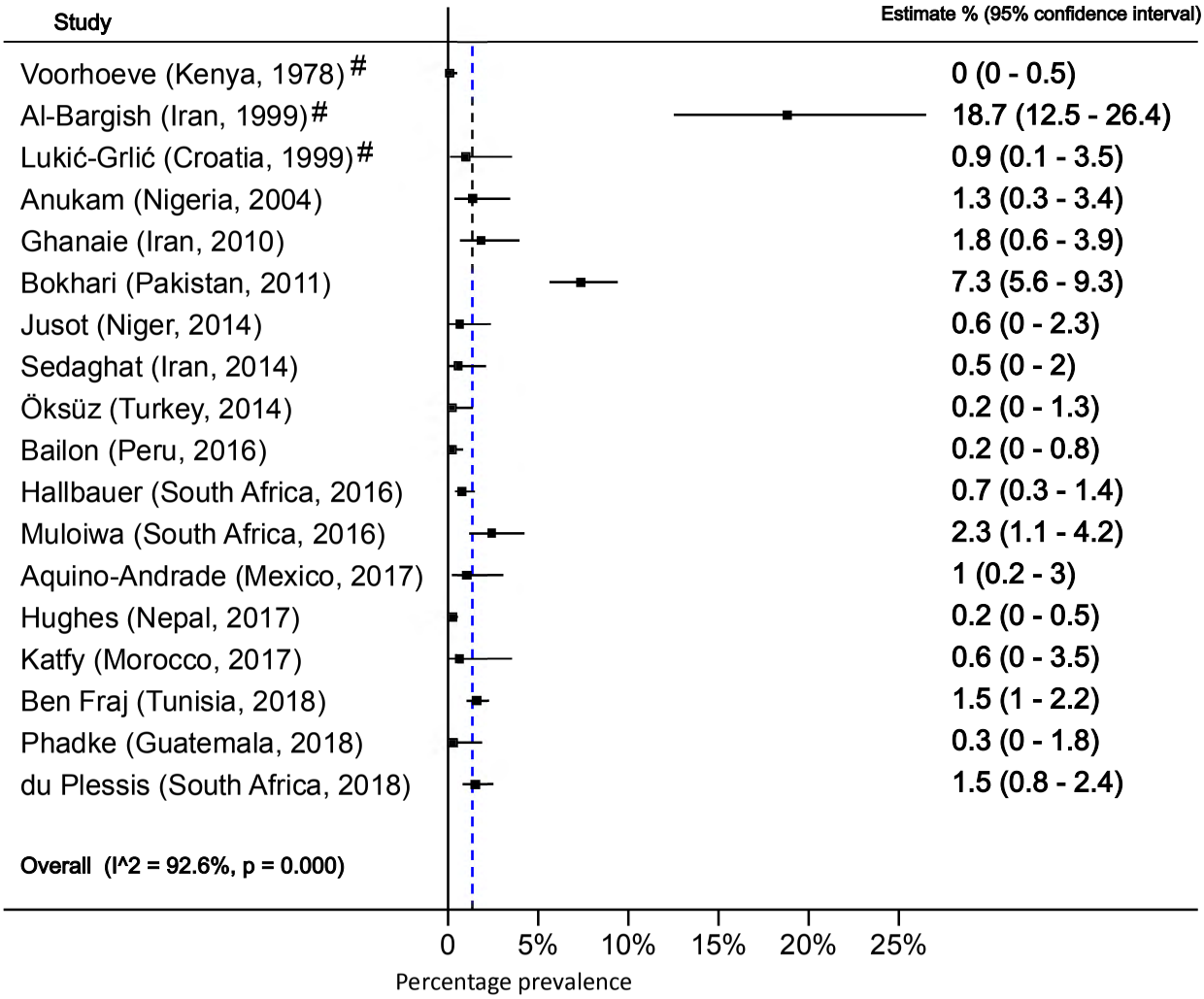
Additional file 1: Country and year of included studies with confirmed pertussis shown by World Health Organization region



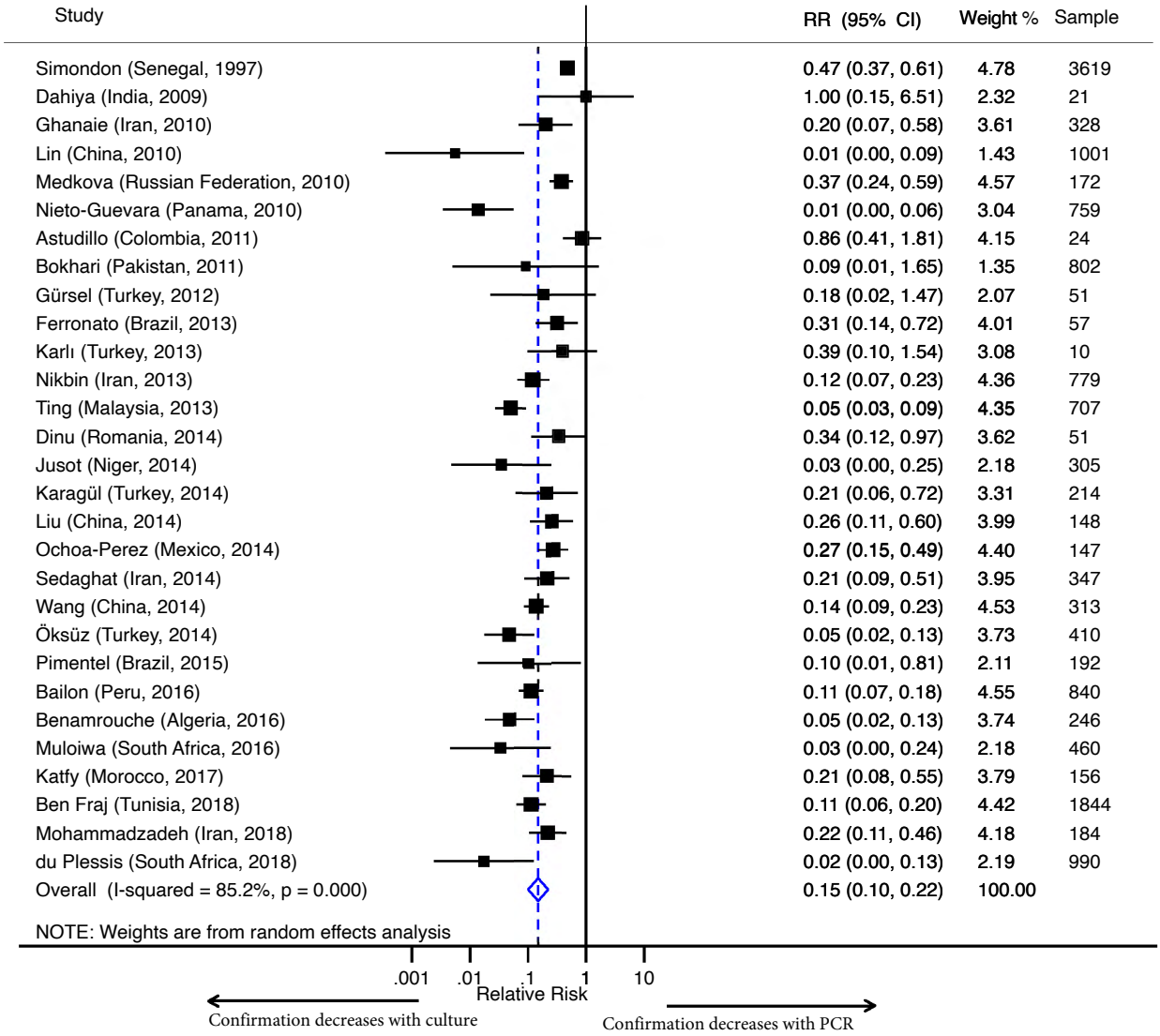
Additional file 2: Distribution of point prevalence of confirmed pertussis by World Health Organization region and confirmation method [PCR = polymerase chain reaction]



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



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



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

Chapter 3

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

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Keywords:

Bordetella pertussis, Bordetella parapertussis, pertussis, respiratory infection, induced sputum

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Cover title:

Pertussis in hospitalised South African children

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Pertussis in hospitalised South African children

Abstract

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

Methods: Children hospitalised with LRTI in Cape Town, South Africa were enrolled over one year. Clinical data were collected. A nasopharyngeal swab (NP) and induced sputum (IS) were taken and PCR specific for *Bordetella pertussis* (IS*481*+/hIS*1001*-) and *Bordetella parapertussis* (IS*1001*+) was performed.

