EVALUATION OF CASE DEFINITIONS TO DETECT *BORDETELLA PERTUSSIS* INFECTIONS IN HOSPITALISED INDIVIDUALS IN SOUTH AFRICA, 2015-2017

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Candidate's declaration

I, Linda Kathleen Erasmus, declare that this research report is my own, unaided work. It is being submitted in partial fulfilment for the degree of Master of Science in Epidemiology and Biostatistics at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other university.

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On this 4 December 2019, in Sandringham, Johannesburg.

Dedication

This research report is dedicated to my husband for his unfailing support and encouragement.

Presentations arising from this study

Oral presentation: National Institute of Communicable Diseases, GERMS-SA Surveillance Review meeting, Johannesburg, July 2019

Oral presentation: Flash abstract presented at 8th FIDSSA (Federation of Infectious Diseases Societies of Southern Africa) Conference, Johannesburg, 7-9 November 2019

Abstract

Background

Despite routine vaccination, pertussis remains a significant public health concern. Robust data on global pertussis burden is lacking. Pertussis surveillance relies on clinical suspicion in cases with classic symptoms as per World Health Organization (WHO) or Centers for Disease Control (CDC) case definitions. Atypical presentation may result in underestimation of disease burden. We evaluated performance of different case definitions to detect *Bordetella pertussis* (*B. pertussis*) illness among children enrolled as part of syndromic, sentinel pneumonia surveillance programme (PSP) in South Africa.

Objectives

To describe the demographic characteristics of all enrolled participants at selected PSP from January 2015 to December 2017. To identify demographic/ clinical predictors of a positive *B. pertussis* polymerase chain reaction (PCR) and to evaluate the sensitivity, specificity, positive (PPV) - and negative predictive value (NPV) of selected individual symptoms and signs and existing pertussis and WHO SARI case definitions for detecting PCR-confirmed *B. pertussis* in patients <5 and ≥5 years of age enrolled in the PSP during this time period.

Methods

This cross-sectional study used secondary data collected from hospitalised patients of all ages enrolled in the PSP at 7 sentinel sites in 5 provinces, January 2015 through December 2017. Patients were systematically enrolled based on broad case definitions (including suspected sepsis [infants aged 2 days - <3 months], physician diagnosed LRTI [<5 years] and acute respiratory infection with fever and cough [≥5 years]). Patients with suspected pulmonary TB and children ≤10 years with suspected pertussis were included at 2 and 1 site, respectively. Enrolled patients were tested for *B. pertussis* and other respiratory pathogens. STATA 15 was used to calculate sensitivity, specificity, PPV and NPV for classic pertussis symptoms and signs and standard pertussis and WHO Severe Acute Respiratory Illness (SARI) (acute respiratory illness with fever, cough and ≤10 days onset) case definitions in

the <5 year and ≥5 year age groups, using *B. pertussis* PCR as the gold standard. Binomial logistic regression investigated demographic/ clinical features predictive of a positive pertussis laboratory result.

Results

We identified *B. pertussis* in 2.5% (188/7504) and 1.1% (54/4866) patients < and ≥ 5 years of age enrolled in PSP respectively. In the < 5 year group, classic symptoms were uncommon (chronic cough 13% (25/187); paroxysmal cough 34% (57/170); whoop 10% (17/170); post-tussive emesis 26% (44/170)) but highly specific (88%-99%) for *B. pertussis*. Forty-five percent of children and 57% of the ≥5 year group had fever. Multivariate analysis of the <5 year age group, revealed age, whoop, paroxysmal cough, post-tussive emesis and cyanosis as significant predictors of a positive pertussis laboratory result. Due to small case numbers, results for the ≥ 5-year-old group should be treated with reserve. However, when controlling for age and race, whoop and HIV infection were significantly associated with a positive pertussis test on multivariate logistic regression. The 2003 WHO pertussis case definitions had low sensitivity (8-14%), high specificity (>97%); and low PPV (3-17%); while WHO SARI case definition had low sensitivity (35-39%) and specificity (51-65%) with low PPV (<2%) for *B. pertussis* across age groups. The updated 2018 WHO pertussis case definitions performed well in infants <6 months; sensitivity increased to 47.5% (95%CI38.4-56.8), while maintaining high specificity (81.2%, [95% CI 79.9-82.5]), PPV remains low (7.7% [95%CI 5.9-9.8]). Sensitivity decreases while specificity and PPV increase with increasing number of case-defined symptoms. Thirty-eight percent (65/170) of pertussis-positive and 14.2% (977/6874) pertussis-negative children <5 years met the 2018 WHO pertussis case definition (cough and at least one symptom). In the ≥5 year group, 3/40 (7.5%) and 2.4% (104/4276) pertussis-positive and negative individuals respectively fulfilled the criteria.

Conclusion

WHO/CDC pertussis case definitions could miss 50-90% of pertussis cases underestimating disease burden, while WHO SARI case definition would systematically exclude afebrile patients. More sensitive case definitions and heightened awareness of atypical presentation are needed for diagnosis of pertussis

in children. The amended WHO (2018) pertussis case definitions improved sensitivity while maintaining specificity in the <6 months age group. Existing syndromic surveillance platforms could provide for pertussis surveillance where routine laboratory testing is unavailable, providing critical information for health policy decisions.

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Nomenclature

aOR	Adjusted Odds Ratio
aP vaccine	Acellular pertussis vaccine
B. pertussis	*Bordetella pertussis*
CBHC	Chris Hani Baragwanath Hospital
CDC	Centers for Disease Control and Prevention
CSTE	Council of State and Territorial Epidemiologists
Ct	Cycle threshold
GPI	Global Pertussis Initiative
HIC	High-income country
HIV	Human immunodeficiency virus
HJH	Helen Joseph Hospital
KTHC	Klerksdorp Tshepong Hospital Complex
KZN	Kwa-Zulu Natal
LMIC	Low-middle income country
LRTI	Lower respiratory tract infection
NICD	National Institute for Communicable Diseases
NMC	Notifiable medical condition
NPV	Negative predictive value
PCR	Polymerase chain reaction
PERCH	Pneumonia Etiology Research for Child Health
PPV	Positive predictive value
RCCH	Red Cross Children's Hospital
RMMCH	Rahima Moosa Mother and Child Hospital
RSV	Respiratory syncytial virus
SARI	Severe Acute Respiratory Illness
WHO	World Health Organization
wP vaccine	Whole cell pertussis vaccine

Chapter 1: Introduction

This chapter describes the microbiology and clinical course of *Bordetella pertussis* (*B. pertussis)* infections as well as providing background on vaccination and burden of disease in South Africa and globally. The importance of, and challenges to surveillance as well as common case definitions are described. Details of the South African Pneumonia Surveillance Programme, as a potential platform for pertussis surveillance, are included. The paucity of robust data from low-and middle-income countries (LMIC) is highlighted and the justification for and aims and objectives of this research are stated.

1.1. Background

Pertussis is a transmissible respiratory tract infection caused by *B. pertussis.* Despite the widespread introduction of vaccination, pertussis remains a significant public health concern, especially in LMICs (1, 2). Infants < 6 months have higher disease and mortality rates compared to older individuals (3, 4). Several high-income counties (HIC) have reported an apparent resurgence in pertussis with age distribution shifts towards adolescents and young adults (1, 5). Vaccination against pertussis was introduced in South Africa in 1950 and in 2009 acellular pertussis (aP) vaccine replaced whole cell pertussis (wP) vaccine (6).

The true global burden of pertussis is uncertain and almost certainly underestimated primarily due to a paucity of data, particularly from LMIC. This is exacerbated by competing health priorities, limited resources, poor surveillance infrastructure and limited/no access to laboratory diagnostic testing. Even in HICs, under-recognition and misdiagnosis contributes to underreporting (7 - 10). Improved surveillance is necessary to define burden of disease and vaccine effectiveness and inform health policy and disease control (1). For LMICs ascertaining the burden of severe early infant pertussis should be prioritised as these cases are associated with higher mortality and are more likely to be hospitalised placing a burden on health care systems (3, 4).

Almost all pertussis surveillance globally is based on clinical criteria of pertussis in patients presenting with typical disease. The World Health Organization (WHO),

Council of State and Territorial Epidemiologists/Centers for Disease Control and Prevention (CSTE/CDC) and other organisations have developed clinical case definitions for pertussis (11 - 14). (Appendix 1 Table A1). These serve to identify potential pertussis cases for appropriate laboratory testing, clinical management and outbreak investigation as well as for surveillance/notification purposes or vaccine efficacy trials. Optimal case definitions depend on the objectives of the study. For estimating disease burden, high sensitivity is desirable to maximise detection of cases (15).

International case definitions are based primarily on typical pertussis symptoms requiring chronic cough, paroxysmal cough, post-tussive emesis or whoop. However, emerging data suggests pertussis presentation is heterogeneous including apnoea or lower respiratory tract infection (LRTI) without classic symptoms or signs. Atypical presentation in very young infants and previously immunised adolescents/adults could result in misdiagnosis and underreporting of disease (16, 17). In addition, clinical presentation of pertussis in Sub-Saharan Africa could be different from other settings. In a study of hospitalised South African children aged <1 year with symptoms or signs of lower respiratory tract infection (LRTI) or neonatal sepsis, only 20.5% (8/42) confirmed pertussis cases reported cough ≥14 days at enrollment and only 33.3% (14/42) had paroxysmal cough. Thirty percent (13/42) failed to meet either the CDC “confirmed” or “probable” case definitions (18).

Recognising that infants may present uniquely, the CSTE/CDC and WHO case definitions were revised in 2013 and 2017 respectively to include apnoea for infants aged <1 year; WHO further expanded the case definition to include cough of any duration in an infant or any person in an outbreak setting and clinician suspicion of pertussis (12, 13, 14). (Appendix1 Table A1). The new WHO case definitions should increase sensitivity, reducing the number of pertussis cases missed, but need to be evaluated in a clinical setting.

Broad case definitions including LRTI may also improve sensitivity for pertussis surveillance. Globally, pertussis surveillance could potentially be linked to surveillance for other respiratory diseases, such as influenza, utilising existing systems and human resources. However, optimal case definitions would need to be

evaluated. The WHO recommends that surveillance for hospitalised influenza should utilise the Severe Acute Respiratory Illness (SARI) case definition (Appendix 1, Table A1). In 2009, the National Institute for Communicable Diseases (NICD) implemented a prospective syndromic, hospital-based, sentinel surveillance system for pneumonia in South Africa (19). Initially developed primarily for influenza surveillance, case definitions for enrollment of patients were expanded over time to allow monitoring of a broader range of respiratory pathogens. In addition, since mid-2012, testing of specimens collected at selected sites has included *B. pertussis* and a variety of bacterial respiratory pathogens. In contrast to most pertussis surveillance which relies on clinician index of suspicion/testing, for this pneumonia surveillance patients are systematically enrolled and tested based on a broad case definition (Appendix 1 Table A3). This platform offers a unique opportunity to evaluate pertussis case definitions in the South African setting and could potentially be used to update South African surveillance case definitions as well as WHO pertussis case definitions.

In addition to syndromic surveillance, notifiable medical condition surveillance systems can also potentially contribute to knowledge of pertussis disease burden in South Africa. Pertussis is a category 1 notifiable medical condition in South Africa as per updated legislation promulgated on 5 December 2017 (National Health Act, 2003, [Act no 61 of 2003]) (20). Health care providers, private and public health laboratories are required to notify all suspected/ confirmed cases in accordance with routine notifiable medical condition notification procedures. Case definitions for notification were developed locally at the NICD, based on international examples (21). (Appendix 1 Table A2).

1.2. Problem statement

Robust data on pertussis burden in LMIC are lacking. In addition, recent studies from LMIC suggest that many cases of pertussis may present without showing classical signs of disease, which could lead to high numbers of missed cases using existing standard case definitions. Syndromic surveillance platforms provide a potential platform for pertussis surveillance in settings where routine laboratory testing for pertussis is not done. However, optimal pertussis case definitions would need to be

evaluated. South Africa is well placed to do this as pneumonia surveillance is already established in the country and unlike many other African countries, confirmatory pertussis laboratory testing is available.

1.3. Justification

This study will improve understanding of how well current case definitions for pertussis and SARI perform for identifying pertussis in hospitalised children and adults in various settings in South Africa. This information may assist in planning/developing appropriate cost-effective pertussis surveillance strategies for South Africa going forward and could potentially inform surveillance in other LMIC countries.

1.4. Literature review

1.4.1. Microbiology and diagnosis

Although *B. pertussis* has been classically associated with whooping cough, other *Bordetella* species, *B. parapertussis* and *B. holmesii* can also cause milder whooping cough–like symptoms in humans. Respiratory tract infections due to *B. bronchiseptica* occur primarily in immunocompromised individuals (9). *B. pertussis* is a fastidious Gram-negative, aerobic coccobacillus that grows optimally on Bordet-Gengou or Regan-Lowe agar between 35 and 37ºC and can be differentiated from other *Bordetella* species by its growth and biochemical characteristics or using polymerase chain reaction (PCR) (9, 22). *B. bronchiseptica* usually shows visible colonies within 1-3 days, *B.parapertussis* has an intermediate growth rate while *B.pertussis* and *B.holmesii* may take up to 12 days to grow in culture. *B.pertussis* is a non-motile, oxidase- and catalase-positive species, while *B.parapertussis* is less fastidious, non-motile, oxidase- negative, urease positive. *B.parapertussis* produces a brown pigment on heart infusion, Regan-Lowe, or Mueller-Hinton agar (9, 22). Once pertussis is clinically suspected, various laboratory tests are available for confirmation (17, 23). For all methods, sensitivity is affected by specimen collection technique, stage of disease and age of the patient (23). Culture and molecular diagnostics are recommended for diagnosis during the early stages of disease (12). Culture is highly specific and is useful for strain identification and antimicrobial susceptibility testing. PCR has higher sensitivity than culture and is more likely to

test positive in infants/ children as well as atypical cases in older or previously vaccinated patients (23). Laboratory testing is usually performed on nasopharyngeal aspirates or swabs or expectorated/induced sputum. Testing induced sputum may increase the diagnostic yield of pertussis but may not always be feasible (22, 24 - 26). Serology, measuring IgG anti-pertussis toxin (Ptx) antibodies in serum, is recommended for patients with longer duration of symptoms, but is affected by previous vaccination or infection (12 ,23).