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

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

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

Introduction

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

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

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

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

Materials and Methods

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

Participants

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

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

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

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

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

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

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

PCR specific for insertion sequences IS*481* for *Bordetella* spp. and IS*1001* for *B. parapertussis* was done with a validated commercial kit (LightMix® Kit *Bordetella pertussis* and *Bordetella parapertussis*, TIB MolBiol, Berlin, Germany) using previously published primers.[16] To exclude *Bordetella holmesii* (IS*481* +, IS*1001* -, hIS*1001* +) before the diagnosis of *B. pertussis* infection was made, all specimens testing positive for IS*481* were tested for the presence of insertion site hIS*1001*.[17]

Controls

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

Ethics

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

Sample size and statistical analysis

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

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

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

Results

Study participants

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

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

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

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

Description of microbiologic confirmation

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

PCR for IS*481* was positive in 17 of 460 NP specimens (3.7 %; 95% CI 2.2-5.9 %) while seven (1.5%; 95% CI 0.6-3.2%) were positive for IS*1001*. PCR was positive for both targets in one participant. Therefore 23 (5%; 95% CI 3.2-7.4%) NP specimens

were positive for either target. Of the 454 IS specimens processed, 25 (5.5%; 95% CI 3.6-8.0%) were positive for insertion site IS*481* and five (1.1%; CI 0.4%-2.5%) positive for IS*1001*, giving a total of 30 (6.6%; CI 4.5-9.3%) IS specimens positive for either *Bordetella* target.

NP specimens from four participants showed bacterial growth compatible with *Bordetella* spp. on culture but only one was positive on PCR for the IS*481* locus. The same participant was also positive for IS*481* on the flocked-swab NP specimen. NP specimens from all 70 controls (table 1) were PCR negative for both IS*481* and IS*1001* insertion sites. As the other three cultures could not be confirmed as *Bordetella pertussis* or *Bordetella parapertussis*, they were not included with positive cases.

None of the IS*481* positive specimens were positive for the hIS*1001 B. holmesii* locus and so all were classified as *B. pertussis* positive while all the IS*1001* positive specimens were classified as *B. parapertussis* positive. In total, 32 (7.0%; 95% CI 4.8-9.7%) participants were *B. pertussis* positive and 11 (2.4%; 95% CI 1.2-4.2%) were *B. parapertussis* positive from NP and IS specimens. Two participants were positive for both organisms. This gave an incidence of 8.9% (95% CI 6.5-11.9%; n=41/460) for either organism. The occurrence of *Bordetella* cases did not show any seasonal pattern although enrolled LRTI cases in general peaked between March and July 2013, figure 1.

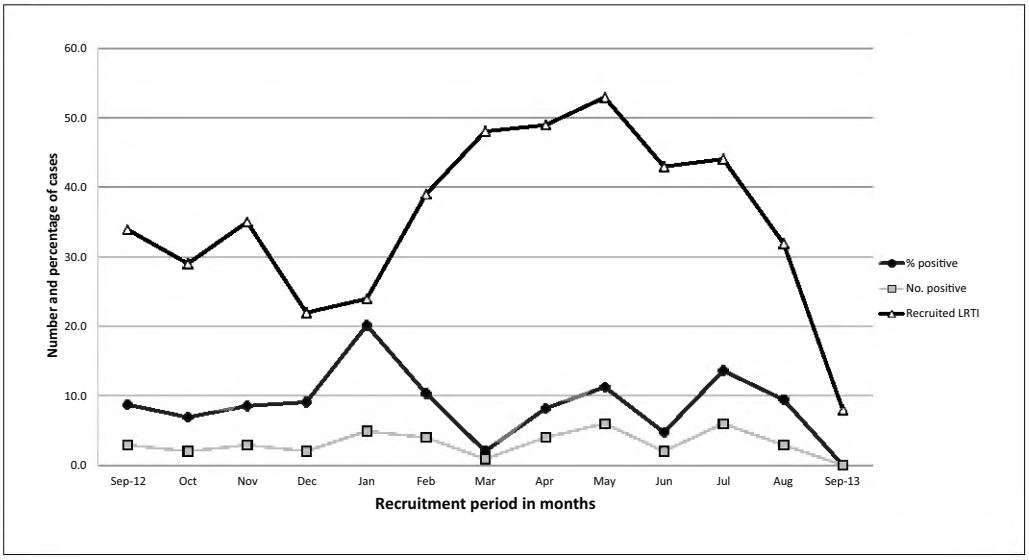

Figure 1: Recruited lower respiratory tract infection cases showing number and percentage of confirmed *Bordetella* per month

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

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

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

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

Stratifying variable	Stratum total (n)	*B. pertussis*	*B. parapertussis*	Total *Bordetella*
		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 unifected	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

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

Use of IS versus NP specimen for diagnostic confirmation

Ten participants had a positive PCR for IS*481* on both NP and IS. In one participant PCR for IS*1001* was positive on both the NP and IS while in another IS*1001* was positive on NP and IS*481* positive on IS. The latter was counted as a single case of *B. pertussis*. In total 30 out of 41 (73.2%) *Bordetella* confirmed cases were detected on IS while only 23/41 (56.1%) were detected on NP (of which six were not identified on IS).

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

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

Outcome

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

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

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

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

Discussion

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

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

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

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

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

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

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

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

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

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

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Chapter 4

Risk factors for *Bordetella pertussis* disease in hospitalised children

Risk factors for pertussis in children

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Abstract

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

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

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

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

Introduction

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

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

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

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

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

Materials and Methods

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

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

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

The mother's HIV status (and that of the primary caregiver if this was not the mother) was established. If the mother or caregiver was HIV infected the latest available CD4 count was recorded and used in the staging of HIV disease according to the Centre for Disease Control (CDC) classification.[19] History was taken on the presence and duration of recent primary caregiver respiratory symptoms as well as presence and numbers of other household members with similar symptoms.
The vaccination status of each child was verified using the national standardized immunisation handheld record, the Road to Health Card (RTHC); specifically, the date and type of each vaccine was copied from the record.

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

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

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

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

A commercially validated duplex real-time PCR assay targeting the insertion sequence IS*481* for *Bordetella* and IS*1001* for *Bordetella parapertussis* (LightMix® *Bordetella pertussis* and *parapertussis* Kit, TIB MolBiol, Berlin, Germany) was used for screening all the respiratory specimens.[22, 23] All specimens testing positive for IS*481* were further tested for the presence of insertion site hIS*1001* in order to exclude *Bordetella holmesii* (IS*481* +, IS*1001-,* hIS*1001* +) before the diagnosis of *B. pertussis* infection was made.[24]

Ethics

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

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

Analysis plan

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

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

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

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

Results

Baseline characteristics of children

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

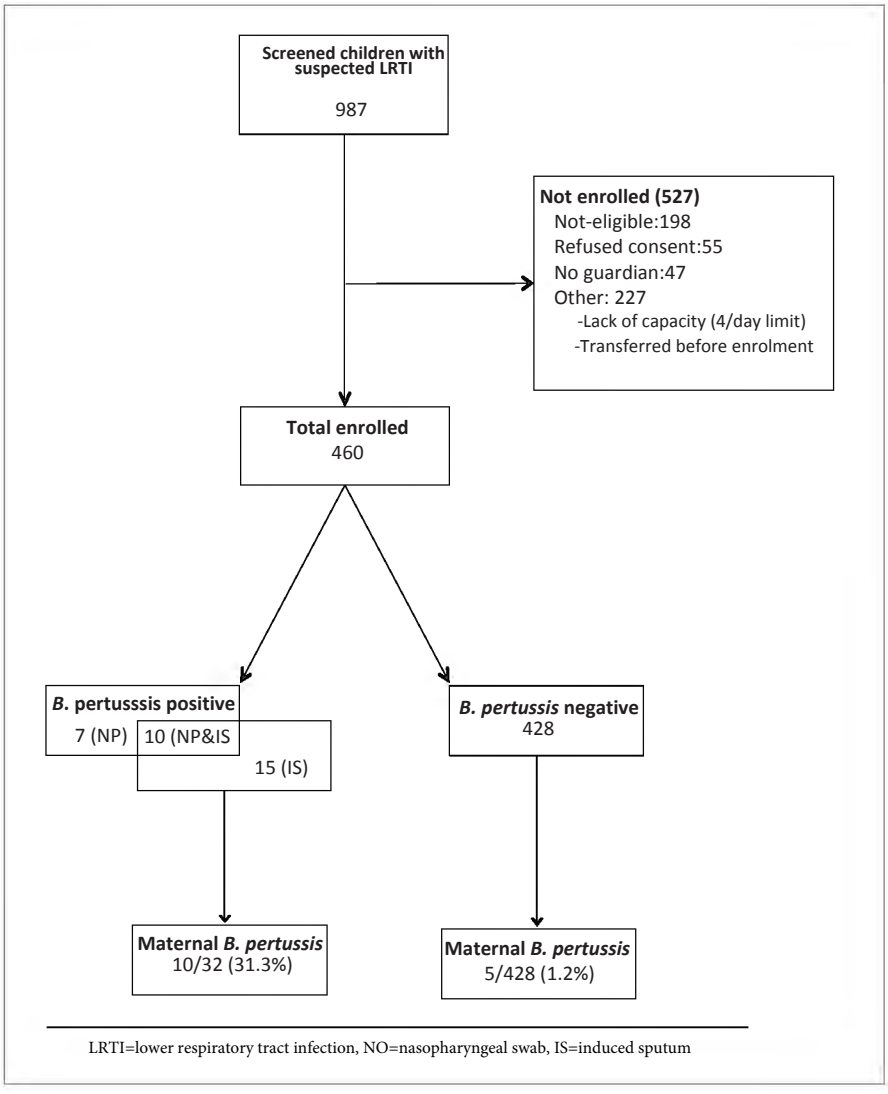

Fig 1. Enrolment flow diagram of study participants showing number of *Bordetella pertussis* positive children and caregivers
LRTI=lower respiratory tract infection, NP=nasopharyngeal swab, IS=induced sputum

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

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

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

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

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

Confirmed *Bordetella pertussis* in children

NP specimens were obtained from all child participants. Four children were transferred or discharged out of the ward before an IS specimen could be obtained. For the remaining 456 children, IS was successfully obtained with no major adverse events, although two were later lost to container leakage.

PCR for IS*481* was positive in 17 NP specimens and 25 IS specimens. There was an overlap of positive NP and IS specimens in 10 participants giving a total of 32 (7.0%; 95% CI 4.8-9.7%) children with confirmed *B. pertussis* infection. *B. parapertussis* (IS1001+) was detected in seven (1.5%; 95% CI 0.6-3.2%) children. *B. holmesii* was excluded in all the positive specimens by the absence of hIS*1001*.

Caregiver baseline characteristics

All 460 primary caregivers took part in the study of whom 450 (97.8%) were mothers. For the remaining 10 children, the caregiver was the father in two (0.4%) instances, grandmother in five (1.1%) and another relative in the other three (0.7%) children. In 451(98.0%) of the recruited pairs, the caregiver slept in the same room as the child. The median age of the caregivers was 28 (IQR 24-33) years. In the week the child presented to hospital, 171 (37.2%) of the caregivers had respiratory symptoms. The symptoms, predominantly of an upper respiratory tract infection, were present in 10 (31.3%) of caregivers whose children had confirmed pertussis and in 161 (37.7) whose children did not have pertussis; p=0.466. Baseline characteristics of the caregivers are summarized in 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)
***B. pertussis* NP**		
PCR positive	5 (1.2)	10 (31.3)

NP = nasopharyngeal swab specimen

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

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

Confirmed *Bordetella pertussis* in caregivers

NP specimens were successfully obtained from all 460 caregivers. IS*481* was positive in 15 (3.3%; 95% CI 1.8-5.3%) of the caregivers, 10 in mothers of children with confirmed *B. pertussis* infection, table 2. All IS*481* positive specimens were negative for the hIS*1001 B. holmesii* locus.

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

Effect of risk factors

Unadjusted and adjusted effects of factors on risk of pertussis disease in children are 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 *B. pertussis*			
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**

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

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

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

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

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

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

Discussion

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

Sub-Saharan Africa, where this study was conducted, carries a high burden of HIV, including a large number of infected or exposed children. In the current study, after adjusting for potential confounding, HIV-infected children had a four-fold increase in the risk of pertussis, possibly due to reduced vaccine effectiveness due to both poor responses to vaccination as well as low persistence of immunoglobulin following vaccination.[8, 9] This increased risk may also reflect increased parental susceptibility. A study Nigerian study showed a 20-fold risk of pertussis in adolescents not yet initiated 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.

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

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

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

Conclusions

There is an urgent need for interventions in LMICs to address modifiable risk factors for pertussis. Such interventions should include nutritional support and immunisation. Immunisation programs should be strengthened to ensure high levels of coverage for children with at least three vaccine doses and include catch-up immunisation for missed doses. A key consideration is to prioritise vaccination of pregnant women, particularly those who are HIV infected, as maternal infection is the greatest risk for disease in infants.[43]

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Chapter 5

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

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Abstract

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

Introduction

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

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

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

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

Methods

Recruitment and specimen collection

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

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

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

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

The children's vaccination status was sourced from their handheld clinic booklets. The primary schedule according to the South African Expanded Program on Immunisation, in addition to other vaccines, contains an acellular (aP) vaccine against *B. pertussis* combined with that against *Haemophilus influenzae type b* at 6, 10 and 14 weeks (with a booster at 18 months), and vaccination with 13-valent pneumococcal conjugate (PCV13) vaccine at 6 and 10 weeks of age (with a booster at 9 months) .[19]

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

Laboratory methods

Diagnosis of *Bordetella pertussis*

To diagnose *Bordetella pertussis* infection, PCR specific for IS*481* for *Bordetella* species was conducted on both NP and IS specimens with a validated commercial kit (Roche LightMix®, Basel) using previously published primers.[21] *Bordetella holmesii* (defined as IS*481* + and hIS*1001* +) infection was excluded by further testing all IS*481* positive specimens for the presence of insertion site hIS*1001*.[22]