1.4.2. Clinical Presentation /Course

B. pertussis is mainly transmitted from person to person by large droplet infection or direct contact with respiratory mucous membranes discharges from infectious people (27). There is some experimental evidence to support airborne transmission (28). Clinical expression of disease is heterogeneous and influenced by patient age, previous vaccination or infection and antibiotic administration. After a 7-10 day incubation period, patients develop non-specific catarrhal symptoms including no/low grade fever, rhinitis and mild cough. This is followed by the paroxysmal stage with typical paroxysmal cough (particularly severe at night), inspiratory whoop and post-tussive vomiting lasting one to three months. The convalescent stage with decreased frequency and severity of symptoms may last several months. Serious complications include pulmonary (secondary bacterial pneumonia or extreme lymphocytosis with pulmonary hypertension), neurological (encephalopathy, seizures) or nutritional deficiencies due to vomiting. Young infants are at highest risk of severe outcomes (9, 17).

Young infants may present atypically with apnoea and cyanosis (1, 17). Similarly, disease in previously immunised adolescents and adults is frequently milder, marked by a prolonged cough without characteristic signs. These atypical presentations contribute to misdiagnosis and underreporting (17).

1.4.3. Pertussis vaccines

The primary objective of pertussis vaccination is to reduce the risk of severe disease in infants (1). Two broad categories of pertussis vaccines are currently available: wP vaccine, based on killed *B. pertussis* organisms and aP vaccine based on one or more highly purified individual pertussis antigens. The incidence of pertussis

decreased markedly following introduction of wP vaccine from the 1940s. However, the manufacturing process of wP vaccine does not allow elimination of bacterial components and use was associated with relatively frequent adverse events including fever, irritability, local reaction at injection site or rare reactions including hypotonia-hyporesponsiveness. A report of a possible association between wP vaccine and permanent neurological damage was subsequently disproven. Concerns around reactogenicity and better understanding of the molecular biology of *B. pertussis* led to development and production of less reactogenic aP vaccines in the early 1980s. Many HICs switched to aP vaccines in their routine vaccination schedules, but increased cost of aP vaccine limits their use in many countries (1, 5). Despite wP vaccine being more commonly associated with local and systemic reactivity, evidence supports that both wP and aP vaccines have excellent safety profiles for serious adverse events. Furthermore, during the first year of life, aP and wP vaccines appear to be initially equivalently effective in preventing disease. However, more rapid waning of immunity is noted with aP vaccines (1). WHO recommends that national programmes currently administering wP vaccination should continue to use them for the primary vaccination series (1). In recent years, additional preventive measures to protect very young infants and adolescents from pertussis have been introduced in some countries including maternal immunisation or booster vaccination for adolescents (1, 7, 29).

1.4.4. Global epidemiology of pertussis

Pertussis remains endemic globally with epidemic cycles occurring in 2-5 year cycles in the global vaccine era (1, 30, 31). In 2018, an estimated 151 074 pertussis cases were reported globally (32). Using vital registration data from WHO Liu *et al* estimate 60 000 (95% uncertainty interval 43000-94 000) deaths from pertussis in children less than five years in 2013 (33). However, the limited reliable surveillance data creates uncertainty about these numbers. As many countries, especially low income countries, lack surveillance systems to monitor pertussis cases, models have been developed to estimate disease burden in children younger than 15 years (34). Using a revised model with 2014 data, WHO and researchers from Hong Kong estimated 24.1 million (range 7 million -40 million) pertussis cases globally in children younger than 5 years with 160 700 (range 38 000 – 670 000 with sensitivity analysis) pertussis related deaths (34). The African region contributed the greatest proportions

and more than fifty percent of pertussis-associated deaths were estimated to occur in infants younger than twelve months of age.
In 2018, an estimated 116.3 million infants globally (86%) received three doses of diphtheria-tetanus-pertussis (DTP3) vaccine; DTP3 vaccine coverage in the WHO African region was estimated at 76% (32, 35). WHO/United Nations Children's Fund estimated 74% DTP3 vaccination coverage in South Africa in 2018 (36).

The WHO/Hong Kong researchers' disease burden model used updated population and vaccine coverage data, took into account the protective effects of incomplete pertussis vaccination, probability of infection in countries with low and high vaccine coverage, as well as case fatality ratios in low and high mortality countries in both infants less than one year of age and children aged 1-4 years (34). Estimates of cases and deaths were not included for the 5-14 year age group, due to a paucity of evidence for duration of vaccine protection beyond 5 years of age. Sensitivity analyses were performed to assess the effects of uncertainty of a range of input parameters on the model outputs.

The paucity and heterogeneity of data preclude clear understanding of global pertussis epidemiology. In addition, the cyclical nature of pertussis disease, different case definitions, vaccine schedules and preparations hinder inter-country data comparisons over time (37, 38). In the pre-antibiotic, pre-immunisation era, pertussis affected mainly ≤5 year olds with very high incidence and case-fatality ratios (5). Following introduction of effective vaccination programmes, there was a significant reduction in the number of pertussis cases and deaths in children. However, recently some HIC have reported an increase in pertussis cases (1, 39). During the early-mid 2000s, there was a shift in age distribution towards adolescents/adults (10). More recently, the incidence appears to be increasing in children of school-age in North America and Western Europe (10, 37). Possible factors contributing to increase reporting of pertussis in HICs remain controversial. These include improved awareness, increased recognition of atypical disease in older patients and improved diagnostics and surveillance systems (9). Reports that immunity conferred by aP vaccines is not as long lasting as for wP vaccines led to hypotheses that the switch to aP vaccines could be the cause of the pertussis resurgence (29, 40). However, several papers report that there is no consistent pattern between changing from wP

to aP vaccines and reported per country pertussis incidence rates (38, 41). Following a reported increase in incidence in a few countries using aP vaccines, WHO reviewed data from 19 HI- and MICs and concluded that natural cyclical changes accounted for the increased case numbers in most countries. However, 5 of 19 countries show evidence of true resurgence and 4 of those use aP vaccines exclusively (1, 39). According to WHO there is currently no evidence that shifts in *B. pertussis* allele frequencies and genomic changes following univeral introduction of vaccination (41-43) would reduce the effectiveness of wP vaccines (1). Similarly, there is currently no evidence to support that detection of antigen-deficient isolates in regions where aP vaccines are used has affected vaccine immunity (1, 41-44). Several recent papers highlight the epidemiological heterogeneity of pertussis incidence across different countries supporting that the resurgence of pertussis is not universal and suggesting that the underlying explanations are probably complex and multifaceted involving host-organism interactions, evolutionary and transmission dynamics and shifting population immunity profiles (38, 41, 42). Lastly, the prevalence of *B. parapertussis* and *B. holmesii* in pertussis-like illness needs to be further investigated (45).

1.4.5. Pertussis in South Africa

Few systematic data are available on the burden and epidemiology of pertussis in South Africa. The published studies of pertussis from South Africa are summarized in Table1.1. Pertussis epidemics occurred in the City of Cape Town in 1947 and between June 1988 and April 1989, despite introduction of vaccine in the area in 1950 (46). Vaccine failures and children too young to be vaccinated were considered drivers of the latter outbreak. National laboratory data from 2008 to 2011 identified 311 confirmed pertussis cases; 67% with documented age were <3 months. Data from KZN were limited to 2011 only (47). A case series of eighteen cases of pertussis were described in Bloemfontein, between April 2008 and March 2009 (48). The last pertussis case described in Free State province prior to this outbreak, was diagnosed in 1998. In a retrospective hospital-based study in Bloemfontein, April 2008 to March 2015, 57% (104/183) of children with PCR-confirmed pertussis were <18 weeks (6). Hospitalisation was significantly associated with young age. Case-fatality ratio among the 154 cases of all ages where clinical data was available, was 5.2%. In a nested case-control study of a birth cohort in Western Cape, 2012 to

2014, *B. pertussis* was detected by PCR in 6/284 (2%) of pneumonia cases in children and 1/412 (0.002%) controls with a strong association between *B. pertussis* and pneumonia (odds ratio (OR) 11.08, 95% CI 1.33-92.54 [7/696 positive]) (24). Muloiwa *et al* (25) detected *B. pertussis* in 32/460 (7%) of children <13 years hospitalised with LRTI in Cape Town between September 2012 and 2013. The median age of pertussis-infected individuals was 8 months. The detection rate was 14.6% in infants aged <2 months and 15.8% (3/19), 10.9% (10/92) and 5.4% (19/349) in human immunodeficiency virus (HIV) infected, exposed-uninfected and unexposed-uninfected children respectively. No in-hospital deaths occurred. Du Plessis *et al*, (49) detected *B. pertussis* in 6.2% (61/992) of children ≤10 years, hospitalized with suspected pertussis at 2 sites in Gauteng (one of which was a pneumonia surveillance site), 2013-2015. The prevalence of pertussis was reportedly higher in incompletely vaccinated children and infants too young for vaccination. HIV prevalence was similar in pertussis-positive and pertussis-negative children (6%, 5/82 and 6%, 55/896 respectively; p=0.90).

In a study at a Soweto hospital in Gauteng (2015), 2.3% (42/1839) of children <1 year, hospitalised for any respiratory illness tested positive for *B. pertussis* (18). The majority (36/42, 86%) were <3 months of age and overall in-hospital case-fatality ratio among cases was 4.8% (2/42). Estimated overall incidence of *B. pertussis*-associated hospitalisations was 2.2 cases per 1000 infants; 2.9 per 1,000 and 1.9 per 1,000 in HIV-exposed and HIV-unexposed infants respectively (p=0.09). This hospital was also a site for the multi-country Pneumonia Etiology Research for Child Health (PERCH) Study that investigated children 1-59 months old, with WHO-defined severe and very severe pneumonia and contributed the highest positivity reported in the 1-5 month age group (4% vs 1.2% prevalence across all sites) (18, 50). Other PERCH study sites included Dhaka and Matlab, Bangladesh; Basse, The Gambia; Kilifi, Kenya; Bakmako, Mali; Thailand; Sa Kaeo and Nakhon and Zambia; Lusaka. All sites other than South Africa used wP vaccine. Respiratory samples collected from infants <6 months in Soweto with symptoms of respiratory illness during a maternal influenza trial in 2011 were tested retrospectively and showed an overall pertussis incidence rate of 5.8/1000 child-months: 7.4/1000 in HIV-exposed infants and 5.5/1000 in HIV-unexposed infants (P=0.47). Six of seventeen (35.3%) cases in infants <2 months required hospitalisation (51).

In 2017, 86/4206 (2%) of patients from the pneumonia surveillance programme tested positive for *B. pertussis*. The highest proportion of cases was in children <3 months (56% 48/86) (52). In January 2018, the NICD reported an increase in the number of pertussis cases detected in the Western Cape pneumonia surveillance amongst children <5 years, particularly <1 year of age; starting from August 2017. No similar increase in surveillance case numbers was noted in other provinces (53).

Table 1.1: Studies on epidemiology and clinical presentation of pertussis in South Africa, 1988-2019 (2)

City/Province	First author, year published	Study period	Study type	Study population	Diagnostic method used
City of Cape Town , Western Cape	Strebel P, 1991(46)	June 1988 - May 1989	Retrospective Descriptive	292 hospitalized children (<5years) with a clinical diagnosis of whooping cough	Most not laboratory confirmed. Limited culture and serology
National (laboratory data)	Archer BLW, 2011 (47)	2008-2011 (KZN 2011 only)	Retrospective data analysis	All patients with clinically suspected pertussis seen at public hospitals in South Africa	PCR and/or laboratory culture
Bloemfontein, Free State	Hallbauer UM, 2011 (48)	April 2008-March 2009	Case series	18 cases diagnosed with pertussis	PCR
Bloemfontein, Free State	Hallbauer UM, 2016 (6)	April 2008-March 2015	Hospital-based, retrospective, descriptive	1259 hospitalised children with suspected pertussis	PCR
Drakenstein, Western Cape	Zar HJ, 2016 (24)	May 2012 to December 2014	Nested case-control	960 children, part of a birth cohort, 314 pneumonia cases, 284 cases, 482 controls analysed	PCR
City of Cape Town, Western Cape	Muloiwa R, 2016 (25)	September 2012-September2013	Prospective hospital-based	460 children (<13 years) with LRTI	PCR
Tshwane, Johannesburg, Gauteng	Du Plessis N, 2018 (49)	August 2013-October 2015	Prospective hospital-based	992 hospitalised children (<10 years), with clinically suspected pertussis	PCR
Soweto, Gauteng	Soofie N, 2016 (18)	January 2015-December 2015	Population-based hospital surveillance	1865 infants with LRTI	PCR
Gauteng (Soweto), part of multi-centre PERCH study	Barger-Kamate B, 2016 (50)	August 2011-January 2014	Prospective, hospital-based, part of multi-centre study at 9 sites in 7 African and Asian countries	Overall, 4200 cases with severe/very severe pneumonia, 5196 controls (1-59 months)	PCR
(Soweto),Gauteng	Nunes MC, 2016 (51)	March 2011-August 2011	Secondary analysis of data from a randomised double-blind, placebo controlled trial of influenza vaccine in pregnant women	194 HIV-infected, 1060 HIV- uninfected women; 188 HIV-exposed and 1028 HIV-unexposed infants	PCR
Gauteng, KZN, North West, Mpumalanga, Western Cape	NICD, 2015-2019 (52, 53)	Annually from 2015	Hospital- and clinic-based, sentinel site, prospective syndromic surveillance	All patients presenting to sentinel sites meeting defined case definitions	PCR

1.4.6. Pertussis case definitions

Many different pertussis case definitions are used internationally. Earlier case definitions were primarily based on typical clinical presentation of childhood pertussis (11,16) and most include a requirement for at least two weeks cough with additional specified symptoms to increase specificity (Appendix 1, Table A1). Laboratory diagnosis of pertussis or epidemiologic linkage further classified probable or confirmed cases (11-14). In 2009, Shakib *et al* (54) suggested that reducing cough duration from ≥2 weeks to >1 week would elevate the number of individuals with positive pertussis PCR meeting the requirements for confirmed pertussis from 88% to 95% and recommended adding apnoea as an additional confirmatory sign in infants. Recognising that infants may present uniquely, the CSTE/CDC case definitions were revised in June 2013 to include apnoea (with or without cyanosis) for infants <1 year (13, 14). The 2018 WHO pertussis case definition includes cough ≥2weeks in a person of any age or of any duration in an infant or any person in an outbreak setting and clinician suspicion of pertussis; and adds apnoea as a case-defining symptom in children aged <1 year (12) (Appendix1 Table A1). In 2011, the Global Pertussis Initiative (GPI) proposed age-specific pertussis clinical case definitions for surveillance purposes (16); including cough without fever with additional age-group specific symptoms (Appendix 1, Figure A1). In the GPI case definitions, cough duration depends on the age of the patient.

1.4.7. Sensitivity and specificity of symptoms and signs and case definitions for pertussis

Optimal case definitions depend on the objectives of the study or surveillance. For estimating disease burden, high sensitivity is desirable while evaluation of vaccine efficiency requires a highly specific case definition. Improved specificity often results in reduced sensitivity (15, 16). Ghanaie *et al* (55) evaluated the performance of the 2003 WHO pertussis case definition in 328 children, 6-14 years of age, in Tehran with cough for ≥2 weeks using culture and PCR for confirmation. Using cough ≥2 weeks and ≥1 additional symptom showed sensitivity and specificity of 95% and 15% respectively. Cough ≥2 weeks with at least 2 and at least 3 WHO criteria increased specificity (60.3% and 91.2% respectively) at the expense of sensitivity (81.0% and 9.5% respectively). In another study of French adults consulting general practitioners for persistent cough without apparent cause, 89% of confirmed cases were reported

to meet the CDC case definition (56). A retrospective review of hospitalised children tested for pertussis in Cape Town 2012- 2013, showed that only 23/75 (33%) laboratory-confirmed cases fulfilled the 2003 WHO clinical case definition for pertussis (57). Among children ≤10 years hospitalised with suspected pertussis at 2 sites in Gauteng, 2013-2015, 9.8% (4/41) of patients testing positive and 5.9% (56/949) testing negative for *B. pertussis* met the 2003 WHO case definitions (49); while a study of hospitalised South African children aged <1 year with any sign or symptom of respiratory illness, 30% (13/42) failed to meet either the CDC "confirmed" or "probable" case definitions (18).

Many heterogeneous clinical studies have tested the diagnostic accuracy of different symptoms and signs for pertussis. Recently Ebell *et al* systematically reviewed the clinical diagnosis of pertussis in all age groups, while Moore *et al* performed a systematic review and meta-analysis to establish the diagnosis accuracy of clinical characteristics of pertussis-associated cough (58, 59). Limitations included heterogeneity in study designs, so only limited clinical characteristics could be included in meta-analysis, risk of misclassification or patient selection bias at study level and variation in reference standards across studies. Clinical evaluation of the new WHO 2018 guidelines will now need to be done.

1.5. Pneumonia surveillance in South Africa

Active, prospective, hospital-based sentinel pneumonia surveillance was implemented in 3 of 9 provinces in South Africa in 2009, primarily to describe the burden and viral aetiology (particularly influenza) of severe respiratory illness in hospitalised patients (19). Initial sites included Chris Hani Baragwanath Hospital (CHBH) in Gauteng, Edendale Hospital in Kwazulu-Natal (KZN) and Matikwana and Mapulaneng hospitals (Agincourt) in Mpumalanga. Klerksdorp/Tshepong Hospital Complex (KTHC) was added in North West Province in 2010. The CHBH site was terminated at the end of 2013 and in July 2014, Helen Joseph (HJH) and Rahima Moosa child and maternal hospitals (RMMH) became the Gauteng sites. In March 2015, surveillance was expanded to Western Cape at Red Cross Children's Hospital (RCCH). Mitchell's Plain Hospital was included a year later. In 2012, surveillance was expanded at Edendale hospital and KTHC to include expanded testing of

specimens (naso- and oropharyngeal swabs and aspirates) for bacterial pathogens, including *B. pertussis*. Additional specimens (induced sputum and oral washes) were also collected. This expanded testing was implemented for all sites in 2015.

1.5.1. WHO SARI and South African pneumonia surveillance case definitions

The WHO SARI case definitions were developed primarily for influenza surveillance and were updated in 2014 (60). (Appendix 1 Table A1). Case definitions for NICD pneumonia surveillance were based on WHO SARI case definitions adapted to the South African context. In June 2012, expanded pneumonia surveillance case definitions were introduced at two enhanced surveillance sites (Edendale Hospital and KTCH) and additional specimens, including induced sputum were collected from enrolled patients. Laboratory testing of specimens from all sites expanded from viral respiratory pathogens to include a number of bacterial respiratory pathogens, notably *B. pertussis.* In 2015, the expanded case definition was implemented at all enhanced surveillance sites (Appendix 1 Table A3). Children aged <5 years with physician diagnosed LRTI and infants aged 2 days to <3 months with suspected sepsis were included as were ≥5 year olds hospitalised with an acute respiratory infection with fever (≥38ºC) or history of fever AND cough. At Edendale Hospital and KTHC, patients with suspected pulmonary TB were also included; while at RMMCH children aged ≤10 years with suspected pertussis as per specified case definitions were enrolled. (Appendix 1, Table 2). Furthermore, a new case investigation form was introduced with specific questions regarding paroxysmal cough, inspiratory whoop, post-tussive emesis and other symptoms classically associated with pertussis infection.

1.6. Research question

How well do existing clinical pertussis and WHO SARI surveillance case definitions perform in identifying laboratory-confirmed *B. pertussis* infection in hospitalised children and adults with LRTI in South Africa?

1.7. Research Aim and Objectives

Aim:

To assess the sensitivity, specificity, positive- (PPV) and negative-predictive value (NPV) of existing pertussis and syndromic severe respiratory infection case definitions for detection of *B. pertussis* in persons aged <5 years and ≥5 years of age enrolled in pneumonia surveillance at seven sentinel surveillance sites in five provinces from January 2015 to December 2017.

Objectives:

1. To describe the demographic characteristics of all enrolled participants at selected pneumonia surveillance sites from January 2015 to December 2017.
2. To identify demographic and clinical predictors of a positive *B. pertussis* PCR in patients <5 and ≥5 years of age enrolled in the pneumonia surveillance programme from January 2015 to December 2017.
3. To evaluate the sensitivity, specificity, PPV and NPV of selected individual symptoms and signs and existing pertussis and WHO SARI case definitions for detecting PCR-confirmed *B. pertussis* in patients <5 years and ≥5 years of age enrolled in the pneumonia surveillance programme from January 2015 to December 2017.

Chapter 2. Methods and materials

This chapter provides an overview of the study design, study population and setting, data collection and laboratory testing. Potential measurement limitations are discussed and statistical analysis methods described in detail.

2.1. Study design

This was a cross-sectional study using secondary data collected by the NICD through the hospital-based sentinel pneumonia surveillance from January 2015 to December 2017.

2.2. Study population

The study population included all hospitalised patients of any age meeting the case definitions and enrolled at all pneumonia surveillance sites from January 2015 to December 2017. This included HJH and RMMCH in Gauteng, Edendale hospital in KZN, Mapulaneng and Matikwana hospitals comprising the Agincourt site in Mpumalanga, the KTHC in North West and RCH and Mitchell's Plain hospitals in Western Cape.

2.3. Study setting

Edendale Hospital and Mitchell's Plain Hospital are peri-urban, while the Gauteng sites are urban. RMMH specialises in maternity, neonatal and paediatric services (61) and RCCH hospital is an urban 273-bed tertiary level paediatric hospital serving poor, peri-urban communities in the Western Cape (62). The KTHC is peri-urban and includes Klerksdorp Hospital that provides paediatric services and Tshepong Hospital for adult patients. The Agincourt site comprises two rural district hospitals (63).

2.4. Data collection

NICD pneumonia surveillance is an active, prospective, hospital-based sentinel surveillance system for severe respiratory illness. On weekdays, surveillance officers (enrolled or professional nurses) based at the pneumonia surveillance sites identified hospitalised patients meeting the specified case definitions. After obtaining informed consent, a standardised case investigation form including demographic and clinical

data was completed through patient interview and medical record review. Specified specimens, including upper respiratory tract samples (naso-/oropharyngeal swabs and nasopharyngeal aspirates and expectorated or induced sputum at selected sites) were collected and submitted to the NICD for laboratory testing for *B. pertussis* and other viral and bacterial pathogens. Details of site specific specimens collected for *B. pertussis* testing each year are summarized in Appendix1 Table A4.

2.5 Laboratory testing for *B. pertussis*

All upper respiratory tract samples were placed in universal transport medium, stored at 4ºC and transported to the NICD on ice within 72 hours of collection. Sputum samples were stored at -20ºC and transported to NICD on dry ice weekly. Specimens were tested for *B. pertussis* at the NICD Centre for Respiratory Disease and Meningitis using a PCR assay adapted from a previously published real-time PCR assay to include all IS481 positive samples, irrespective of the ptx result, as pertussis-positive cases (22, 64). The IS481 gene target is a multi-gene target (150-200 copies per bacterial genome) and therefore is more sensitive than the ptx result. In addition, *B. holmesii*, the only other pathogen that would test IS481 positive, was excluded by testing for hIS1001.

Multiple controls, at all stages of the testing process were used to check for contamination and false-positives. As per WHO recommendations, the multiplex PCR assay used a combination of PCR targets which detected and excluded other Bordetella species and the testing laboratory subscribed to EQA panels (QCMD) for pertussis external quality assessment of the test.

For this analysis, a specimen was considered pertussis-positive if the naso-/oropharyngeal sample and/or the expectorated or induced sputum samples tested positive for IS481 (and hIS1001 negative) with a cycle threshold (ct) of <45.

2.6 Statistical analysis methods

Data analysis was performed using STATA 15. (StataCorp. 2017).

Categorical variables were described using frequency or proportion, normally distributed continuous variables were described by mean with standard deviation. If distribution was not normal, median with inter-quartile range was used. The chi-squared test was used to compare the percentage of patients presenting with typical

clinical characteristics and meeting WHO case definitions between groups testing positive and negative for *B. pertussis*.

Demographic and clinical predictors of a positive *B. pertussis* PCR result was investigated using logistic regression modelling, as the outcome (pertussis infection positive or negative, based on the laboratory PCR result) was binary. Univariate logistic regression was used to establish the unadjusted odds ratio, 95% confidence interval and p value for each individual demographic and clinical variable. Variables with p value <0.1 were evaluated in a stepwise multivariable logistic regression where p value <0.05 was considered significant. Likelihood ratio test was used to select the best model.

Explanatory variables included chronic cough, inspiratory whoop, paroxysmal cough, fever, wheezing, nocturnal cough, difficulty breathing and fever for all ages; as well as post-tussive vomiting, cyanosis, chest in-drawing, stridor, apnoea and convulsions for the <5 year age group and chest pain for the ≥5 year group. Nocturnal cough was considered a proxy for worsening of symptoms at night. Demographic factors (province, year, age, race, gender) and HIV status were included as possible confounders. For logistic regression analysis, age groups <5 and ≥5 years were further sub-categorised to more accurately evaluate disease associations. For multivariate analysis, chronic cough was evaluated rather than cough as this is the basis for most standard case definitions. Full vaccination coverage according to age was not included in the final model as it is not considered a demographic or clinical feature. However, the reported full vaccination coverage for age for pertussis-negative and -positive patients is described in the results section (3.3). A sub-analysis of infants <6 months was also performed.

Sensitivity, specificity, PPV and NPV was calculated for each sign or symptom included in the standard pertussis case definitions or found to be significantly associated with a positive pertussis PCR; as well as for the WHO SARI and WHO/CDC pertussis case definitions, using pertussis PCR as the gold standard. Two by two tables were used to establish false positive and negative and true positive and negative values for each variable. These values were then used in the STATA15 “diagt” command to calculate sensitivity, specificity, PPV and NPV.

Evaluation of performance of pertussis case definitions was limited to the subset of cases for which all required variables were completed. For classification of sensitivity and specificity, values <50% were considered low sensitivity or specificity; 50-79%, moderate sensitivity or specificity and ≥80% was classified as high sensitivity or specificity.
The case definitions are detailed in Appendix1 Table A1.

In addition to the specific study objectives, to assess for representivity and bias, demographic characteristics (age, gender, race, site, year) and HIV status of patients tested and not tested for pertussis were described using chi-squared test and Wilcoxin rank sum t-test for categorical and continuous data respectively.

2.7. Ethical considerations

This study was approved by the University of the Witwatersrand Human Research Ethics Committee (Medical). Clearance certificate number M170664 (Appendix 2).

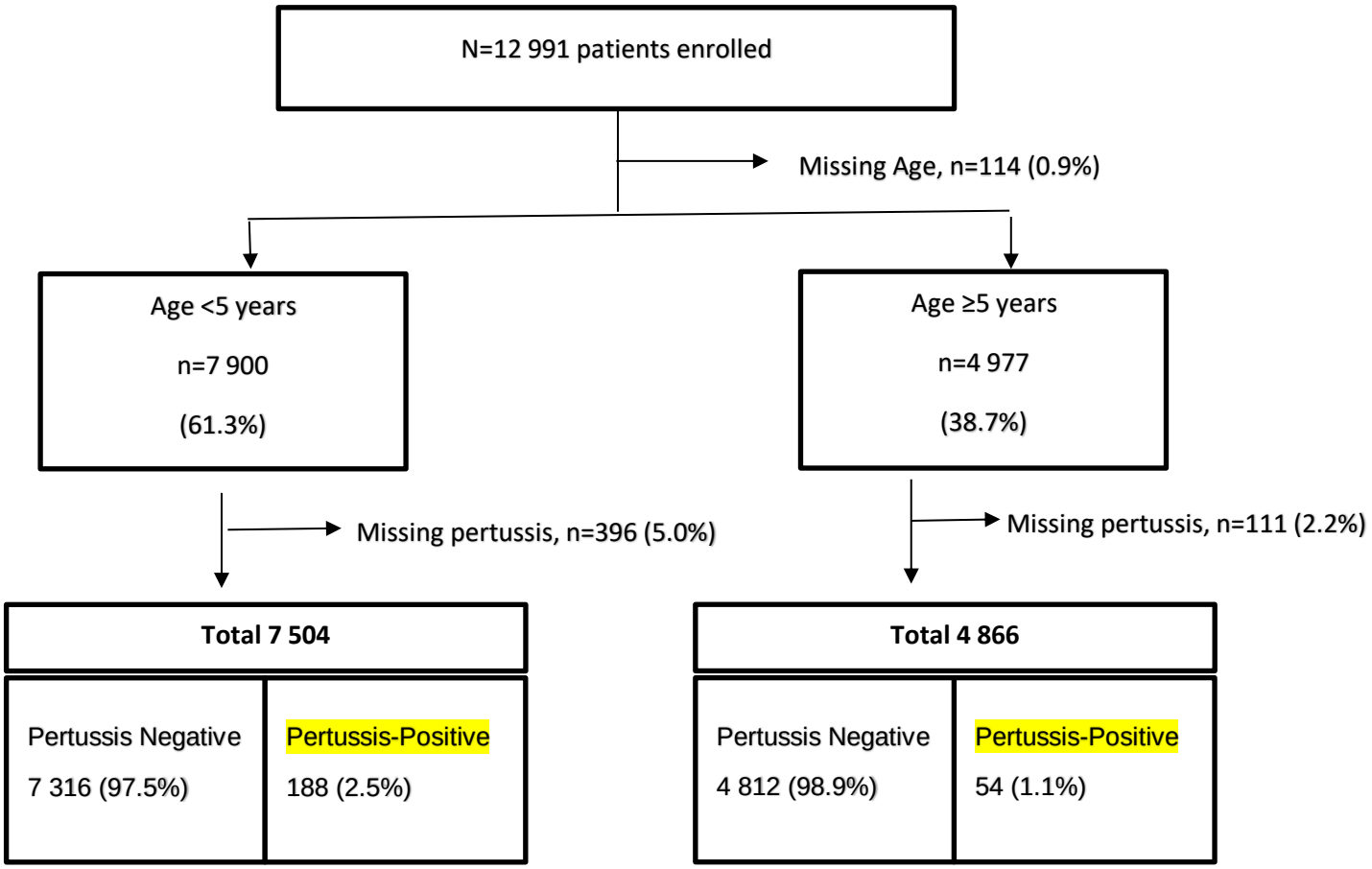
Chapter 3: Results

The section describes the demographic characteristics of the patients enrolled in the pneumonia surveillance in South Africa, 2015-2017. The results of multivariate analysis to evaluate demographic and clinical predictors of a positive *B. pertussis* and the sensitivity and specificity of individual classic symptoms and signs and existing case definitions for detecting pertussis cases are reported.