Diagnosis of co-infections

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

Statistical analysis

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

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

Statement on ethics approval

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

Results

Baseline data

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

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

Table 1: Baseline characteristics of study participants

Baseline character	*N=454*
Age	
Median (IQR) months	8 (4-18)
	n (%)
Male sex	253 (55.7.)
Pertussis/*H. influenza type b* 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 Bordetella pertussis	31(6.8)

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

Confirmed *Bordetella pertussis* infection

PCR for insertion site IS*481* was positive in 16 NP specimens and 25 IS specimens on LightMix®. Ten participants had a positive PCR on both NP and IS specimens, therefore 31 (6.8%; 95% CI 4.7-9.6%) participants were confirmed as having *B. pertussis* infection. The *B. holmesii* insertion site hIS*1001,* was not identified in any of the IS*481* positive specimens. Only 10 (2.2%) samples, all also found to be

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

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

General description of PCR detected pathogens

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

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

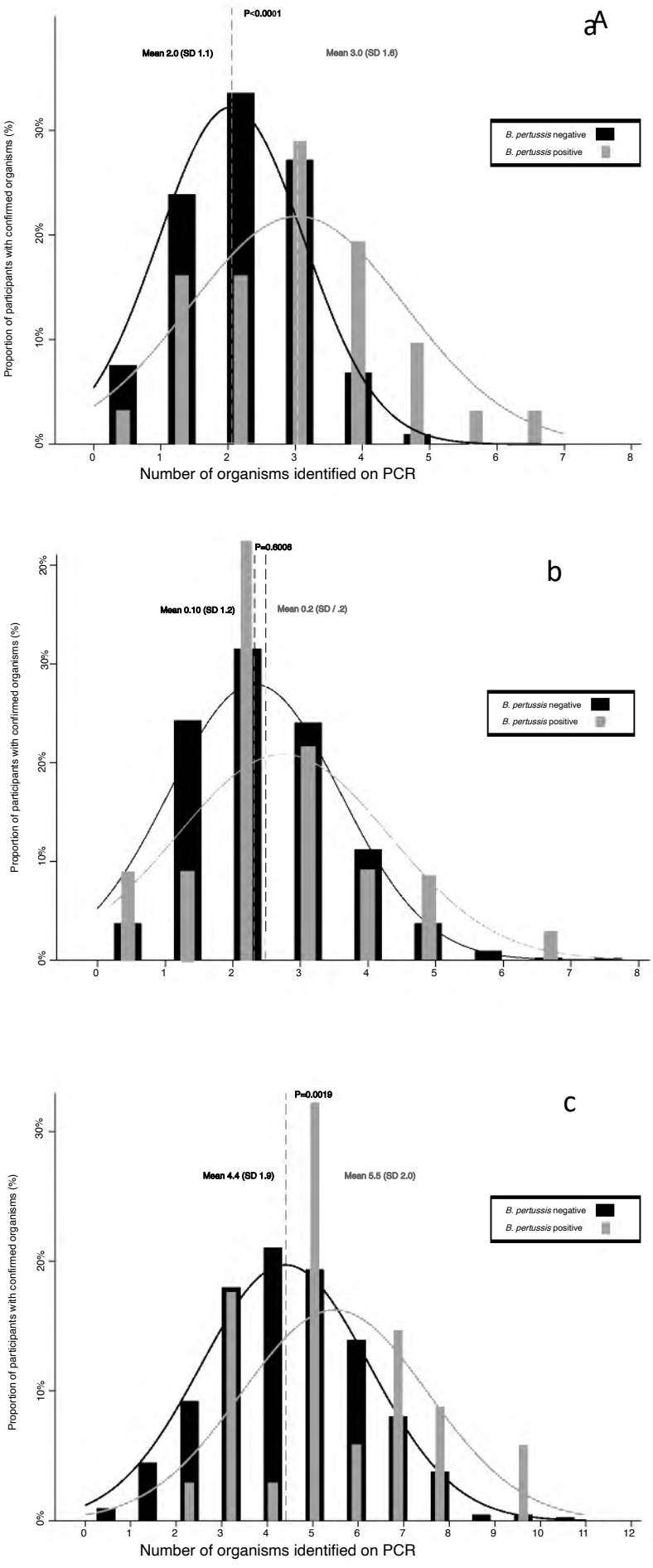

Figure 1. Distribution of number of bacteria **(a) ,** viruses **(b)** and bacteria + viruses **(c)** identified on polymerase chain reaction (PCR) in participants with and without *Bordetella pertussis*

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

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

Pathogen¶	Total n (%)	*Bordetella pertussis* 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				
Moraxella catarrhalis	295 (65.0)	18 (58.1)	277 (65.5)	0.403
Streptococcus pneumoniae	240 (52.9)	17 (54.8)	223 (52.7)	0.819
Haemophilus influenzae	231 (50.9)	18 (58.1)	213 (50.4)	0.407
Staphylococcus aureus	136 (30.0)	9 (29.0)	127 (30.0)	0.907
Haemophilus influenzae B	16 (3.5)	2 (6.5)	14 (3.3)	0.299
Mycoplasma pneumoniae	10 (2.2)	3 (9.7)	7 (1.7)	**0.025**
Chlamydia pneumoniae	7 (1.2)	2 (6.5)	5 (1.2)	**0.076**
Fungal organism				
Pneumocystis jirovecii	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

Co-detection of *Bordetella pertussis* with specific organisms

Overall, *Moraxella catarrhalis* was the commonest bacterium identified with 295 (65.0%) samples testing PCR positive for the organism. *Mycoplasma pneumoniae* showed association with *B. pertussis* infection (P=0.025) while *Chlamydia pneumoniae* displayed weak evidence of association with *B. pertussis* (P=0.076). No other bacteria were significantly associated with confirmed pertussis. Table 2. Cytomegalovirus was the commonest virus, identified in 253 (55.7%) of the participants. Parainfluenza viruses (1, 2, 3 &4) were weakly associated (P=0.052) with the presence of *B. pertussis*, while Respiratory Syncytial Virus (RSV) was

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

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

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

Co-infection	*Bordetella pertussis* PCR n (%)		RR (95% Confidence interval)	
	Positive n=31	Negative n=423	Crude	Adjusted[#]
Chlamydia pneumoniae	2 (6.5)	5 (1.2)	**4.40 (1.29-14.98)**	**4.01 (1.03-15.64)**
Mycoplasma pneumoniae	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.