3.1 Overview

From January 2015 to end December 2017, 12 991 hospitalised patients were enrolled in the pneumonia surveillance programme in five provinces in South Africa. Of these, age was known for 99.2% (12 877); 61.3% (7 900) were children <5 years of age and 38.7% (4 977) were ≥5 years. (Figure 3.1). *B. pertussis* laboratory results were available for 12 370 (95.2%) patients, 60.7% (7 504) <5 years and 39.3% (4 866) ≥5 years, who were included in the final analysis.

Figure 3.1: Flowchart of patients enrolled in the pneumonia surveillance programme, South Africa, 2015-2017



3.2. Demographic characteristics of patients enrolled in pneumonia surveillance in South Africa, 2015-2017, tested and not tested for *B. pertussis*

For both age groups, more than 95% of enrolled patients were tested for *B. pertussis.* The demographics of patients tested and not tested for *B. pertussis* differed significantly for several characteristics (Table 3.1).
Among children aged <5 years, compared to tested individuals, untested individuals were more likely to be black (76.9%; 296/385 vs 71.7%; 5 314/7 408, p=0.028), more likely to be recruited in North West or Mpumalanga (14.4%; 57/396 vs 8.7%; 654/7504 and 16.2% and 64/394 vs 7.4%; 554/7504 respectively, p=<0.001) and more likely to be recruited in 2015 or 2017 (32.8%; 130/396 vs 28.0%; 2 097/7504 and 41.2%; 163/396 vs 39.9%; 2 995/7504 respectively; p=0.021. Children tested and not tested for *B. pertussis* were similar in all other characteristics.

In the ≥5 years age group, compared to tested individuals, untested individuals were more likely to be aged 5-14 years (18.9%; 21/111 vs 6.8%; 329/4866, p<0.001); younger (median age 36.45 years (inter-quartile range 22.7-50.2 years) vs 39.64 years (inter-quartile range 30.3-52.7 years, p=0.038), to be recruited in Mpumalanga or Western Cape (31.5%; 35/111 vs 7.6%; 369/4 866 and 16.2%; 18/111 vs 6.2%; 301/4 866 respectively, p<0.001) and less likely to be male (34%; 36/706 vs 47.7%; 2 302/4 826, p=0.005) or HIV positive (56.2%; 50/89 vs 66.2%; 3 132/4730,p=0.048); but were similar in all other aspects (Table 3.1).

Table 3.1: Demographic characteristics of patients enrolled in pneumonia surveillance, tested or not tested for *B. pertussis*, South Africa, 2015-2017.

	< 5 years n=7900			**≥ 5 years** n=4977		
	Not tested	**Tested**	**P value**	**Not tested**	**Tested**	**P value**
	n=396	n=7 504		n=111	n=4 866	
Province			<0.001			<0.001
North West	57 (14.4)	654 (8.7)		11 (9.9)	1 484 (30.5)	
KZN	46 (11.6)	1003 (13.4)		11 (9.9)	1 117 (23.0)	
Mpumalanga	64 (16.2)	554 (7.4)		35 (31.5)	369 (7.6)	
Western Cape	160 (40.4)	3 411 (45.5)		18 (16.2)	301 (6.2)	
Gauteng	69 (17.4)	1 882 (25.1)		36 (32.4)	1 595 (32.8)	
Year			0.021			0.079
2015	130 (32.8)	2 097 (28.0)		46 (41.4)	1 597 (32.8)	
2016	103 (26.0)	2 412 (32.1)		35 (31.5)	1 499 (30.8)	
2017	163 (41.2)	2 995 (39.9)		30 (27.0)	1770 (36.4)	
Age group			0.608			<0.001
<6months	210 (53.0)	4 072 (54.3)		N/A	N/A	
6-11 months	81 (20.5)	1 385 (18.5)		N/A	N/A	
1-4 years	105 (26.5)	2 047 (27.3)		N/A	N/A	
5-14 years	N/A	N/A		21 (18.9)	329 (6.8)	
15-23 years	N/A	N/A		7 (6.3)	289 (5.9)	
24-44 years	N/A	N/A		45 (40.5)	2 424 (49.8)	
≥45 years	N/A	N/A		38 (34.23)	1 824 (37.5)	
Age in years Median(IQR)	0.44 (0.2-1.0)	0.42 (0.2-1.1)	0.961	36.45 (22.7-50.2)	39.64 (30.3-52.7)	0.038
Gender			0.104			0.005
Male	232 (60.1)	4 158 (55.9)		36 (34.0)	2 302 (47.7)	
Race			0.028			0.657
Black	296 (76.9)	5 314 (71.7)		95 (89.6)	4 374 (90.9)	
Non-Black	89 (23.1)	2 094 (28.3)		11 (10.4)	439 (9.1)	
HIV status			0.426			0.048
Positive	16 (4.6)	269 (3.8)		50 (56.2)	3 132 (66.2)	

3.3. Clinical characteristics of patients enrolled in pneumonia surveillance in South Africa, 2015-2017, testing positive and negative for *B. pertussis*

Among children aged <5 years, compared to pertussis-negative individuals, pertussis-positive individuals presented more commonly with cough (95.1%, [174/183] vs 84.2% [6049/7186], p<0.001), chronic cough (13.4% [25/187] vs 6.0% [438/7255], p<0.001), paroxysmal cough (33.5%[57/170] vs 11.8% [811/6887], p<0.001), whoop (10.0% [17/170] vs 1.4% [93/6883], p<0.001), post-tussive vomiting (25.9% [44/170] vs 10.0% [690/6880], p<0.001), apnoea (11.8% [20/170] vs 4.2% [290/6884], p<0.001) and cyanosis (12.9% [22/170] vs 2.6%; [176/6886], p<0.001) (Table 3.2.1). Although cough was the commonest presenting clinical feature, only 14% of pertussis-positive children with cough, reported chronic cough lasting 14 days or more. Fever was more common in pertussis-uninfected than pertussis-infected children (59.2% [4281/7238] and 45.4% [84/185] respectively, p<0.001). In our study, fever was medically documented in 47% of patients with this symptom, the remainder were based on maternal reporting. Compared to pertussis uninfected children, pertussis-infected children were significantly less likely to have reported full vaccination coverage for age (38.3% [69/180] vs 56.5% [3972/7029], p<0.001) and were more likely to be too young for vaccination (aged< 6 weeks); (24.5% [46/188] and 18.6% [1357/7 316] respectively, p<0.001) (data not shown).

For the ≥ 5 year age group; whoop was significantly more common in pertussis-positive compared with pertussis-negative patients (7.5% [3/40] vs 1.6% [68/4279], p=0.031) (Table 3.3). Chronic cough and fever was seen more frequently in pertussis-negative than pertussis-positive patients but this failed to reach statistical significance (51.5% [2453/4768] vs 39.6% [21/53], p=0.086 and 62.8% [2984/4754] vs 56.6% [30/53], p=0.361 respectively). Other clinical features did not differ significantly between the pertussis-infected and uninfected groups.

Amongst children aged <5 years, significantly more pertussis-positive individuals met the WHO 2018 pertussis case definitions (cough and at least one other symptom) compared with pertussis- negative individuals (38.2% [65/170] vs 14.2% [977/6874], p=<0.001 respectively) (Table 3.2.1). Similarly, significantly more pertussis-infected

than uninfected infants <6 months met the WHO 2018 pertussis case definitions (47.5% [58/122] and 18.8% [898/3716] respectively, p<0.001) (Table 3.2.2). Among the ≥5 year age group, 7.5% (3/40) pertussis-positive and 2.4% (104/4276) pertussis-negative individuals fulfilled the WHO 2018 pertussis case definitions (p=0.053) of chronic cough and paroxysmal cough and/or inspiratory whoop (Table 3.3).

3.4: Multivariate analysis of characteristics associated with a positive *B. pertussis* laboratory result in the <5 year and <6 months age groups

3.4.1: Multivariate analysis of characteristics associated with a positive *B. pertussis* laboratory result in the <5 year age group

Among children aged <5 years, on multivariate analysis, compared to children who tested pertussis negative, children testing pertussis-positive were more likely to be identified in Mpumalanga (12.8% [24/188] vs 7.2% [530/7316], adjusted OR (aOR) 4.7, 95% CI 2.6 -8.4) and KZN (14.9% [28/188] vs 13.3% [975/7316], aOR 2.5, 95% CI 1.4-4.4) compared to Gauteng, and to be diagnosed in 2015 (41.5% [78/188] vs 27.6% [2019/7316], aOR 3.2, 95% CI 2.0 – 5.0) and 2017 (42.0% [79/188] vs 39.9 [2916/7316], aOR 1.8, 95% CI 1.1 - 2.7) compared to 2016 and more likely to be aged <6 months compared with 1 -<5 years (70.2% [132/188] vs 53.9% [3940/7316], aOR 2.5, 95% CI 1.6-4.0)(Table 3.2.1) Paroxysmal cough (33.5% [57/170] vs 11.8% [811/6 887], aOR 2.7, 95% CI 1.7-3.4), inspiratory whoop (10.0% [17/170] vs 1.4% [93/6 883], aOR 3.2, 95% CI 1.7 -6.3), post-tussive vomiting (25.9% [44/170] vs 10% [690/6 880], aOR 2.1, 95% CI 1.4-3.4) and cyanosis (12.9% [22/170] vs 2.6% [176/6 886], aOR 3.7, 95% CI 2.2-6.3), were significantly more common in *B. pertussis*-positive patients compared to *B. pertussis* negative patients. Children with pertussis were significantly less likely to report fever compared to those testing pertussis negative (45.4% [84/185] vs 59.2% [4 281/7 238], aOR 0.65, 95% CI 0.47-0.91)

Table 3.2.1: Demographic and clinical characteristics associated with laboratory-confirmed *B. pertussis* in individuals aged <5 years enrolled in pneumonia surveillance, South Africa, 2015-2017.

Demographic /Symptom/Sign	Pertussis negative n/N (%)	Pertussis positive n/N (%)	Unadjusted odds ratio (95% CI)	P value	Adjusted odds ratio (95% CI)	P value
Province				**0.079**		**<0.001**
North West	639/7316 (8.7)	15/188 (8.0)	1.1 (0.6-2.0)	0.376	1.8 (0.8 -3.7)	0.139
KZN	975/7316 (13.3)	28/188 (14.9)	1.4 (0.8-2.2)	0.223	2.5 (1.4 -4.4)	0.002
Mpumalanga	530/7316 (7.2)	24/188 (12.8)	2.1 (1.3 -3.6)	0.004	4.7 (2.6 -8.4)	<0.001
Western Cape	3329/7316 (45.50	82/188 (43.6)	1.1 (0.8 -1.7)	0.440	1.4 (0.9 -2.1)	0.142
Gauteng	1843/7316 (25.2)	39/188 (20.7)	1 (ref)		1 (ref)	
Year				**<0.001**		**<0.001**
2015	2019/7316 (27.6)	78/188 (41.5)	3.0 (2.0-4.5)	<0.001	3.2 (2.0-5.0)	<0.001
2016	2381/7316 (32.6)	31/188 (16.5)	1 (ref)		1 (ref)	
2017	2916/7316 (39.9)	79/188 (42.0)	2.1 (1.4 -3.2)	<0.001	1.8 (1.1 -2.7)	0.013
Age				**<0.001**		**<0.001**
<6 months	3940/7316 (53.9)	132/188 (70.2)	2.3 (1.5-3.4)	<0.001	2.5 (1.6-4.0)	<0.001
6-11 months	1359/7316 (18.6)	26/188 (13.8)	1.3 (0.8 -2.2)	0.352	1.4 (0.8 -2.5)	0.276
≥ 1 year	2017/7316 (27.6)	30/188 (16.0)	1 (ref)		1 (ref)	
Female	3201/7254 (44.1)	81/186 (43.6)	1.0 (0.7-1.3)	0.875		
Non-Black[a]	2033/7221 (28.2)	61/187 (32.6)	1.2 (0.9-1.7)	0.187		
HIV-infected	260/6954 (3.7)	9/176 (5.1)	1.4 (0.7-2.7)	0.368		
Cough	6049/7186 (84.2)	174/183 (95.1)	3.6 (1.9 -7.1)	**<0.001**		NE
Chronic cough[b]	438/7255 (6.0)	25/187 (13.4)	2.4 (1.6-3.7)	**<0.001**		
Paroxysmal cough	811/6887 (11.8)	51/170 (33.5)	3.8 (2.7-5.2)	**<0.001**	2.7 (1.7-3.4)	**<0.001**
Nocturnal cough	1692/6886(24.6)	53/170 (31.2)	1.4 (1.0-1.9)	**0.055**		
Inspiratory whoop	93/6883 (1.4)	17/170 (10.0)	8.1 (4.7-13.9)	**<0.001**	3.2 (1.7 -6.3)	**<0.001**
Post-tussive vomiting	690/6880 (10.0)	44/170(25.9)	3.1 (2.2-4.5)	**<0.001**	2.1 (1.4 -3.4)	**<0.001**
Apnoea	290/6884 (4.2)	20/170 (11.8)	3.0 (1.9 -4.9)	**<0.001**		
Cyanosis	176/6886 (2.6)	22/170 (12.9)	5. 7 (3.5-9.1)	**<0.001**	3.7 (2.2 -6.3)	**<0.001**
Fever[c]	4281/7238 (59.2)	84/185 (45.4)	0.6 (0.4-0.8)	**<0.001**	0.7 (0.5 -0.9)	**0.026**
Difficulty breathing	4375/7186 (60.9)	117/183 (63.9)	1.1 (0.8-1.6)	0.401		
Wheezing	2937/7191 (40.8)	61/183 (33.3)	0.7 (0.5-1.0).	**0.039**		
Stridor	845/7176 (11.8)	28/183 (15.3)	1.4 (0.9-2.0)	0.160		
Tachypnoea[d]	2246/6546 (34.3)	48/172 (27.9)	0.7 (0.5-1.0)	**0.076**		
Chest recession	3341/7184 (46.5)	89/183 (48.6)	1.1 (0.8-1.5)	0.569		
Hypoxia[e]	796/7045 (11.3)	31/180 (17.2)	1.6 (1.1-2.4)	**0.020**		
Convulsions	167/7184 (2.3)	1/183 (0.6)	0.2 (0.3-1.7)	**0.058**		
Any/chronic cough & at least 1 symptom[f]	977/6874 (14.2)	65/170 (38.2)	3.7 (2.7-5.1)	<0.001		**NE**
Any/chronic cough & 3 symptoms[f]	38/6874 (0.6)	16/170 (9.4)	18.7 (10.2-34.2)	<0.001		**NE**

Denominators exclude unknown responses

Column percentages may not sum to 100 due to rounding

CI: Confidence interval

Variables statistically significant on univariate regression analysis (p<0.1) or multivariate regression analysis (p<0.05), presented in boldface

NE (not evaluated): variables statistically significant on univariate analysis but omitted in the multivariate analysis

Ref (reference)

[a]Non-black: includes whites, coloureds, asians/indian

[b]Chronic cough: cough for ≥14 days

[c]Fever: recorded ≥38 ºC degrees C and/ or reported on history

[d]Tachypnoea: respiratory rate >50 breaths/minute for 2-12months, >40 breaths/minute1-<5 years

[e]Hypoxia: oxygen saturation ≤88 at room temperature

[f]For WHO (2018)

<1 year: Any cough & paroxysmal cough/ inspiratory whoop/ post-tussive vomiting/ apnoea

1-5 years: Chronic cough & paroxysmal cough/ inspiratory whoop/ post-tussive vomiting

3.4.2: Multivariate analysis of characteristics associated with a positive *B. pertussis* laboratory result in the <6 month age group

Among infants aged <6 months, on multivariate analysis, compared to children who tested pertussis negative, children testing pertussis-positive were more likely to be identified in Mpumalanga (12.1% [16/132] vs 4.9% [191/3940], aOR 7.0, 95% CI 3.5-13.9) and KZN (9.9% [13/132] vs 10.7% [420/3940], aOR 2.5, 95% CI 1.2-5.4) compared to Gauteng, and to be diagnosed in 2015 (38.6% [51/132] vs 28.1% [1105/3940], aOR 2.7, 95% CI 1.6 -4.5) compared with 2016 and more likely to be aged <3 months compared with 3 -<6 months (82.6% [109/132] vs 67.0% [2639/3940], aOR 3.0, 95% CI 1.8 -4.9) (Table 3.2.2). Chronic cough (16.8% [22/131] vs 5.0% [194/3 914], aOR 2.2, 95% CI 1.2-3.9), paroxysmal cough (39.3% [48/122] vs 11.4% [423/3 725], aOR 3.2, 95% CI 1.9-5.4), inspiratory whoop (12.3% [15/122] vs 1.1% [42/3722], aOR 4.4, 95% CI 2.1 -9.4), post-tussive vomiting (28.7% [35/122] vs 9.4% [348/3720], aOR 2.1, 95% CI 1.2-3.6) and cyanosis (16.4% [20/122] vs 3.4% [127/3 724], aOR 3.5, 95% CI 1.9-6.3) were significantly more common in *B. pertussis*-positive patients compared to *B. pertussis*-negative patients.

Table 3.2.2: Demographic and clinical characteristics associated with laboratory-confirmed *B. pertussis* in individuals aged <6 months enrolled in pneumonia surveillance, South Africa, 2015-2017.

Demographic /Symptom/ Sign	Pertussis Negative n/N (%)	Pertussis Positive n/N (%)	Unadjusted Odds Ratio (95%CI)	P value	Adjusted Odds Ratio (95%CI)	P value
Province				**0.026**		**<0.001**
North West	264/ 3940 (6.7)	8/132 (6.1)	1.1 (0.5-2.4)	0.822	1.9 (0.7-5.0)	0.186
KZN	420/ 3940 (10.7)	13/132 (9.9)	1.1 (0.6 -2.2)	0.738	2.5 (1.2 -5.4)	0.016
Mpumalanga	191/3940 (4.9)	16/132 (12.1)	3.0 (1.6-5.6)	<0.001	7.0 (3.5-13.9)	<0.001
Western Cape	1909/3940 (48.5)	62/132 (47.7)	1.2 (0.8 -1.8)	0.425	1.4 (0.9 -2.3)	0.189
Gauteng	1156/3940 (29.3)	32/132 (24.2)	1 (ref)		1 (ref)	
Year				**<0.001**		**0.001**
2015	1105/3940 (28.1)	51/132 (38.6)	2.4 (1.5 -4.0)	<0.001	2.7 (1.6 -4.5)	<0.001
2016	1271/3940 (32.3)	24/132 (18.2)	1 (ref)		1 (ref)	
2017	1564/3940 (39.7)	57/132 (43.2)	1.9 (1.2 -3.1)	0.008	1.5 (0.9-2.5)	0.108
Age				**<0.001**		**<0.001**
<3 months	2639/3940 (67.0)	109/132 (82.6)	2.3 (1.5 -3.7)	<0.001	3.0 (1.8 -4.89)	<0.001
Female	1727/3916 (44.1)	60/130 (46.2)	1.1 (0.8 -1.5)	0.643		
Non-Black[a]	1210/3896 (31.1)	50/131 (38.2)	1.4 (1.0-2.0)	0.090		
HIV-infected	101/3736 (2.7)	5/124 (4.0)	1.5 (0.6-3.8)	0.403		
Cough	2919/3877 (75.3)	121/130 (93.1)	4.4 (2.2-8.7)	**<0.001**		**NE**
Chronic cough[b]	194/3914 (5.0)	22/131 (16.8)	3.9 (2.4 -6.3)	**<0.001**	2.2 (1.2-3.9)	**0.013**
Paroxysmal cough	423/3725 (11.4)	48/122 (39.3)	5.1 (0.0-3.5)	**<0.001**	3.2 (1.9-5.4)	**<0.001**
Nocturnal cough	790/3723 (21.2)	40/122 (32.8)	1.8 (1.2-2.7)	**0.004**		
Whoop	42/3722 (1.1)	15/122 (12.3)	12.3 (6.6-22.8)	**<0.001**	4.4 (2.1 -9.4)	**<0.001**
Post-tussive vomiting	348/3720 (9.4)	35/122 (28.7)	3.9 (2.6 -6.0)	**<0.001**	2.1 (1.2-3.6)	**0.008**
Apnoea	251/3724 (6.7)	20/122(16.4)	2.7 (1.7 -4.5)	**<0.001**		
Cyanosis	127/3724 (3.4)	20/122 (16.4)	5.6 (3.3-9.3)	**<0.001**	3.5 (1.9-6.3)	**<0.001**
Fever[c]	1945/3904 (49.8)	50/129 (38.8)	0.6 (0.4-0.9)	**0.013**		
Difficulty breathing	2213/3877 (57.1)	76/130 (58.5)	1.1 (0.7-1.5)	0.754		
Wheezing	1304/3878 (33.6)	35/130 (26.9)	0.7 (0.5 -1.1)	0.105		
Stridor	366/3870 (9.5)	15/130 (11.5)	1.3 (0.7-2.2)	0.440		
Tachypnoea[d]	979/3449 (28.40)	28/117 (23.9)	0.8 (0.5-1.2)	0.285		
Chest recession	1745/3876 (45.0)	58/130 (44.6)	1.0 (0.7 -1.4)	0.927		
Hypoxia[e]	448/3792 (11.8)	26/127 (20.50	1.9 (1.2-3.0)	**0.007**		
Convulsions	63/3876 (1.6)	1/130 (0.8)	0.5 (0.1 -3.4)	0.396		
Any cough & at least 1 symptom[f]	898/3716 (18.8)	58/122 (47.5)	3.9 (2.7-5.6)	**<0.001**		NE
Any cough & 4 symptoms[f]	2/3716 (0.1)	4/122 (3.3)	62.9 (11.4-347.1)	**<0.001**		NE

Denominators exclude unknown responses

Column percentages may not sum to 100 due to rounding

CI: Confidence interval

Variables statistically significant on univariate regression analysis (p<0.1) or on multivariate regression analysis (p<0.05), presented in boldface

NE (not evaluated): variables statistically significant on univariate analysis but omitted in the multivariate analysis

Ref (reference)

[a]Non-black: includes whites, coloureds, asians/indian

[b]Chronic cough: cough for ≥14 days

[c]Fever: recorded ≥38 ºC and/ or reported on history

[d]Tachypnoea: respiratory rate >60 breaths/minute in <2 months, >50 breaths/minute in <2-12 months
[e]Hypoxia: oxygen saturation ≤88 at room temperature
[f] For WHO (2018)
<1 year: Any cough & paroxysmal cough/ inspiratory whoop/ post-tussive vomiting/ apnoea

3.5: Multivariate analysis of characteristics associated with a positive *B. pertussis* laboratory result in the ≥5 year age group

In the ≥5 year age group, on multivariate analysis, compared to individuals who tested pertussis negative, individuals testing pertussis-positive were more likely to be aged 5-14 years compared with 15-23 years of age (24.1% [13/54] vs 6.6% [316/4812], aOR 14.80, 95% CI 1.9-118.4), and to be people of non-black compared with black race (17.0% [9/54] vs 9.0% [430/4760], aOR 3.2, 95% CI 1.3-8.0) (Table 3.3). HIV-infection (79.3% [42/53] vs 66.1% [3090/4677], aOR 5.1, 95% CI 2.0-12.8) and inspiratory whoop (7.5% [3/40] vs 1.6% [68/4279], aOR 4.7, 95% CI 1.3-16.6) were significantly more common in *B. pertussis*-positive patients compared to *B. pertussis*-negative patients (Table 3.3)

Table 3.3: Demographic and clinical characteristics associated with laboratory-confirmed *B. pertussis* in individuals aged ≥ 5years enrolled in pneumonia surveillance, South Africa, 2015-2017.

Demographic/ Symptom/ Sign	Pertussis negative n/N (%)	Pertussis-positive n/N (%)	Unadjusted Odds Ratio (95% CI)	P value	Adjusted Odds Ratio (95% CI)	P value
Province				**0.349**		
North West	1461/4812 (30.4)	23/54 (42.6)	1.9 (1.0-3.8)	0.062		
KZN	1107/4812 (23.0)	10/54 (18.5)	1.1 (0.5-2.5)	0.823		
Mpumalanga	364/ 4812 (7.6)	5/54 (9.3)	1.7 (0.6-4.7)	0.332		
Western Cape	298/4812 (6.2)	3/54 (5.6)	1.2 (0.4-4.3)	0.752		
Gauteng	1582/4812 (32.9)	13/54 (24.1)	1 (ref)			
Year				**0.003**		
2015	1567/4812 (32.6)	30/54 (55.6)	2.6 (1.3 -5.2)	0.007		
2016	1488/4812 (30.9)	11/54 (20.4)	1 (ref)			
2017	1757/4812 (36.5)	13/54 (24.1)	1.0 (0.5-2.2)	0.998		
Age				**<0.001**		**<0.001**
5-14 years	316/4812 (6.6)	13/54 (24.1)	5.9 (1.3-26.4)	0.020	14.8 (1.9-118.4)	0.011
15-23 years	287/4812 (6.0)	2/54 (3.7)	1 (ref)		1 (ref)	
24-44 years	2402/4812 (49.9)	22/54 (40.7)	1.3 (0.3-5.6)	0.712	1.5 (0.2-11.0)	0.720
≥45 years	1807/4812 (37.6)	17/54 (31.5)	1.4 (0.3-5.9)	0.689	1.8 (0.2-13.9)	0.585
Female	2496/4773 (52.3)	28/54 (52.8)	1.0 (0.6 -1.8)	0.938		
Non-Black[a]	430/4760 (9.0)	9/54 (17.0)	2.1 (1.0-4.3)	0.071	3.2 (1.3-8.0)	0.012
HIV -infected	3090/4677 (66.1)	42/53 (79.3)	2.0 (1.0-3.8)	**0.036**	5.1 (2.0-12.8)	**<0.001**
Cough	4465/4698 (95.0)	49/53 (92.5)	0.6 (0.2-1.8)	0.422		
Chronic cough[b]	2453/4768 (51.5)	21/53 (39.6)	0.6 (0.4-1.1)	**0.086**		NS
Paroxysmal cough	163/4278 (3.8)	3/40 (7.5)	2.1 (0.6-6.7)	0.283		
Nocturnal cough	1782/4279 (41.7)	17/40 (42.5)	1.0 (0.6-1.9)	0.913		
Whoop	68/4279 (1.6)	3/40 (7.5)	5.0 (1.5-16.7)	**0.031**	4.7 (1.3-16.6)	**0.042**
Fever[c]	2984/4754 (62.8)	30/53 (56.6)	0.8 (0.5-1.3)	0.361		
Difficulty breathing	2825/4697 (60.1)	29/53 (54.7)	0.8 (0.46-1.4)	0.425		
Wheezing	892/4698 (19.0)	13/53 (24.5)	1.4 (0.7-2.6)	0.323		
Tachypnea[d]	2004/4628 (43.3)	24/51 (47.1)	1.2 (0.7-2.0)	0.591		
Hypoxia[e]	829/4561 (18.2)	6/51 (11.8)	0.6 (0.3-1.4)	0.213		
Chest pain	3419/4698 (72.8)	37/53 (69.8)	0.9 (0.5 -1.6)	0.633		
Chronic cough & at least 1 symptom[f]	104/4276 (2.4)	3/40 (7.5)	3.2 (1.0-10.7)	0.053		

Denominators exclude unknown responses
Column percentages may not sum to 100 due to rounding
CI: Confidence interval
Variables statistically significant on univariate regression analysis (p<0.1) or multivariate regression analysis (p<0.05), presented in boldface
NE (not evaluated): variables statistically significant on univariate analysis but omitted in the multivariate analysis
Ref (reference)
[a]Non-black: includes whites, coloureds, asians/indian
[b]Chronic cough: cough for ≥14 days
[c]Fever: recorded ≥38 ºC and/ or reported on history
[d]Tachypnoea: respiratory rate >20 breaths/minute
[e]Hypoxia: oxygen saturation ≤ 88 at room temperature
[f]For WHO(2018): other symptoms include paroxysmal cough and inspiratory whoop

3.6: Sensitivity, specificity, positive– and negative-predictive values in <5 years and <6 months age groups

Performance of symptoms and signs was similar for both age groups although sensitivity and PPV was generally higher in the <6 month group.