Clinical presentation and outcome

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

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

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

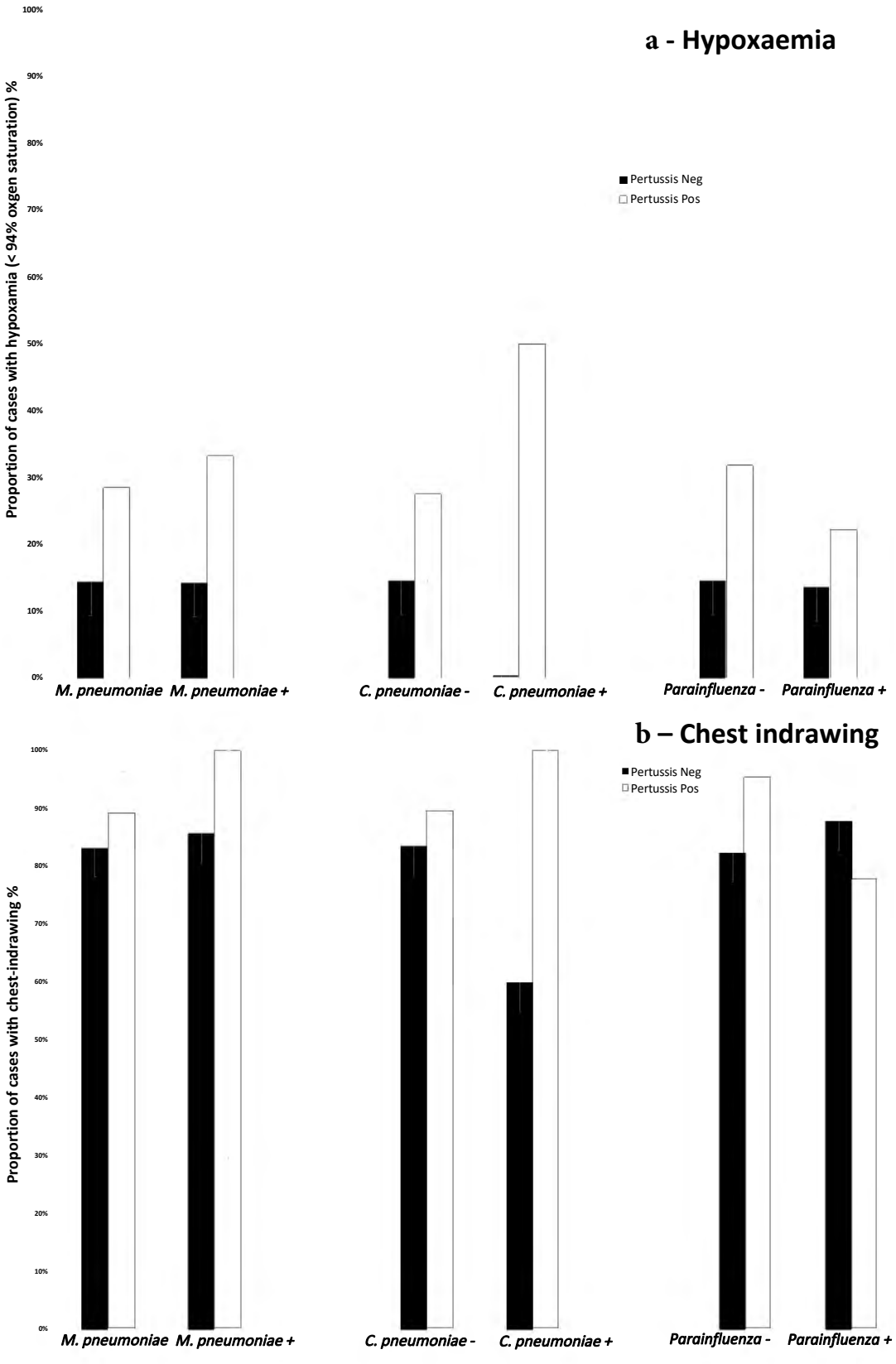

Figure 2. Proportion of children with hypoxaemia and chest indrawing by presence of co-detected organisms that were strongly associated with pertussis

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

Discussion

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

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

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

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

Children with pertussis generally showed more severe clinical illness (hypoxaemia, chest-indrawing and need for high dependency care). Although these findings were not all statistically significant due to the small numbers, the pattern was consistent. Children who had both strongly associated organisms and *B. pertussis* detected showed additional risk of severe disease, again without statistical significance. It is of note that the three organisms independently associated with *B. pertussis* are associated with 'atypical' or interstitial pneumonia which commonly presents with impaired oxygenation. There was no difference in mortality (study registered no deaths) and

length of hospital stay.

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

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

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

Partial and waning immunity, especially in individuals vaccinated with acellular

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

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

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

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Author contribution statement

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

Competing interests statement

The authors declare no competing interests.

Data availability

All data analysed during the current study have been presented in this manuscript, but the corresponding datasets generated are available from the corresponding authors on reasonable request.

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Chapter 6

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

Limited diagnostic accuracy of pertussis clinical case definitions

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Abstract

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

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

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

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

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

Introduction

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

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

Acknowledging shortcomings of WHO clinical case definition, the Global Pertussis

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

Table 1: Clinical features for diagnosis of pertussis cases

World Health Organization	**Global Pertussis Initiative**	
▪ **A case diagnosed as pertussis by a physician, OR**	▪ **Cough or illness in a person without or with only minimal fever AND:**	
▪ **A person with a cough lasting ≥2 weeks with ≥1 of the following: symptoms:**	**0 – 3 months**	**4 months to 9 years**
	Cough and coryza **PLUS** any of	Paroxysmal cough **PLUS** any of
• Paroxysms (i.e., fits) of coughing • Inspiratory "whooping" • Post-tussive vomiting (i.e., vomiting immediately after coughing) without other apparent cause	• 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	• 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

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

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

lymphocytosis to support a clinical diagnosis.[11]

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

Materials and Methods

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

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

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

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

Pertussis PCR

PCR targeting the IS*481* common Bordetella insertion site and IS*1001* for *Bordetella parapertussis* was done after which specimens testing positive for the former were further tested with PCR for hIS*1001* to exclude *Bordetella holmesii* which shares the same IS*481* insertion site. IS*481+/* hIS*1001*- samples were classified as confirmed *Bordetella pertussis* infection. The methods and epidemiological findings of this study have been published elsewhere.[12] Only assessment of *Bordetella pertussis* was included in the current analysis to allow for comparison with other studies.

Analysis of data

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

Proportions were summarized as percentages with a 95% interval of confidence, as 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

Results

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

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

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

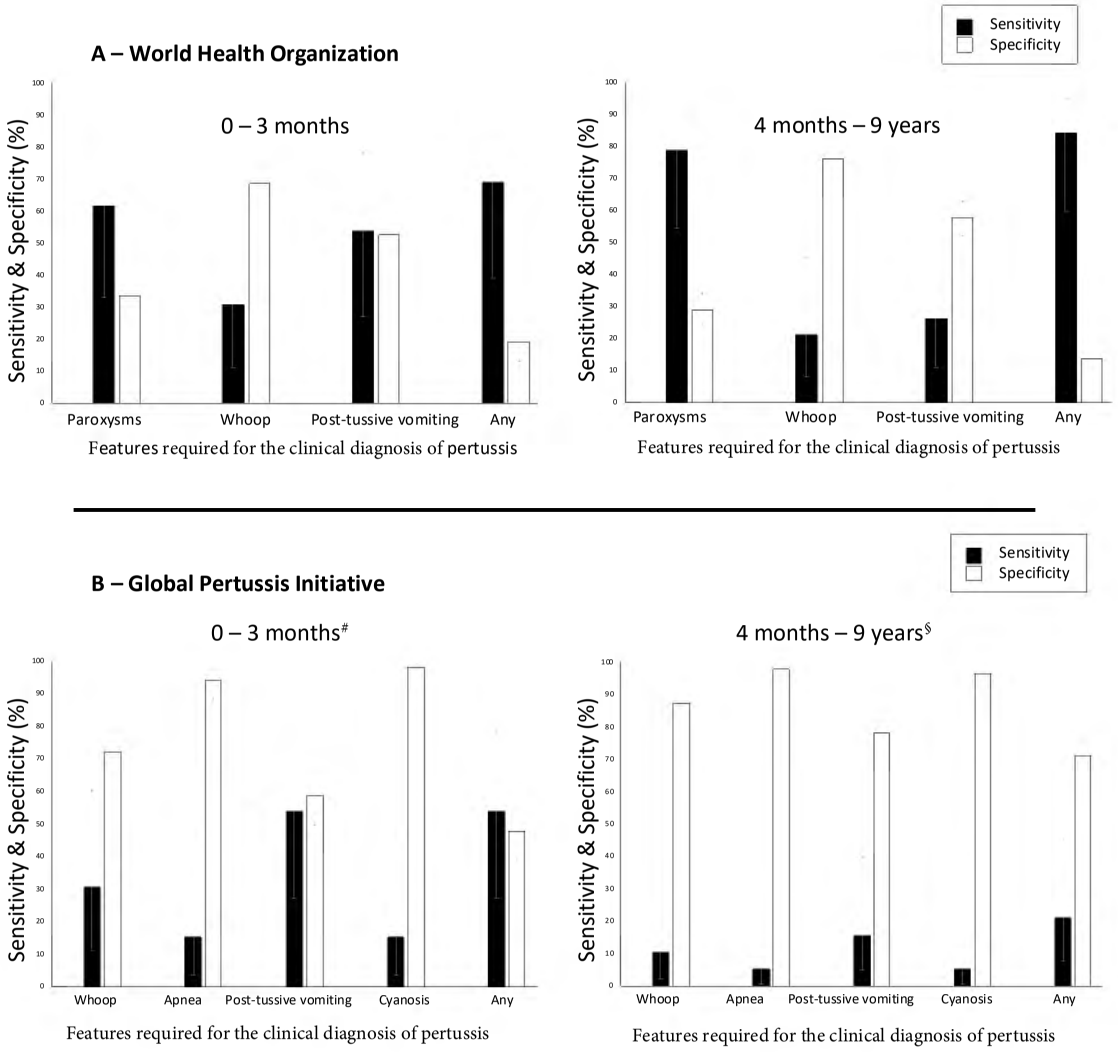
The median duration of symptoms was 3 (IQR 2-5) days. Paroxysmal cough was reported as a presenting feature in 398 (69.9%) children; similar in confirmed pertussis cases and those with negative PCR under four months of age with 8/13 (61.5 %) and 79/119 (66.4%) respectively; p=0.726. Similarly, paroxysmal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

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

	Clinical Feature	PCR+[n (%)]	PCR- [n (%)]	P Value
		n=13	**n=119**	
0 - 3 months	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
		n=19	**n=307**	
4 months - 9 years	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

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



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

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

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

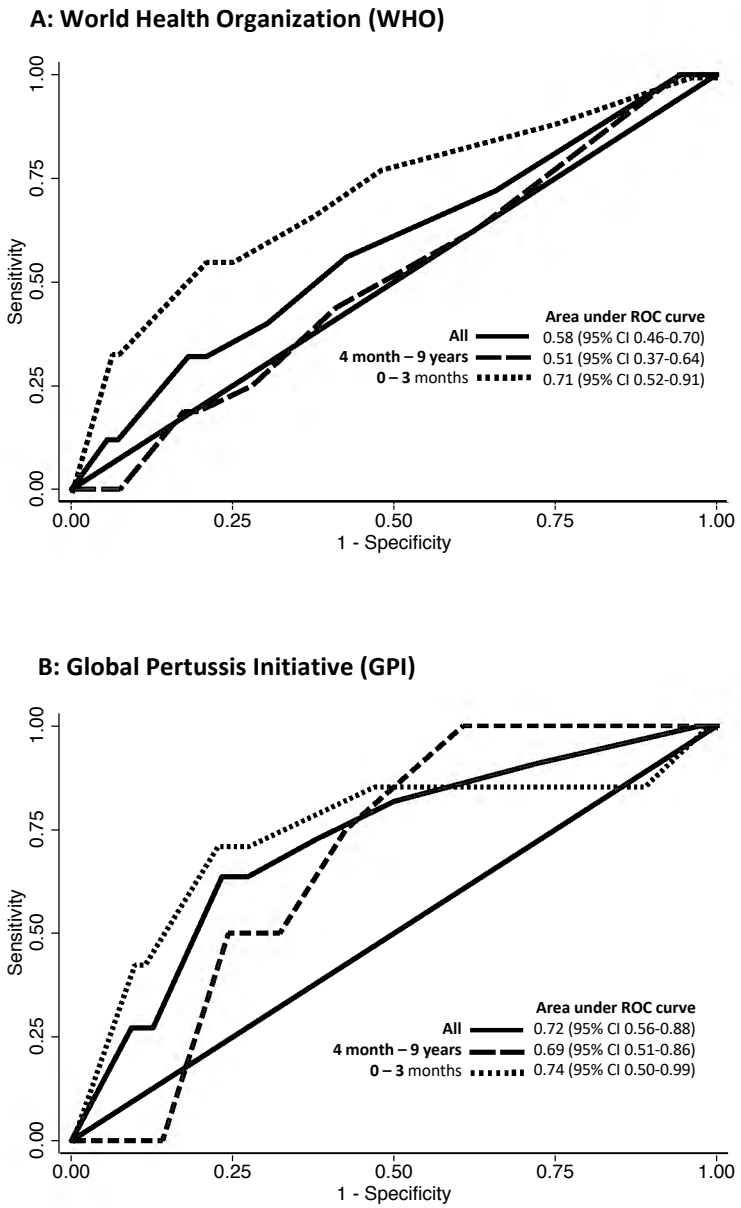


Figure 2. Receiver operating characteristics (ROC) curves for duration of symptoms

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

decline in sensitivity occurred earlier and steeper when using WHO clinical criteria than when GPI criteria were utilized. Sensitivity was 12.0% and 27.3% at ≥14-day duration for WHO and GPI criteria respectively, while specificity was 94.0% and 90.7% respectively for WHO and GPI criteria at a similar duration cut-off.

In general, sensitivity declined, and specificity increased with increase in duration of symptoms. When any symptom was considered, the highest combination of sensitivity and specificity with the use of GPI clinical features was seen between five and seven days with sensitivity ranging between 63.6% and 72.7% and specificity ranging between 62.0% and 76.7%. Criteria recommended by WHO showed their highest combination of sensitivity and specificity between three and five days duration of symptoms with sensitivity ranging between 40.0% and 72.0%, and specificity ranging between 34.3% and 69.6%. Figure 3. The highest likelihood ratio for sensitivity was seen in the young infant group. This ranged between 1.00 and 5.33 using WHO based criteria and between 0.96 and 4.42 when GPI based criteria were applied.

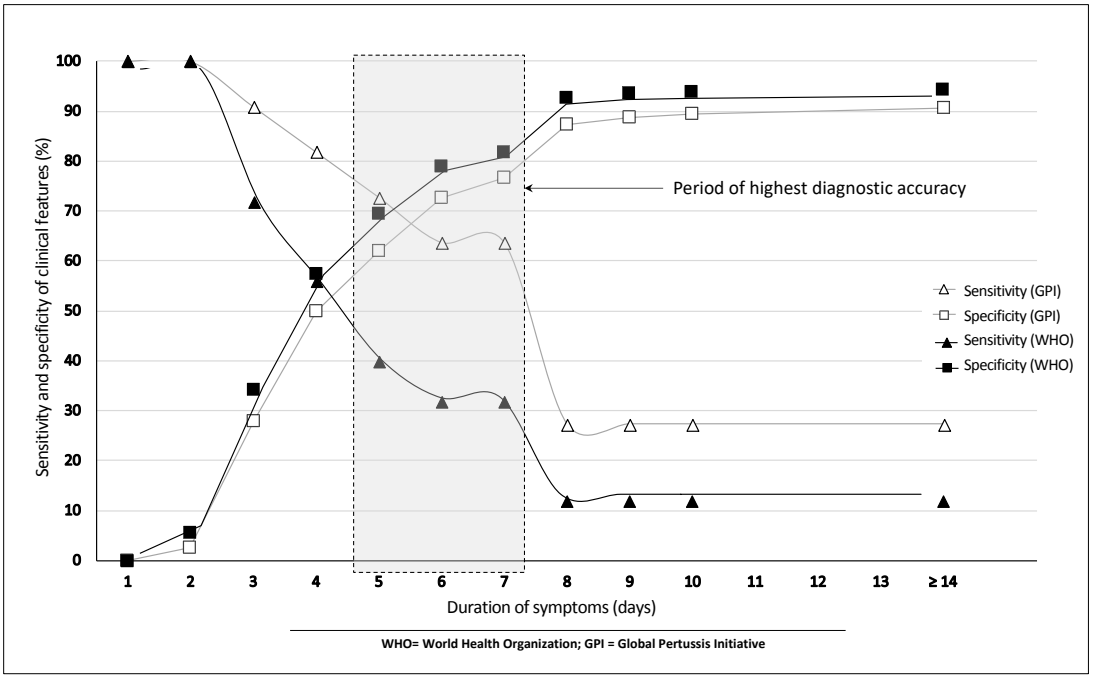


Figure 3. Sensitivity and specificity of clinical features in the diagnosis of pertussis with changing duration

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

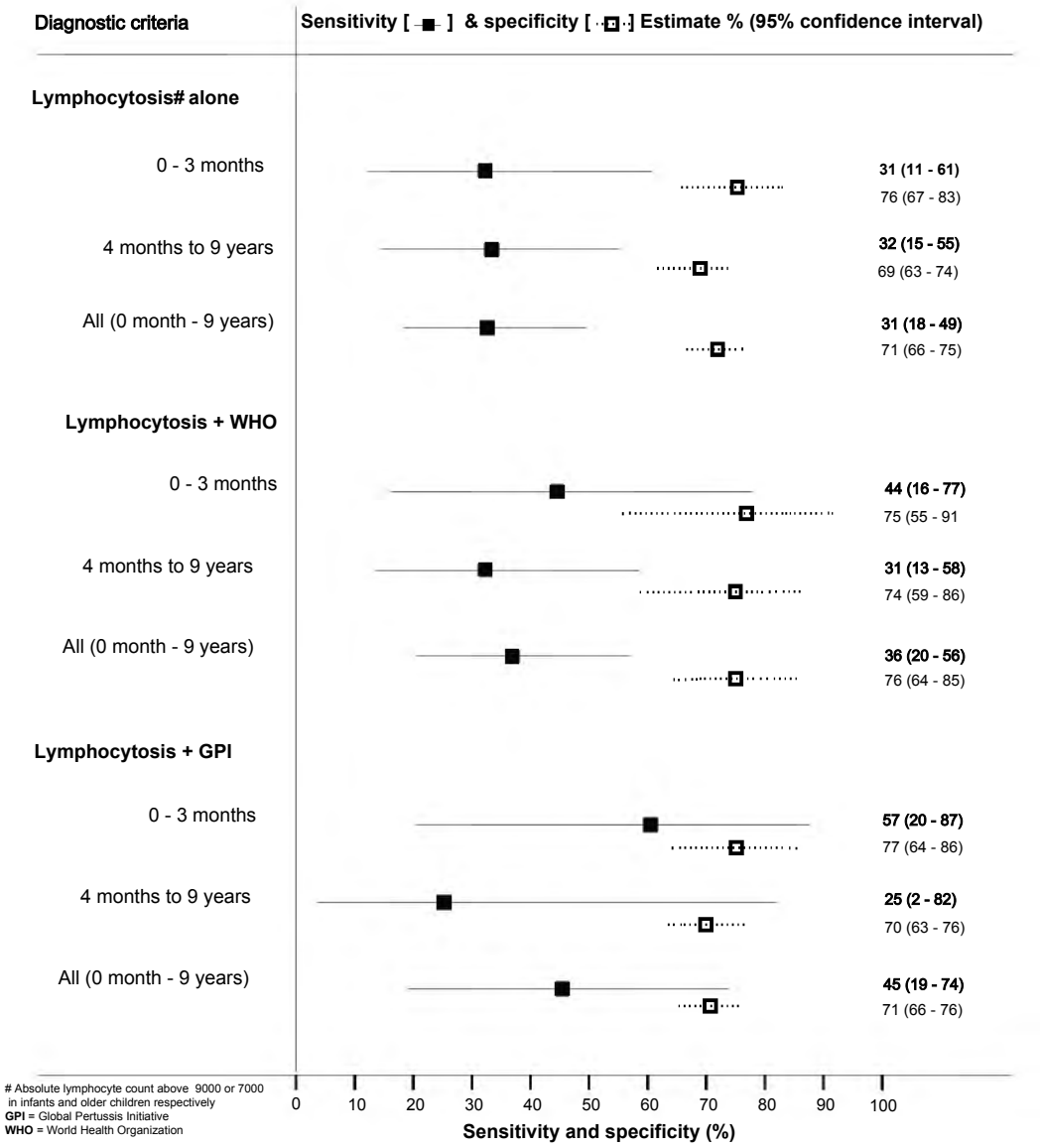


Figure 4. Sensitivity and specificity of lymphocytosis in the diagnosis of pertussis

Discussion

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

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

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

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

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

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

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

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

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

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

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

Conclusions

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

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Chapter 7

Highlights and Conclusions

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

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

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

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

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

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

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

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

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

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]*

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

References

1. Muloiwa R, Wolter N, Mupere E, Tan T, Chitkara AJ, Forsyth KD, et al. Pertussis in Africa: Findings and recommendations of the Global Pertussis Initiative (GPI). Vaccine. 2018;36(18):2385-93. Epub 2018/04/01. doi: 10.1016/j.vaccine.2018.03.025.

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 bythe 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 LameesEmjedi

Research Ethics Committee
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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

<u>If illiterate</u>

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

2. RTHC / Vaccination History

Pertussis study number: __ __ __ __: __ __ __

1.	Does the child have a road to health card available?	☐ Yes *(Skip to 2)* ☐ No
1.1	*If No*, Has the child ever received any vaccines other than the vaccines *he or she* received at birth?	☐ Yes ☐ No ☐ Unknown

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

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

If the RTHC is available, please complete ☐ **No RTHC available**

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

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	☐ *Male* ☐ *Female*
2.	Date of admission	___ ___ / ___ ___ / 2 0 ___ ___ *(dd/mm/yyyy)*
3.	Time of admission	___ ___ : ___ ___ ☐ *Am* ☐ *Pm* ☐ *Unknown*
4.	Race	☐ *Asian/Indian* ☐ *Black* ☐ *White* ☐ *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* ☐ *Not recorded*
11.	Respiratory rate	___ ___ ___ *breaths / min* ☐ *Not recorded*
12.	Oxygen saturation	___ ___ ___ *%* ☐ *Room air* ___ ___ ___ *%* ☐ *On oxygen* ☐ *Not recorded*
13.	Presence of oedema	☐ *Yes* ☐ *No* ☐ *Not recorded*
14.	Did the child receive any of: Bronchodilators Antibiotics *If Yes*, name(s) of antibiotic(s)	 ☐ *Yes* ☐ *No* ☐ *Yes* ☐ *No* _____
15.	HIV Stage of the child	☐ *1* ☐ *2* ☐ *3* ☐ *4* ☐ *Not Applicable*

16.	Lower chest wall indrawing:	☐ *Yes* ☐ *No* ☐ *Not recorded*
17.	Crackles/crepitations	☐ *Yes* ☐ *No* ☐ *Not recorded*
18.	Wheezing	☐ *Yes* ☐ *No* ☐ *Not recorded*
19.	Clubbing	☐ *Yes* ☐ *No* ☐ *Not recorded*

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

20.	Did child receive supplemental oxygen	☐ *Yes* ☐ *No* ☐ *Not recorded*
21.	Admission to a High Care unit or ICU If admitted to above, number of days	☐ *ICU* ☐ *High Care* ☐ *None* ☐ *Unknown* *ICU days:* ____ *High Care days:* ____ ☐ *Unknown*
22.	Assisted ventilation If Yes, Duration Type of support Number of days	☐ *Yes* ☐ *No* ☐ *Not recorded* _____ *days* ☐ *Not recorded* ☐ *CPAP* ☐ *IPPV* _____ *days* _____ *days* ☐ *Not recorded*
23.	Outcome of child	☐ *Discharged* ☐ *Refused Hospital Treatment (RHT)* ☐ *Died* ☐ *Transferred to another hospital*
24.	Date of discharge/ death/ transfer/RHT	___ ___ / ___ ___ / 2 0 ___ ___ *(dd/mm/yyyy)*
25.	If discharged, on what treatment was child discharged? *(mark all that apply)*	☐ *Antibiotics, specify:*_____ ☐ *Bronchodilators* ☐ *Inhaled steroids* ☐ *Anti TB treatment* ☐ *Other, specify* _____
26.	*Discharge diagnosis (mark all that apply)*	☐ *Bronchopneumonia* ☐ *Bronchiolitis* ☐ *Lobar Pneumonia* ☐ *Tuberculosis* ☐ *Pertussis* ☐ *Pneumocystis pneumonia* ☐ *Chronic lung disease* ☐ *Sepsis* ☐ *Underweight/Kwashiorkor/Marasmus* ☐ *Not recorded* ☐ *Other, specify*_____

Staff initials: __ __ __ Date completed: __ __ /__ __ / 20 __ __

4. Care-Giver Information

Pertussis study number: __ __ __ __: __ __ __

1.	Sex	☐ *Male* ☐ *Female*
2.	Date of birth	___ ___ / ___ ___ / 19 ___ ___ *(dd/mm/yyyy)*
3.	What is your relationship to child	☐ Mother ☐ Father ☐ Grandparent ☐ Other relative ☐ Caregiver ☐ Other: _____
4.	Race	☐ *Asian/Indian* ☐ *Black* ☐ *White* ☐ *Coloured*
5.	Do you sleep in the same room as child?	☐ *Yes* ☐ *No*
6.	Do you smoke? If Yes, do you smoke inside the house?	☐ *Yes* ☐ *No (skip to next question if No)* ☐ *Yes* ☐ *No*
	Did you experience any of these symptoms before or during the course of the child's illness?	
7.	Fever?	☐ *Yes* ☐ *No* ☐ *Unknown*
8.	Wheezing?	☐ *Yes* ☐ *No* ☐ *Unknown*
9.	Runny or congested nose?	☐ *Yes* ☐ *No* ☐ *Unknown*
10.	Cough?	☐ *Yes* ☐ *No* ☐ *Unknown* *(skip to 11 if No or Unknown)*
	If Yes, duration?	_____ *days* _____ *weeks* ____ *months* ☐ *Unknown*
10.1	Night time cough	☐ Yes ☐ No ☐ Unknown
10.2	Paroxysms of cough	☐ Yes ☐ No ☐ Unknown
10.3	Whooping cough	☐ Yes ☐ No ☐ Unknown
10.4	Vomiting after cough	☐ Yes ☐ No ☐ Unknown
10.5	Barking cough	☐ Yes ☐ No ☐ Unknown
10.6	Normal state between cough	☐ Yes ☐ No ☐ Unknown
10.7	Did you receive any of the following: Bronchodilators Antibiotics *If Yes*, Name of antibiotic	☐ *Yes* ☐ *No* ☐ *Yes* ☐ *No* _____
11.	Have you ever been diagnosed with one of the following chronic medical conditions?	☐ TB Disease: *If yes*: ☐ On treatment ☐ Completed treatment ☐ Defaulted ☐ Asthma ☐ Other lung problems ☐ Pertussis/Whooping cough ☐ Heart problems ☐ None ☐ Other, S*pecify* _____
12.	Have you ever had an HIV test?	☐ Yes ☐ No *(skip to end if No)*
13.	*If Yes,* what was the result of the latest test?	☐ Positive ☐ Negative *(skip to end if Negative)*
14.	Are you currently receiving Antiretroviral Therapy (ART)?	☐ Yes ☐ No *(skip to 14.2 if No)*
14.1	If *Yes,* how long have you received it?	_____ months
14.2	Most recent CD4 count Date __ __ / __ __ / *20* __ __	_____ ☐ *Unknown*
14.3	Most recent viral load Date __ __ / __ __ / *20* __ __	_____ ☐ *Unknown*

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** ☐ **Not done** *(skip to 2)*		
	Test	Date of test	Result
1.1	☐ ELISA – child	__ __ / __ __ / 2 0 __ __	☐ Reactive ☐ Non reactive
1.2	☐ ELISA - mother	__ __ / __ __ / 2 0 __ __	☐ Reactive ☐ Non reactive
1.3	☐ PCR - child	__ __ / __ __ / 2 0 __ __	☐ Positive ☐ Negative
1.4	☐ Most recent CD4 count - child	__ __ / __ __ / 2 0 __ __	__ __ __ __ Absolute CD4 __ __.__ % of lymphocytes
1.5	☐ Most recent viral load - child:	__ __ / __ __ / 2 0 __ __	__ __ __ __ __ __ copies/ml ☐ >750 000

2.	**Micro/Virology results** - *Obtained in the first 48 hours of admission* ☐ **Not done** *(skip to 3)*			
	Lab number	Date of test	Site	Organism
2.1		__ __ / __ __ / 2 0 __ __		
2.2		__ __ / __ __ / 2 0 __ __		
2.3		__ __ / __ __ / 2 0 __ __		
2.4		__ __ / __ __ / 2 0 __ __		

3.	**CRP (C-reactive protein)** *(<72 hrs of admission)* ☐ **Not done** *(skip to 4)*			
	Date __ __ / __ __ / 2 0 __ __	Time__ __ : __ __ ☐ Am ☐ Pm ☐ Not recorded		Result___ ___ ___ mg/l
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 __ __ / __ __ / 2 0 __ __ ☐ NP Swab for culture ☐ NP Swab for PCR (Child) ☐ Induced sputum ☐ NP Swab for PCR (Care-Giver)		

6.	**TB Results** ☐ **Not done** *(skip to 6)*			
	Date	Specimen	Result –AFB smear	Culture requested
6.1	__ __ / __ __ / 2 0 __ __	☐ Induced sputum ☐ Gastric Washings ☐ Not recorded	☐ Positive ☐ Negative	☐ Yes ☐ No

7.	**Other** *(skip to 8 if not done)*		
	Test	Date of test	Result
7.1	☐ Albumin	__ __ / __ __ / 2 0 __ __	___ ___ . ___ g/dL
7.2	☐ Total protein	__ __ / __ __ / 2 0 __ __	___ ___ . ___ g/dL
7.3	☐ Chemistry	__ __ / __ __ / 2 0 __ __	Na ____ K ____ Urea ____ Creatine ____

8.	**Radiology**		
8.1	Date of chest x-ray	__ __ / __ __ / 2 0 __ __	
8.2	X-ray findings	☐ Normal ☐ Pleural effusion ☐ Cavitations ☐ Sub-optimal quality	☐ Interstitial infiltrate ☐ Consolidation or air bronchograms ☐ Lobar consolidation ☐ Other _____

Staff initials: __ __ __ Date completed: __ __ / __ __ / 2 0 __ __