Among children aged <5 years, only cough showed high sensitivity but low specificity (95.1% [95% CI 90.0 - 97.7] and 15.8% [95% CI 15.0-16.7], respectively) (Table 3.4.1). Symptoms included in the WHO and CDC pertussis case definitions showed low sensitivity with high specificity; these included chronic cough (13.4% [95% CI 8.8-19.1] and 94.0% [95% CI 93.4-94.5]), whoop (10% [95% CI 5.9-15.5] and 98.6% [95% CI 98.3-98.9]), post-tussive vomiting (25.9% [95% CI 19.5-33.1] and 90% [95% CI 89.2-90.7]), paroxysmal cough (33.5% [95% CI 26.5-41.2] and 88.2% [95% CI 87.4-89.0]), cyanosis (12.9% [95% CI 8.3-18.9] and 97.4% [95% CI 97.0-97.8]) and apnoea (11.8% [95% CI 7.3-17.6] and 95.8 [95% CI 95.3-96.2]). Whoop had higher PPV than other symptoms (15.5 [95% CI 9.3-23.6] (Table 3.4.2). Nocturnal cough showed moderate specificity 75.4% [95% CI 74.4-76.4]) with low sensitivity (31.2 [95% CI 24.3-38.7]) and fever had low sensitivity (45.4% [95% CI 38.1-52.9]) and specificity (40.9% [95% CI 39.7-42.0]) in children <5 years of age.

For the <5 year age group, the WHO SARI case definition had low sensitivity and moderate specificity (39.3% [95% CI 32.1- 46.9] and 51.8 [95% CI 50.7-53.0]) with low PPV (2.1%, [95% CI 1.6-2.1]). The 2003 WHO pertussis case definition showed high specificity (97.8, [95% CI 97.5-98.2]) and low sensitivity (10.6 [95% CI 6.4-16.2]) for predicting pertussis infection. However, the 2018 WHO case definitions increased sensitivity to 38% (95%CI 30.9-46.0) in the <5 year olds while specificity 85.8% (95% CI 84.9-86.6) was maintained, PPV remained low 6.2% (95% CI 4.9-7.9). Generally, as the number of case-defined symptoms increased, sensitivity decreased while specificity and PPV increased: for the WHO 2018 pertussis case definition; chronic cough (or any cough for <1 year) with at least 3 defined symptoms, PPV was 29.6% (95% CI 18.0 -43.6) but sensitivity dropped to 9.4% (95% CI 5.5-14.8) with high specificity (99.4 [95% CI 99.2-99.6]).

Among infants aged <6 months, for any cough (WHO 2018) and 4 additional classic symptoms, PPV is 66.7% (95% CI 22.3-95.7), with very low sensitivity (3.3% [95% CI 0.9-8.2]) and high specificity (99.9% [95% CI 99.8-100.0]) (Table 3.4.2). Similar trends were noted for the CDC case definitions for infants <1 year, which includes apnoea with or without cyanosis. In the <6 month age group PPV reached 60% (95% CI 14.7-94.7) for chronic cough and 4 additional symptoms; with low sensitivity (2.5% [95% CI 0.5-7.0]) and high specificity (99.9% [95% CI 99.8-100.0]).

Table 3.4.1: Sensitivity, specificity, positive and negative predictive values of signs, symptoms and case definitions for detecting a positive *B. pertussis* result in individuals <5 years enrolled in pneumonia surveillance, South Africa, 2015-2017

Symptom/Sign Case definition	Sensitivity % (95%CI)	Specificity % (95%CI)	Positive predictive value % (95%CI)	Negative predictive value % (95%CI)
Cough	95.1 (90.0-97.7)	15.8 (15.0-16.7)	2.8 (24.0-3.2)	99.2 (98.5-99.6)
Chronic cough[a]	13.4 (8.8-19.1)	94.0 (93.4-94.5)	5.4 (3.5-7.9)	97.7 (97.3-98.0)
Paroxysmal cough	33.5 (26.5-41.2)	88.2 (87.4-89.0)	6.6 (5.0-8.4)	98.2 (97.8-98.5)
Nocturnal cough	31.2 (24.3-38.7)	75.4 (74.4-76.4)	3.0 (2.3-4.0)	97.8 (97.4-98.2)
Inspiratory whoop	10 (5.9-15.5)	98.6 (98.3-98.9)	15.5 (9.3-23.6)	97.8 (97.4-98.1)
Post-tussive vomiting	25.9 (19.5-33.1)	90.0 (89.2-90.7)	6.0 (4.9-8.0)	98.0 (97.6-98.3)
Apnoea	11.8 (7.3-17.6)	95.8 (95.3-96.2)	6.5 (4.0-9.8)	97.8 (97.4-98.1)
Cyanosis	12.9 (8.29-18.9)	97.4 (97.0-97.8)	11.1 (7.1-16.3)	97.8 (97.5-98.2)
Fever[b]	45.4 (38.1-52.9)	40.9 (39.7-42.0)	1.9 (1.5-2.4)	96.7 (96-97.3)
Wheezing	33.3 (26.6-40.7)	59.2 (58.0-60.3)	2.0 (1.6-2.6)	97.2 (96.7-97.7)
CASE DEFINITIONS:				
WHO SARI	39.3 (32.1-46.9)	51.8 (50.7-53.0)	2.1 (1.6-2.6)	97.1 (96.5-97.6)
WHO (2003)[c]				
Chronic cough and at least 1 symptom	10.6 (6.4-16.2)	97.8 (97.5-98.2)	10.8 (6.5-16.5)	97.8 (97.4-98.1)
Chronic cough and at least 2 symptoms	7.65 (4.13-1.7)	98.9 (98.7-99.2)	15.1 (8.3-24.5)	97.7 (97.4-98.1)
Chronic cough and 3 symptoms	1.8 (0.4-5.1)	99.9 (99.8-99.9)	25 (5.5-57.2)	97.6 (97.2-98.0)
WHO (2018)[d]				
Cough and at least 1 symptom	38.2 (30.9-46.0)	85.8 (84.9-86.6)	6.24 (4.9-7.9)	98.3 (97.9-98.6)
Cough and at least 2 symptoms	25.3 (19.0-32.5)	95.1 (94.6-95.6)	11.4(8.4-15.0)	98.1 (97.7-98.4)
Cough and 3 symptoms	9.4 (5.5-14.8)	99.4 (99.2-99.6)	29.6 (18.0-43.6)	97.8 (97.4-98.1)
CDC 2014[e]				
Chronic cough and at least 1 symptom	11.2 (6.9-16.9)	97.8 (97.4-98.1)	11.0 (6.8-16.7)	97.8 (97.4-98.1)
Chronic cough and at least 2 symptoms	8.2 (4.6-13.4)	98.9 (98.6-99.1)	15.2 (8.6-24.2)	97.8 (97.4-98.1)
Chronic cough and 3 symptoms	4.7 (2.1-9.1)	99.8 (99.7-99.9)	38.1 (18.1-61.6)	97.7 (97.3-98.0)

[a] Chronic cough: cough for ≥14 days

[b]Fever: recorded ≥38 ºC and/ or reported on history

[c]For WHO (2003) other symptoms include paroxysmal cough, inspiratory whoop and post-tussive vomiting

[d]For WHO (2018)

< 1 year: Any cough & paroxysmal cough/ inspiratory whoop/ post-tussive vomiting/ apnoea

1-5 years: Chronic cough & paroxysmal cough/ inspiratory whoop/ post-tussive vomiting

[e]For CDC (2014) other symptoms include paroxysmal cough, inspiratory whoop and post-tussive vomiting and apnea with or without cyanosis (infants <1 year only)

Table 3.4.2: Sensitivity, specificity, positive and negative predictive values of signs, symptoms and case definitions for detecting a positive *B. pertussis* result in individuals <6months enrolled in pneumonia surveillance, South Africa, 2015-2017

Symptom/Sign Case definition	Sensitivity % (95%CI)	Specificity% (95%CI)	Positive Predictive Value% (95%CI)	Negative Predictive Value% (95%CI)
Cough	93.1 (87.3-96.8)	24.7 (23.4-26.1)	4.0 (3.31-4.74)	99.1 (98.2-99.6)
Chronic cough[a]	16.8 (10.8-24.3)	95.0 (94.3-95.7)	10.2 (6.5-15)	97.2 (96.6-97.7)
Paroxysmal Cough	39.3 (30.6-48.6)	88.6 (87.6-89.6)	10.2 (7.6-13.3)	97.8 (97.3-98.3)
Nocturnal cough	32.8 (24.6-41.9)	78.8 (77.4-80.1)	4.8 (3.5 -6.5)	97.3 (96.6-97.8)
Whoop	12.3 (7.1-19.5)	98.9 (98.5-99.2)	26.3 (15.5-39.7)	97.2 (96.6-97.7)
Post-tussive vomiting	28.7 (20.9-37.6)	90.6 (89.7-91.6)	9.14 (6.5-12.5)	97.5 (96.9-98.0)
Apnoea	16.4 (10.3-24.2)	93.3 (92.4-94.0)	7.38 (4.6-11.2)	97.1 (96.5-97.7)
Cyanosis	16.4 (10.3-24.2)	96.6 (96.0-97.1)	13.6 (8.5-20.2)	97.2 (96.7-97.7)
Fever[b]	38.8 (30.3-47.7)	50.2 (48.6-51.8)	2.5 (1.87-3.29)	96.1 (95.2-96.9)
Wheezing	26.9 (19.5-35.4)	66.4 (64.9-67.9)	2.6 (1.83-3.62)	96.4 (95.7-97.1)
CASE DEFINITIONS:				
SARI	30.4 (22.5-39.3)	63.7 (62.1-65.2)	2.7 (1.92-3.69)	96.5 (95.7-97.2)
WHO (2003) [c]				
Chronic cough and at least 1 symptom	13.1 (7.7-20.4)	97.8 (97.3-98.3)	16.5 (9.73-25.4)	97.2 (96.6-97.7)
WHO (2018) [d]				
Cough and at least 1 symptom	47.5 (38.4-56.8)	81.2 (79.9-82.5)	7.7 (5.88-9.81)	97.9 (97.4-98.4)
Cough and at least 2 symptoms	32.8 (24.6-41.9)	93.8 (92.9-94.5)	14.7 (10.7-19.5)	97.7 (97.2-98.2)
Cough and 3 symptoms	12.3 (7.1-19.5)	99.3 (99.0-99.6)	37.5 (22.7-54.2)	97.2 (96.6-97.7)
Cough and 4 symptoms	3.3 (0.9-8.2)	99.9 (99.8-100.0)	66.7 (22.3-95.7)	96.9 (96.3-97.4)
CDC (< 1year)[e]				
Chronic cough at least 1 symptom	13.9 (8.33-21.4)	97.7 (97.2-98.2)	16.7 (10-25.3)	97.2 (96.6-97.7)
Chronic cough and least 2 symptoms	11.5 (6.4-18.5)	99.1 (98.8-99.4)	30.4 (17.7-45.8)	97.1 (96.6-97.6)
Chronic cough and 3 symptoms	6.6 (2.7-12.5)	99.8 (99.6-99.9)	53.3 (26.6-87.7)	97 (96.4-97.5)
Chronic cough and 4 symptoms	2.5 (0.5-7.0)	99.9 (99.8-100.0)	60 (14.7-94.7)	96.9 (96.3-97.4)

[a] Chronic cough: cough for ≥14 days

[b]Fever: recorded ≥38 ºC and/ or reported on history

[c]For WHO(2003) case definitions, other symptoms include paroxysmal cough, inspiratory whoop and post-tussive vomiting

[d]For WHO (2018)

< 1 year: Any cough & paroxysmal cough/ inspiratory whoop/ post-tussive vomiting/ apnoea

1-5 years: Chronic cough & paroxysmal cough/ inspiratory whoop/ post-tussive vomiting

[e]For CDC 2014 (for < 1 year) other symptoms include paroxysmal cough, inspiratory whoop and post-tussive vomiting and apnoea with or without cyanosis

3.7: Sensitivity, specificity, positive – and negative predictive value ≥ 5 years age group

Similarly to the <5 year age group, cough showed high sensitivity with low specificity (92.5%, 95% CI 81.8-97.9 and 5.0%, 95%CI 4.4-5.6) but PPV was low (1.1%, 95%CI 0.8-1.4) (Table 3.5). Furthermore, whoop had low sensitivity and high specificity (7.5%, 95%CI 1.6-20.4 and 98.4%, 95%CI 98.0-98.8) and fever had moderate sensitivity and low specificity (56.6%, 95%CI 42.3-70.2 and 37.2%, 95%CI 35.9-38.6). In contrast to the high specificity in younger children, chronic cough had low sensitivity and specificity (39.6%, 95%CI 26.5-54.0 and 48.6%, 95%CI 47.1-50.0).

As in the <5 year group, the WHO SARI case definition showed low sensitivity and moderate specificity (35.6%, 95%CI 21.9-51.2 and 65.2%, 95%CI 0.6 -1.7) while WHO/CDC pertussis case definitions showed low sensitivity, high specificity (7.5%, 95%CI 1.57-20.4 and 97.6%, 95%CI 97.1- 98.0) and low PPV (2.8%, 95%CI 0.6-8.0). The criteria for this age group remained the same for the 2003 and 2018 WHO case definitions.

Table 3.5: Sensitivity, specificity, positive and negative predictive values of signs, symptoms and case definitions for detecting a positive *B. pertussis* result in individuals ≥5 years enrolled in pneumonia surveillance, South Africa, 2015-2017

Symptom/ Sign/Case definition	Sensitivity % (95%CI)	Specificity % (95%CI)	Positive predictive value % (95%CI)	Negative predictive value % (95%CI)
Cough	92.5 (81.8-97.9)	5.0 (4.4-5.6)	1.1 (0.8-1.4)	98.3 (95.7-99.5)
Chronic cough[a]	39.6 (26.5-54.0)	48.6 (47.1-50.0)	0.8 (0.5-1.3)	98.6 (98.1-99.1)
Paroxysmal cough	7.5 (1.6-20.4)	96.2 (95.6-96.7)	1.8 (0.4-5.2)	99.1 (98.8-99.4)
Nocturnal cough	42.5 (27.0-59.1)	58.4 (56.9-59.8)	0.9 (0.6-1.5)	99.1 (98.6-99.4)
Whoop	7.5 (1.6-20.4)	98.4 (98.0-98.8)	4.2 (0.9-11.9)	99.1 (98.8-99.4)
Fever[b]	56.6 (42.3-70.2)	37.2 (35.9-38.6)	1.0 (0.7-1.4)	98.7 (98.1-99.2)
Wheezing	24.5 (13.8-38.3)	81(79.9-82.1)	1.4 (0.8-2.4)	99.0 (98.6-99.3)
CASE DEFINITIONS:				
SARI	35.6 (21.9-51.2)	65.2 (63.8-66.6)	1.1 (0.6-1.7)	99.0 (98.5-99.3)
WHO/CDC[c]				
Chronic cough and at least 1 symptom	7.5 (1.57-20.4)	97.6 (97.1-98)	2.8 (0.6-8.0)	99.1 (98.8-99.4)
Chronic cough and 2 symptoms	5 (0.6-16.9)	99.7 (99.5-99.8)	13.3 (1.7-40.5)	99.1 (98.8- 99.4)

[a]Chronic cough: cough for ≥14 days
[b]Fever: recorded ≥38 ºC and/ or reported on history
[c]For WHO/CDC case definitions, other symptoms include paroxysmal cough and inspiratory whoop

Chapter 4: Discussion

Standard WHO and CDC pertussis case definitions showed low sensitivity, high specificity and low PPV for detecting pertussis disease across all age groups and would result in underestimation of disease burden. Incorporation of apnoea and cough of any duration for infants <1 year of age improved sensitivity in infants <6 months of age (47.5%, 95%CI 38.4-56.8) while maintaining high specificity (81.2%, 95%CI 79.9-82.5), with low PPV (7.67%, CI 5.88-9.81). WHO SARI case definitions demonstrated low sensitivity and moderate specificity for identifying pertussis. Classic pertussis symptoms were relatively uncommon presenting features but were highly specific for pertussis. Multivariate analysis of the <5 year age group, revealed age, whoop, paroxysmal cough, post-tussive emesis and cyanosis as significant predictors of a positive pertussis laboratory result. Fever was less common in pertussis- positive than pertussis-negative individuals, but 45% and 57% of pertussis-infected children and adults (≥5 years) respectively, presented with fever. This finding would impact on the performance of case definitions that specifically include or exclude fever as a criteria in our setting.

Optimal case definitions for estimating burden of disease require a balance between high sensitivity (to ensure cases are not missed) and specificity (to contain costs of testing too many cases). Current standard pertussis case definitions would miss 60-90% of cases. In addition, PPV for these case definitions is low, influenced by the low incidence of pertussis in hospitalised patients with respiratory symptoms.
The 2018 WHO pertussis case definition performed well in infants <6 months of age, although PPV remained low. While PPV increases to 37.5% (95% CI 22.7-54.2) and 66.7% (95% CI 22.3-95.7) with cough and at least 3 and 4 additional symptoms, respectively, in the <6 month age group, this was counteracted by a reduction in sensitivity. In this analysis, only 3% (4/122) of infants with pertussis detected would have fulfilled the requirement for cough and 4 additional symptoms.

In South Africa, pertussis surveillance was introduced into existing surveillance platforms that utilise case definitions intended for other diseases. This principle could be applied in other LMIC countries to optimise use of human resource and existing systems. However, the need to test many patients in order to identify pertussis cases

does have cost implications for the programmes. In our study, 2.5% (188/7504) of children <5 years, meeting the broad case definitions for pneumonia surveillance, tested positive for *B. pertussis* and 6.2% (65/1042) that fulfilled the 2018 WHO pertussis case definitions of chronic/any cough and at least one other symptom, were pertussis-positive. For WHO SARI case definition, 2.1% (70/420) children screened were positive for *B. pertussis* on laboratory testing. For ≥5 year age group 1.1% (54/4812) meeting pneumonia surveillance case definitions; 2.8% (3/107) meeting WHO 2018 pertussis case definitions and 1.0% (16/1529) fulfilling WHO SARI case definition, tested positive for *B. pertussis*.

The WHO SARI case definition showed moderate sensitivity (30-39%) and specificity (52-65%) with low PPV for detection of pertussis across all age groups. Using this case definition for pertussis surveillance could miss 60-70% of cases. In addition, using a case definition that includes fever as a criterion could systematically exclude a significant proportion of pertussis-infected patients. WHO have recently completed a multi-site two year pilot study to assess utilising existing mechanisms and infrastructure of the WHO Global Influenza Surveillance and Response System for respiratory syncytial virus (RSV) surveillance. Extended SARI and Acute Respiratory Illness case definitions that did not require fever as a criterion were used for hospital and community-based infections respectively (65). These extended case definitions could potentially perform well for pertussis surveillance.

There is a paucity of literature assessing the sensitivity and specificity of WHO 2003 pertussis case definitions and the inclusion criteria may differ from the broad case definitions used for pneumonia surveillance making direct comparisons difficult. In this analysis, 10.6% (18/170) pertussis-positive, 2.2% (149/6873) pertussis-negative <5 year olds met the 2003 WHO pertussis case definition (with at least one symptom). A retrospective review of hospitalised children tested for pertussis in Cape Town 2012- 2013, showed that only 23/75 (33%) laboratory-confirmed cases fulfilled the 2003 WHO clinical case definition for pertussis. (57). Among children ≤10 years hospitalised with suspected pertussis at 2 sites in Gauteng, 2013-2015, 9.8% (4/41) of patients testing positive and 5.9% (56/949) testing negative for *B. pertussis* met the 2003 WHO case definitions (49). A higher percentage of children in our study met the criteria for the WHO 2018 case definitions (38.2% [65/170] pertussis-

positive and 14.2% [977/6874] pertussis-negative) met the criteria for the WHO 2018 case definitions, but these remain to be evaluated in other settings. Ghanaie *et al* (55) showed sensitivity and specificity of 95% and 15% respectively for the 2003 WHO pertussis case definition when evaluating children 6-14 years of age in Tehran with cough for ≥2 weeks. In another study of French adults consulting general practitioners for persistent cough, 89% of confirmed cases were reported to meet the CDC case definition (56). However, in our analysis only 7.5% (3/40) pertussis-positive and 2.4% (104/4276) pertussis-negative ≥ 5 years would have fulfilled the criteria. In contrast to our study, both the Tehran and French studies pre-select for chronic cough and were done in an outpatient setting where patients may have presented with milder/atypical disease.

Many studies have evaluated the performance of individual signs and symptoms for predicting pertussis but heterogeneity in inclusion criteria and setting limits comparisons. In a systematic review and meta-analysis, unrestricted by patient age, Ebell *et al* (58) concluded that that overall clinical impression was the most accurate predictor of *B.* pertussis (positive likelihood ratio, 3,3; negative likelihood ratio *0.63).*Furthermore, the presence of whoop (positive likelihood ratio, 2.1) or post-tussive vomiting (positive likelihood ratio, 1.7) make a diagnosis of pertussis somewhat more likely, but have lower sensitivity in adults. Absence of paroxysmal cough (negative likelihood ratio, 0.58) and absence of sputum (negative likelihood ratio, 0.63) reduced the likelihood of pertussis. In a separate systematic review and meta-analysis, Moore *et al* (59), suggested the presence of whoop or post-tussive vomiting in adults made a diagnosis of pertussis more likely, while absence of paroxysmal cough and presence of fever should rule out pertussis. This analysis found post-tussive vomiting less helpful as a clinical diagnostic test in children.

Our findings concur with the low sensitivity and high specificity for whoop in all ages and post-tussive emesis for children. However, paroxysmal cough did not have high sensitivity for pertussis in our setting. Ebell's conclusions regarding the accuracy of overall clinical impression to predict *B. pertussis,* supported the inclusion of "clinical suspicion of pertussis" in the 2018 WHO pertussis case definition. Studies included were from multiple setting and many included prolonged cough or suspicion of pertussis as inclusion criteria. This analysis did not include evaluation of the GPI

clinical case definitions for pertussis. However, a study from Serbia (66) reported that in patients aged 0-3 months, whoop and apnoea were associated with a positive laboratory test for pertussis; for children aged 4 months to 9 years, whoop combined with apnoea or post-tussive emesis; and in individuals ≥10 years of age, various different combinations of symptoms and signs, were predictive of pertussis. The Serbian study only included patients who fulfilled at least one criteria of the case definitions for their age group and covered both inpatients and outpatients.

Although pertussis-positive children <5 year were significantly more likely to present with classic pertussis symptoms compared to pertussis-negative group; only ~one third of pertussis-positive children had paroxysmal cough or post-tussive vomiting and <14% presented with whoop, apnoea or cyanosis. These classic symptoms were highly specific for pertussis (≥88%) but had low PPV. The use of a non-specific pneumonia surveillance platform which focused on severe respiratory infections of any duration would have enriched for non-pertussis clinical features. Cough was the commonest presenting symptom (95%), but only 14% of pertussis-positive children with cough reported chronic cough lasting 14 days or more. These findings support the 2018 amendment of the WHO pertussis case definitions to include any cough for infants < 1 year of age. However, this case definition would still miss up to 60% of pertussis cases and PPV remains low. Our observations concurred with another study of hospitalised South African children aged <1 year with LRTI or neonatal sepsis, where only 20.5% (8/42) confirmed pertussis cases reported cough ≥ 14 days at enrollment and only 33.3% (14/42) had paroxysmal cough (18). Similarly, a study from Rome of 53 infants ≤90 days testing positive for pertussis reported that 30% (16/53) presented with paroxysmal cough, 8% (4/53) with whoop and 19% (10/53) with emesis (67). Apnoea was more common (57%, 30/53) (67). In our study, multivariate analysis identified whoop, paroxysmal cough, post-tussive emesis and cyanosis as significant predictors of pertussis in the <5 year age group; but not apnoea or chronic cough.

Patients infected with *B. pertussis* classically do not present with fever (16, 68). However, in our study, 45% of pertussis-positive children <5 years and 57% of ≥5 year old had fever, compared with 59% and 63% respectively in pertussis-negative patients. On multivariate analysis, fever was significantly negatively associated with

B. pertussis in the <5 year group but not in infants <6 months of age and adults ≥5 years. Reliance on maternal/self-reporting or co-infection with other viral/bacterial pathogens could increase incidence of fever, while in adults, high rates of HIV infection could potentially change disease presentation. The presentation of other common age-group specific causes of severe respiratory diseases could also impact, such as tuberculosis or RSV which is more common than pertussis in infants and also typically not associated with fever.
Case definitions commonly used for surveillance such as WHO SARI, which require fever or the GPI pertussis case definition that specify absence of fever could systematically exclude pertussis-infected patients in our setting. The PERCH study, which included a South African site, also noted a high prevalence of fever in pertussis cases 1-5 months of age; possible explanations included co-infecting pathogens and/or reliance on maternal reporting of fever (50). A study from Peru of children <5 years hospitalised with acute respiratory infection reported fever as a common symptom in patients with confirmed *B. pertussis* (69). Fourteen of the 114 patients that tested positive for *B. pertussis* in that study, were co-infected with RSV-A and one patient was co-infected with RSV-B. In contrast, in an Italian study of infants ≤90 days, fever was uncommon in pertussis-positive infants, 2% (1/53) (67).

Due to small case numbers, findings in the ≥5 year age group should be treated with reserve. However, points of interest included that cough was the commonest presenting feature but chronic cough was not significantly more common in pertussis patients. This may reflect high prevalence of tuberculosis in our setting, particularly among HIV-infected individuals. Fever was less common in pertussis-positive compared to pertussis-negative patients, however, 57% of pertussis-infected individuals had fever. Both whoop and paroxysmal cough had high specificity for pertussis but only 8% (3/40) of pertussis-positive patients presented with these symptoms. When controlling for age and race, whoop and HIV infection were significantly associated with a positive pertussis test on multivariate logistic regression. Low case numbers prevented/precluded an analysis restricted to HIV-infected patients, but this would be of interest in a future study.

Rather than relying on clinical suspicion of pertussis, the strength of this study was that patients were systematically enrolled based on broad case definitions not

restricted by typical symptoms of pertussis or fever (in children), making it less likely to miss cases. The limitations included restriction to hospitalised patients so results may not apply in an outpatient setting where patients present with milder/atypical disease. Only 54 cases of pertussis were identified in the ≥5 year age group and findings would need to be confirmed in a larger study and small numbers of HIV co-infected patients prevented a sub-analysis of this group in <5 year children.
The primary case definition for ≥5 year group included fever as a requirement which could have systematically excluded patients with *B. pertussis*. However, for 2015-2016 at two sites, the case definitions for pneumonia surveillance included patients with suspected TB.

Not every patient enrolled in the pneumonia surveillance was tested for *B. pertussis;* possible reasons include patients not consenting, being too sick or being discharged prior to specimen collection, prioritization of RSV and influenza testing over pertussis or specimens not reaching the laboratory. Although comparison of demographic features of patients tested and not tested for pertussis revealed some significant differences, these were unlikely to have systematically biased the results due to the small proportion not tested (5% and 2% in the <5 and ≥ 5 year age groups respectively). Furthermore, year and province were unlikely to have inherently affected test results. Different dates of initiation/termination or differential uptake of testing may have influenced year and province.

For this analysis, *IS481*-positive specimens with a Ct value of <45 cycles were considered a positive outcome for pertussis. However, the interpretation and clinical relevance of high Ct values (>35) is not clearly understood (22). It is possible that cases identified as PCR-positive included patients with residual pertussis DNA following disease or *B. pertussis* carriage, as well as active disease in patients presenting atypically. Lack of understanding, recognition or recall may result in misclassification of clinical symptoms. Furthermore, duration of symptoms/cough depends on how soon the patient seeks medical care, so may be influenced by health seeking practices, age group or severity of illness. Vaccination history was poorly collected in children and vaccination history and post-tussive emesis data were unavailable for adults. Evaluation of performance of pertussis case definitions was limited to the subset of cases for which all required variables were completed.

Chapter 5: Conclusions and Recommendations

The WHO have identified an urgent need to improve pertussis surveillance particularly in LMIC to assess burden of disease and impact of infant immunisation. Current WHO/CDC case definitions could miss 50 - 90% of pertussis cases underestimating disease burden, while WHO SARI case definition would systematically exclude afebrile patients. More sensitive case definitions and heightened awareness of atypical presentation are needed for diagnosis of pertussis in children. The amended WHO (2018) pertussis case definitions improved sensitivity while maintaining specificity in the <6 months age group.

In South Africa, pertussis surveillance has been successfully implemented for children using existing pneumonia surveillance platforms with broad case definitions that do not specify fever. However, only ~2.5% of patients enrolled were confirmed pertussis-positive on laboratory testing. The new RSV global surveillance programmes would potentially also provide opportunity for incorporation of pertussis surveillance providing critical information for health policy decisions.

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APPENDIX 1: Case definitions and Pneumonia surveillance, South Africa

Table A1: Pertussis case definitions: World Health Organization (WHO)(2003, 2018) (11,12) and Council of State and Territorial Epidemiologists (CSTE) /Centers for Disease Control and Prevention (CDC) (2014) (13,14) and WHO Severe Acute Respiratory Illness (SARI) case definition (2014)(60)

Organisation, Year	Clinical Criteria	Laboratory and Epidemiologic Criteria	Comment
Pertussis case definition: WHO 2003	A case diagnosed as pertussis by a physician or A person with a cough lasting at least 2 weeks with at least 1 of the following symptoms: -Paroxysms of coughing -Inspiratory whooping -Post-tussive vomiting without other apparent cause	Isolation of *B. pertussis* or Detection of genomic sequences by PCR or Positive paired serology	Case classification: *Clinically confirmed*: A case that meets the clinical case definition but is not laboratory-confirmed *Laboratory-confirmed*: A case that meets the clinical case definition and is laboratory-confirmed
Pertussis case definition: WHO 2018	A person of any age with a cough lasting ≥ 2 weeks or of any duration in an infant or any person in an outbreak setting, without a more likely diagnosis and with at least one of the following symptoms, based on observation or parental report: -paroxysms (fits) of coughing -inspiratory whooping -post-tussive vomiting, or vomiting without other apparent cause -apnea (only in <1year of age) OR -clinical suspicion of pertussis	Isolation of *B. pertussis* or Detection of genomic sequences by PCR assay, if PCR meets performance criteria outlined in WHO 2018 Surveillance Standards for vaccine preventable diseases (6) or elevated IgG antibodies to pertussis toxin in an individual ≥11 years of age, one year or longer after last vaccine dose. Culture and PCR detection of acute pertussis infection have high specificity and are preferred diagnostic methodologies over serology. Serology	Confirmed case: A confirmed case may be determined by laboratory-confirmation or epidemiological linkage. Laboratory-confirmation. A laboratory-confirmed case is a person who meets the suspected case definition with laboratory-confirmation Epidemiologically linked. An epidemiologically linked case is a person meeting the suspected case definition with close contact to a laboratory-confirmed case (or another epidemiologically linked case in an outbreak setting) in the three weeks prior to onset of cough. -Close contact is defined as having face–to-face exposure to a case, which includes household or family contact, people having stayed overnight in the same

	Note that pertussis in immunized or previously infected individuals can present without the classic signs of pertussis and therefore might not be captured by the above case definition	should be reserved for cases ≥ 4 weeks from cough onset; however, IgG can sometimes remain elevated for more than a year after infection or vaccination, leading to potential false positives	room with a case, and people having direct contact with respiratory, oral or nasal secretions with a laboratory-confirmed case. Possible A person who meets the suspected case definition but does not meet confirmed classification as defined above should be considered a possible case. This includes suspected cases who did not have laboratory testing done and those who tested negative
Pertussis case definition: CSTE2013/CDC 2014	In the absence of a more likely diagnosis, a cough lasting ≥2 weeks with at least 1 of the following signs or symptoms: -Paroxysms of coughing, OR -Inspiratory "whoop", OR -Post-tussive vomiting, OR -Apnea (with or without cyanosis (FOR INFANTS AGED<1 YEAR ONLY)	Isolation of *B. pertussis* from clinical specimen Positive PCR for *B. pertussis* Contact with a laboratory-confirmed case of pertussis*	Case classification: Probable: Meets the clinical case definition And Absence of laboratory-confirmation And No epidemiological linkage to a laboratory-confirmed case of pertussis, OR, FOR INFANTS < 1 YEAR ONLY: Acute cough illness of any duration with at least one of the following signs or symptoms: -Paroxysms of cough, OR -Inspiratory "whoop", OR -Post-tussive vomiting, OR -Apnea (with or without cyanosis) AND PCR positive for pertussis , OR, FOR INFANTS < 1 YEAR ONLY: Acute cough illness of any duration, with at least one of the following signs or symptoms: -Paroxysms of coughing, or -Inspiratory "whoop", or

			-Post-tussive vomiting, or -Apnea (with or without cyanosis) And Contact with a laboratory-confirmed case of pertussis **Confirmed:** Acute cough illness of any duration with isolation of *B. pertussis* from a clinical specimen , OR Meets the clinical case definition And is PCR positive for pertussis OR Meets the clinical case definition And had contact with a laboratory-confirmed case of pertussis Case classification comments: *Note: An illness meeting the clinical case definition should be classified as “probable” rather than “confirmed” if it occurs in a patient who has contact with an infant <1 year who is PCR positive for pertussis and has ≥ 1 sign or symptom and cough duration <14 days (classified as a “probable” case)
SARI case definition: WHO 2014	An acute respiratory infection with: History of fever or measured fever of ≥ 38ºC and cough; with onset in the last 10 days; and requires hospitalisation		

Figure A1: Clinical case definition of pertussis for surveillance purposes (Global Pertussis Initiative meeting 2011) (4)

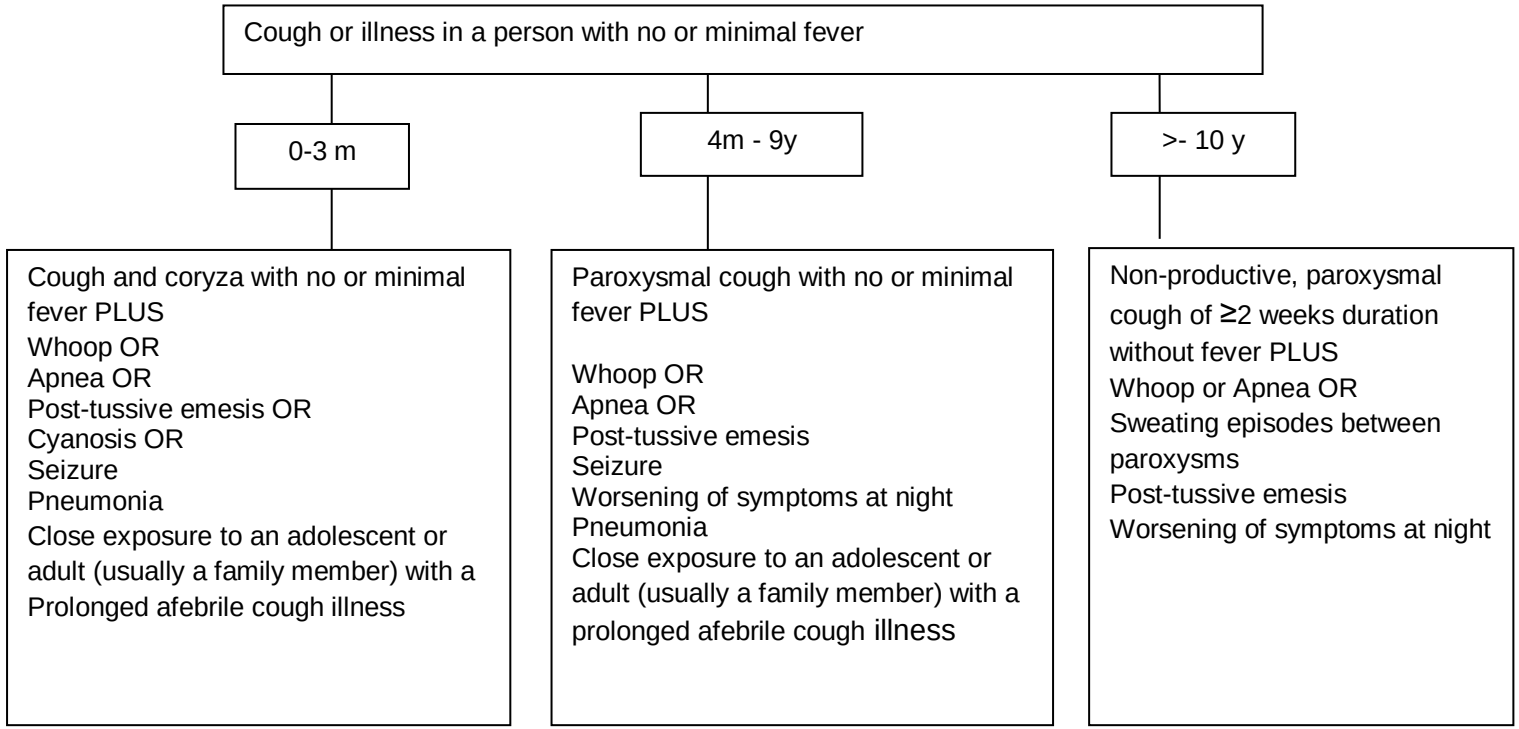

Table A2: Case definition for Notifiable Medical Condition (pertussis), South Africa, 2017.

Clinical case definition (suspected case)	**Probable case definition**	**Confirmed case definition**
Any person with an acute cough illness lasting ≥14 days (cough illness of any duration for children <1 year), without a more likely diagnosis AND one or more of the following signs or symptoms: paroxysms of coughing, or inspiratory "whoop", or post-tussive vomiting or apnoea in children <1 year; OR Any person in whom a clinician suspects pertussis.	Any person meeting the clinical case definition **AND** An epidemiologic linkage to a laboratory-confirmed case of pertussis in the 21 days before symptom onset.	Any person meeting the clinical case definition **AND** Isolation *of B. pertussis* from a clinical respiratory specimen OR polymerase chain reaction positive for pertussis OR specific antibody response (anti-pertussis toxin IgG response in older children and adults, and ≥ 1 year after last vaccine dose. Interpret with caution in younger children).

Table A3: Pneumonia surveillance case definitions January 2015 to December 2017 (South Africa)

Case definition – 2015-2017	Criteria
Severe respiratory illness (SRI)[a,b]	**2 days-<3months** Any child hospitalized with diagnosis of suspected sepsis, or physician diagnosed LRTI irrespective of signs and symptoms.
	3 months-<5 years Any child ≥3 months to <5 years hospitalized with physician-diagnosed LRTI including bronchiolitis, pneumonia, bronchitis and pleural effusion.
	≥5 years Any person hospitalized with an acute respiratory infection with fever (≥38ºC) or history of fever AND cough.
Suspected TB	Any patient with a clinical diagnosis of suspected pulmonary TB AND not meeting any of the above criteria (EDH, KTHC only)[c]
Suspected pertussis(only children ≤10years at RMMCH [d]	• Any infant ≤12 months with apnoea OR • Any patient ≤10 years not meeting above and presenting with cough and any of the following: Cough for ≥7days Paroxysms of coughing Inspiratory whoop Apnoea Cyanosis Pulse oximeter in room air ≤88% Gagging with coughing

EDH=Edendale Hospital, KTHC=Klerksdorp-Tshepong Hospital Complex, RMMCH= Rahima Moosa Mother and Child Hospital

[a] Severe respiratory illness includes both severe acute respiratory illness where patients present ≤10 days of onset and illness AND Severe chronic respiratory illness for children and adults meeting the same case definitions presenting with symptoms >10 days

[b] Mitchells Plain hospital was initiated in April 2016

[c] this case definition was excluded in 2017

[d]this case definition was excluded in December 2015

Table A4: Specimen type collected by site and year, transport medium and test conducted for *B. pertussis* in pneumonia surveillance, South Africa (2015-2017)

Pathogen	Year	Surveillance Site	Specimen collected	
B. pertussis	2015	Agincourt, RMMCH/HJH, RCH	NP* and OP* flocked swabs >5 years. NPA* ≤ 5 years	Multiplex real time PCR
		EDH, KTHC	NP and OP flocked swabs >5 years. NPA ≤5 years Induced/expectorated sputum	Multiplex real time PCR
	2016	Agincourt, RMMCH/HJH, RCH/MPH, EDH, KTHC	NP and OP flocked swabs ≥5 years. NPA <5 years until June 2016, changed to NPA <1year in September 2016 and NPS for≥1year	Multiplex real time PCR
Suspected TB patients only	2016	EDH, KTHC	Induced/expectorated sputum	
	2017	RMMC/HJH,RCH/MPH	NP and OP flocked swabs (all ages)	Multiplex real time PCR
	2017	EDH, KTHC,Agincourt	NP and OP flocked swabs ≥1year, NPA <1year Induced/expectorated sputum Plus NPS in Regan Lowe medium Induced/expectorated sputum	Multiplex real time PCR Culture
EDH=Edendale hospital, KTHC=Klerksdorp-Tshepong Hospital Complex, RMMC/HJH=Rahima Moosa Mother and Child hospital/Helen Joseph Hospital, RCH/MPH=Red Cross War MemorialChildren's Hospital/Mitchell's Plan Hospital NPA= nasopharyngeal aspirate, NPS=nasopharyngeal swab NP=nasopharyngeal OP=oropharyngeal *NP,OP,NPA,NPS in universal transport medium				

APPENDIX 2: University of the Witwatersrand. Human Research Ethics Committee (Medical) Ethics Clearance Certificate No. M170664

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Dr Linda Kathleen Erasmus

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M170664

NAME: (Principal Investigator)	Dr Linda Kathleen Erasmus
DEPARTMENT:	School of Public Health National Institute for Communicable Diseases
PROJECT TITLE:	Evaluation of Case Definitions to Detect Bordetella Pertussis Infections in Hospitalised Individuals in South Africa, 2015-2017
DATE CONSIDERED:	30/06/2017
DECISION:	Approved unconditionally
CONDITIONS:	Title change 30/11/2018
SUPERVISOR:	Prof Cheryl Cohen and Prof Anne Von Gottberg
APPROVED BY:	Dr C Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	03/07/2017

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 301, Third floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in June and will therefore be due in the month of June each year. Unreported changes to the application November invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature _____ Date _____

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES