PREVALENCE AND MOLECULAR EPIDEMIOLOGY OF *BORDETELLA PERTUSSIS* INFECTION IN SOUTH AFRICA

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702185

A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Doctor of Philosophy.

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Johannesburg, 2024

Student declaration

I, Fahima Moosa (student number: 702185), declare that the thesis is my own, unaided work (no plagiarism). It is being submitted for the Degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

The experimental work described in this thesis was conducted under the supervision of Dr Nicole Wolter, Dr Mignon du Plessis and Prof. Anne von Gottberg, in the Center for Respiratory Diseases and Meningitis at the National Institute for Communicable Diseases.

Specimens and data used in this thesis were from the prospective household observational cohort study of influenza, respiratory syncytial virus and other respiratory pathogens community burden and transmission dynamic study (PHIRST) as well as Pneumonia Surveillance. I (Fahima Moosa), have been involved in setting up laboratory processes for both surveillance platforms. Data arising from these studies (detailed in this thesis), which included laboratory testing, troubleshooting, retesting of specimens, data management (including results upload and data cleaning), data analysis (including statistical analysis), data interpretation and drafting/revising/finalizing of manuscripts were conducted by Fahima Moosa. Chapter 3 and Chapter 5 of this thesis have been published and all co-authors have agreed to the use of the results as part of this thesis.

28th March 2024, Johannesburg

"In the name of the Allah, the most Gracious, the most Merciful"

For my guardian angel

Rookmany Pillay

(1938 – 2001)

"I am because you were"

Presentations

Fahima Moosa. Incidence and transmission dynamics of *Bordetella pertussis* infection in a rural and urban community in South Africa, 2016 – 2018 (PHIRST): a community cohort study. **Oral presentation**. Wits Faculty of Health Sciences Biennial Research Day, University of Witwatersrand, Johannesburg: 15th September 2022.

Fahima Moosa. Incidence and transmission dynamics of *Bordetella pertussis* infection in a rural and urban community in South Africa, 2016 – 2018 (PHIRST): a community cohort study. **Oral presentation**. 13th International *Bordetella* Symposium. Vancouver, Canada: 26th – 30th June 2022.

Moosa F, du Plessis M, Tempia S, McMorrow M.L, Moyes J, Treurnicht FK, Carrim M, Hellferscee O, Mkhencele T, Mathunjwa A, Kleynhans J, Martinson N.A, Kahn K, Lebina L, Mothlaoleng K, Wafawanaka F, Gómez-Olivé F.X, Wagner R G, Mathee A, Piketh S, Cohen C, von Gottberg A, and Wolter N. Incidence and transmission dynamics of *Bordetella pertussis* in an urban and rural community in South Africa, PHIRST study, 2016 – 2018. **Poster presentation**. 12th International *Bordetella* Symposium. Brussels, Belgium: 09th – 12th April 2019.

List of publications

Moosa F, Kleynhans J, Makhathini L, du Plessis M, Tempia S, McMorrow M, et al. *Bordetella pertussis* antibody dynamics in household cohorts in two South African communities, 2016 – 2018. – *In preparation*.

Moosa F, du Plessis M, Weigand MR, Peng Y, Mogale D, de Gouveia L, et al. Genomic characterization of *Bordetella pertussis* in South Africa, 2015 – 2019. Microb Genomics. 2023 Dec 1;9(12). doi: 10.1099/mgen.0.001162.

Moosa F, Tempia S, Kleynhans J, McMorrow M, Moyes J, du Plessis M, et al. Incidence and Transmission Dynamics of *Bordetella pertussis* Infection in Rural and Urban Communities, South Africa, 2016 – 2018. Emerg Infect Dis. 2023 Feb 1. doi: 10.3201/eid2902.221125.

Abstract

Pertussis remains a public health concern in South Africa, with increases in cases and outbreaks in recent years. We determined the incidence, transmission dynamics, serological attack rates and molecular epidemiology of *B. pertussis* in South Africa.

Data from a longitudinal study enrolling individuals each year in 2016–2018 from two communities were used. Nasopharyngeal swabs were collected from participants twice-weekly and tested by real-time PCR. Serum was collected at 8 time points and tested using the anti-pertussis toxin IgG ELISA kit. Whole genome sequencing was performed on all available cultures (n=32) sourced from three additional surveillance programs between 2015–2019. Data were described and analyzed using univariate and multivariable regression models.

Among 1684 participants, the incidence of *B. pertussis* was 0.21 (95% confidence interval 0.17–0.25) per 100 person-weeks. The mean duration of infection was 12 days (±standard deviation 19.1). Transmission of infection was more likely to occur from male index cases [adjusted odd ratio 12.20 (95%CI 1.57–94.96)], and individuals with ≥7 day's infection duration [aOR 24.80 (95%CI 2.74–224.30)].

B. pertussis seroprevalence ranged from 1.8% to 5.2% across eight blood draws. The serological attack rate was 5.8% (87/1509), which was similar to the PCR attack rate (6.2%, 94/1509) (p=0.64). PCR-positive individuals aged 5–18 years (vs 19-44, aOR 6.8, 95% CI 1.3-35.1) and with episode duration of ≥7 days (vs <7 days, aOR 13.3, 95%

CI 3.4-51.1) were more likely to seroconvert. For all individuals that seroconverted, the ≥4-fold rise in anti-PT IgG titer was detected by the next blood draw (mean: 2.9 months (range 3 weeks – 5.9 months).

Using genome data, all isolates were identified as the globally-disseminated sequence type 2 and harbored the pertussis toxin promoter *ptxP3*. The dominant genotype was *ptxP*3-*ptxA*1-*ptxB2*-*prn*2-*fimH*2 (31/32, 96.9%), with no pertactin-deficient or other mutations in vaccine antigen genes identified.

Within the community, despite a high incidence of *B. pertussis*, there was an overall low seroprevalence. Our data highlighted that increases in cases in South Africa are not likely due to evolutionary changes in the genome but potentially waning immunity due to the use of acellular vaccines and/or population immunity gaps.

Acknowledgements

Academic acknowledgements

Dr Nicole Wolter, Dr Mignon du Plessis and Prof. Anne von Gottberg – It has been five years of standing on the shoulders of giants. Thank you for sharing with me your wealth of knowledge, providing me with invaluable opportunities and for your unwavering guidance and support. It has been a pleasure and privilege to work with you.

Prof Anne von Gottberg and Prof Lee Harrison – It has been an honor and privilege to be chosen by you to be part of the SAPPHGenE mentorship program. Thank you for allowing me this opportunity.

My SAPPHGenE mentors – Dr Lucia Tondella and Dr Michael Weigand – Thank you for mentoring me. The wealth of knowledge that you have imparted me with during this journey is truly invaluable.

Dr Cheryl Cohen, Dr Stefano Tempia, Dr Sibongile Walaza, Dr Jackie Kleynhans and the rest of the Epidemiology team – Thank you for the additional supervision and for guiding me though my statistical analyses.

Linda de Gouveia and Dineo Mogale – Thank you for sharing your time and expertise with me and for helping me grow the *B. pertussis* cultures. The genomics aspect of this work would have been impossible without your contributions.

My colleagues at CRDM – Thank you for all your contributions and words of encouragement during this period.

Dr Marta Nunes and Dr Shabir Madhi – Thank you for providing me with the additional *B. pertussis* strains that allowed me to strengthen the genomics aspect of this PhD.

Dr Lucia Tondella, Dr Michael Weigand, Dr Yanhui Scott, Dr Margaret Williams, Dr Lucia Pawloski, Pam Cassidy, Marissa Fraire, Janessa Aneke and the rest of the Pertussis and Diphtheria lab team at CDC Atlanta **–** The time spent with each of you during my visit to the lab in March 2020 (start of the COVID-19 pandemic) is invaluable to me both professionally and personally.

Personal acknowledgements

Rookmany Pillay – I have finally made your dream come true. I hope you are smiling down at me from heaven. I will miss you forever.

Mobeen Emmamally – My darling husband – no words can express how much I love and appreciate you. Your unwavering presence, love, guidance and constant words of encouragement is what brought me through this degree. Thank you for always pushing me to be the best version of myself.

Mohamed Ziyaad Emmamally – My sweet boy – mummy did this for you.

My parents (Joomun Moosa, Naseema Moosa and Ayesha Emmamally) – Thank you for being the guiding lights in my life. Your guidance, love and support has allowed me to always soar to great heights and achieve more than I ever dreamed of.

Family and friends – Thank you to each of you for your constant love and support.

Research funding

Thank you to the following entities for funding this project:

Sanofi Pasteur – cooperative agreement number PER00059.

National Institute for Communicable Diseases of the National Health Laboratory Service and the US Centers for Disease Control and Prevention – cooperative agreement number 5U51IP000155.

Fogarty International Center Global Infectious Disease research training grant, National Institutes of Health, to the University of Pittsburgh and National Institute for Communicable Diseases – cooperative agreement number D43TW011255.

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List of Abbreviations

%	–	percentage
>	–	greater than
<	–	less than
≤	–	less than or equal to
≥	–	greater than or equal to
±	–	plus/minus
°C	–	degrees Celsius
µl	–	microliter
aHR	–	adjusted hazard ratio
ACT	–	adenylate cyclase toxin
aIRR	–	adjusted incidence rate ratio
aOR	–	adjusted odds ratio
BMI	–	body mass index
bp	–	base pair
BLASTn	–	basic local alignment search
CI	–	confidence interval
C_t	–	cycle threshold
CDC	–	Centers for Disease Control and Prevention
CHBAH	–	Chris Hani Baragwanath Academic Hospital
DFA	–	direct fluorescent antibody
DNA	–	deoxyribonucleic acid
DTaP	–	diphtheria tetanus acellular pertussis-containing vaccine
e.g.	–	example

ELISA	–	enzyme-linked immunosorbent assay
FIM	–	fimbriae
HR	–	hazard ratio
HIV	–	human immunodeficiency virus
HCIR	–	household community infection risk
HREC	–	Human Research Ethics Committee
i.e.	–	that is
IRR	–	incidence rate ratio
IgG	–	immunoglobulin G
kb	–	kilo base
LCB	–	localized co-linear block
LMIC	–	low-to-middle-income countries
LRTI	–	lower respiratory tract infection
MTM	–	molecular transport medium
MB	–	mega base
MP96	–	MagNA Pure 96
MLST	–	multilocus sequence typing
MALDI-TOF	–	matrix-assisted laser desorption/ionization time of flight mass spectrometry
n	–	number
N	–	total number
NA SV	–	nucleic acid small volume
NCBI	–	National Center for Biotechnology Information
NHLS	–	National Health Laboratory Service

NMC	–	notifiable medical condition
NICD	–	National Institute for Communicable Diseases
OR	–	odds ratio
PCR	–	polymerase chain reaction
PT	–	pertussis toxin
ptxP	–	pertussis toxin promotor
PHIRST	–	prospective household observational cohort study of influenza, respiratory syncytial virus and other respiratory pathogens community burden and transmission dynamic study
PRN	–	pertactin
RSV	–	respiratory syncytial virus
rpm	–	revolutions per minute
RTHC	–	road-to-health card
SD	–	standard deviation
Spp.	–	species
SNP	–	single nucleotide polymorphism
SRI	–	severe respiratory illness
ST	–	sequence type
TCT	–	tracheal cytotoxin
VE	–	vaccine effectiveness
WHO	–	World Health Organization
Wits	–	University of the Witwatersrand
WGS	–	whole genome sequencing

Chapter One – Literature review

1. Introduction

1.1. The microorganism – *Bordetella pertussis*

Pertussis, caused by the bacterium *Bordetella pertussis*, is a vaccine-preventable respiratory disease affecting persons of all ages (1). The organism belongs to the genus *Bordetellae* and is one of eight other *Bordetella* species i.e. *B. parapertussis*, *B. bronchiseptica*, *B. holmesii*, *B. avium*, *B. trematum*, *B. hinzii*, *B. petrii* and *B. ansorpii* (2). *B. pertussis*, *B. parapertussis*, *B. bronchiseptica* and *B. holmesii* are known causative agents of disease in humans.

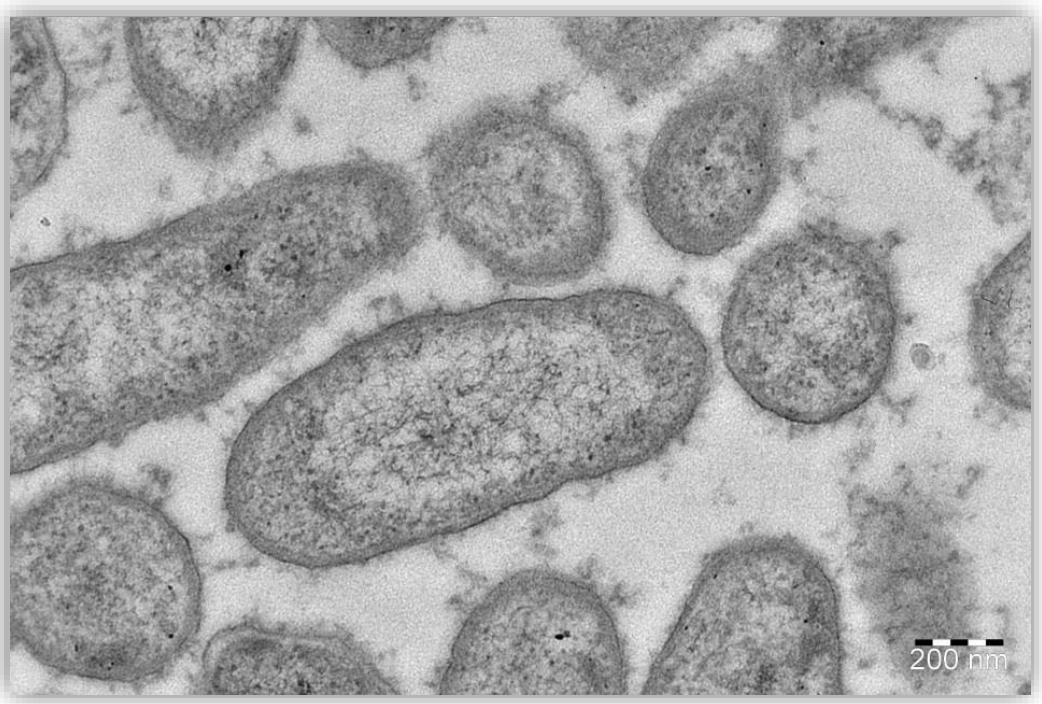


Figure 1.1: Electron-micrograph showing the coco-bacillus structure of *Bordetella pertussis* ATCC 9797 (image courtesy of Monica Birkhead, Electron Microscopy Unit, NICD).

The bacterium caused its first known outbreak of pertussis in 1578, and in 1679 the disease was named whooping cough (2). *B. pertussis* was discovered by Jules Bordet and Octave Gengou in 1900 after examining sputum from a 6-month-old baby diagnosed with whooping cough. The microorganism is a Gram-negative coccobacillus that is oxidase and catalase positive (2,3). *B. pertussis* is aerobic and requires optimum temperatures between 35 – 37°C for growth on specialized culture media such as Bordet-Gengou and Regan-Lowe charcoal medium, where colonies appear as mercury grey-like droplets (Figure 1.2).

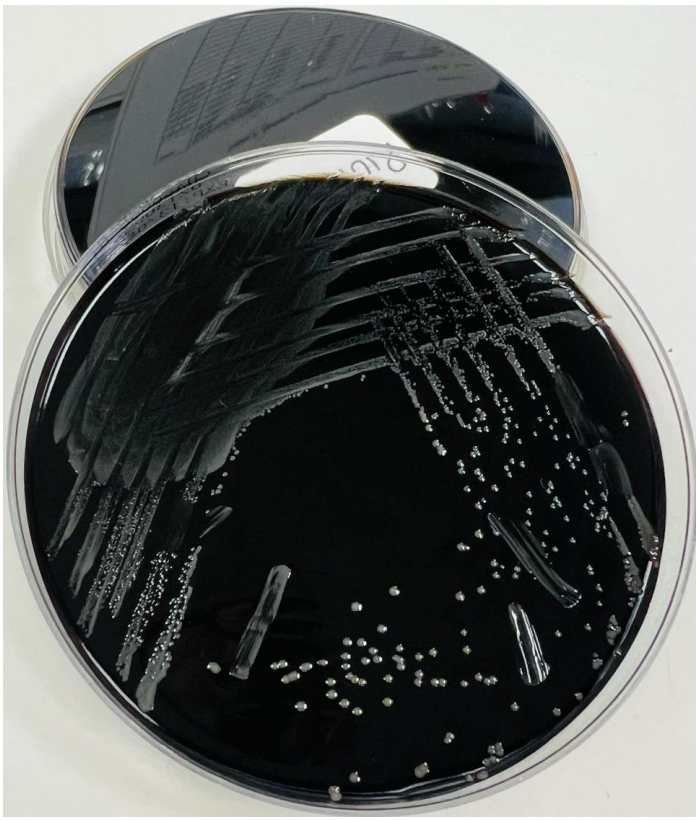


Figure 1.2: *Bordetella pertussis* ATCC 9797 grown on charcoal agar for *Bordetella*, highlighting the mercury grey colonies (image courtesy of Dineo Mogale, Centre for Respiratory Diseases and Meningitis, NICD).

1.2. Clinical manifestation of disease

Pathogen transmission occurs from person-to-person via respiratory droplets from an infected person (4). *B. pertussis* infects the ciliated epithelial cells of the human upper respiratory tract (1). Children are at greatest risk for severe disease with increased morbidity and mortality observed in infants too young to be vaccinated. Adolescents and adults may have asymptomatic infection and are known to be a common source of transmission of *B. pertussis* to infants too young to be fully vaccinated (2,5,6). The disease is toxin mediated and the organism expresses a number of toxins which aid in attachment to the epithelial cells (filamentous hemagglutinin (FHA), pertactin (PRN) and fimbriae (fim)), evade the host immune system and facilitate destruction of epithelial cells (pertussis toxin (PT), tracheal cytotoxin (TCT) and adenylate cyclase toxin (ACT)) (5). Disease is broken up into four stages; the initial catarrhal phase that is characterized by cold-like symptoms and occasional cough. This is followed by the paroxysmal phase where patients have the typical pertussis symptoms including whooping cough, paroxysms and post-tussive vomiting. Patients are most infectious during the catarrhal and paroxysmal phases of disease. The last phase of disease is the convalescent phase where patients are left with a long lasting cough which is not often diagnosed.

1.3. Laboratory diagnosis

Together with clinical presentation, culture, direct fluorescent antibody (DFA), serology and real-time PCR are used for *B. pertussis* diagnostics (2). Culture of respiratory specimens, the gold standard, is specific and recommended during the initial catarrhal stage of illness. However, *B. pertussis* is a fastidious organism and requires up to 10 days of incubation, making culture challenging. DFA offers a more rapid diagnosis and

can be used as a screening method (confirmed by another diagnostic test); however, DFA requires specialized trained staff and is prone to false positives (2,5).

Serology is used for detecting antibodies against *B. pertussis* in the convalescent phase of infection and is recommended as a diagnostic method when older individuals present with prolonged, undiagnosed atypical symptoms e.g. prolonged cough (1,6). However, a limitation of serology is that it sometimes requires paired sera for diagnosis and the data generated could be indicative of either infection or recent vaccination, which makes diagnosis time consuming and inaccurate (unable to differentiate between natural infection and vaccination). However, the European Union (EU) Perstrain has suggested that the use of serology in older children/adults and other household members who have coughed for at least 2 weeks should be sufficient for diagnosis (8). Serology is also recommended during outbreaks to determine the true burden of infection amongst the population (9).

Due to the limitations associated with culture, DFA and serology, real-time PCR is the recommended laboratory method (7,10–12). PCR is recommended during the first three phases of pertussis infection. However, this methodology is hampered by the lack of availability of validated and organism-specific gene targets. The more common target used is the insertion sequence IS*481* which is present in multiple copies (between 50-238) in the *B. pertussis* genome. However, IS*481* is not species specific making diagnosis non-specific (4). A qualitative assessment of pertussis diagnostics in the USA revealed that 5% of laboratories reported incorrect results using IS*481* detection only, without a secondary PCR target for confirmation (13). A European proficiency testing

study found that all laboratories that used IS*481* for diagnosis reported specimens positive for *B. bronchiseptica* and *B. holmesii* as *B. pertussis* (14). Pseudo-outbreaks linked to patient clinic surfaces contaminated by *IS481*, resulting in contamination of clinical specimens, have been reported in the United States (15). These limitations can be overcome by incorporating an additional PCR target such as the pertussis toxin subunit gene (*ptxS1)* which is present as a single copy in *B. pertussis*. It is also important to have sufficient and stringent laboratory control measures in place so that contamination can be minimized and easily detected (12).

1.4. Disease intervention

The whole-cell pertussis vaccine was introduced in the 1940's (1–3). It was later found to be reactogenic and associated with rare adverse side effects including chronic neurologic damage, sudden infant death syndrome, infantile spasms and hypsarrhythmia (3,16). The acellular pertussis vaccine was developed and introduced in the 1980's (1). However, due to the increased cost associated with this vaccine, not all countries have switched from the whole-cell vaccine. Acellular vaccines are composed of up to four *B. pertussis* purified protein antigens (fim, PRN, FHA, and PT) in various combinations and concentrations (2). The whole-cell pertussis vaccine was introduced in South Africa in 1950 and was replaced by the pentavalent diphtheria, tetanus, acellular pertussis, inactivated polio and *Haemophilus influenzae* type b (DTaP-IPV/Hib) vaccine in April 2009. The pentavalent vaccine is given to infants as part of the routine immunization program [Pentaxim (2009 – 2014) replaced by Hexaxim (2015 to date), Sanofi Pasteur, Lyon, France] at 6, 10 and 14 weeks of age with a booster dose at 18 months of life (April 2009 – December 2023) (17). Both Pentaxim and Hexaxim contain the acellular components PT and FHA antigens and do not contain

PRN. In 2019, according to World Health Organization (WHO)/UNICEF, the coverage for the first and third dose of the DTaP vaccine in South Africa was 84% and 77%, respectively, which is less than the 95% coverage that is recommended by the WHO (18).

Data have shown that the use of either the whole-cell or acellular vaccines have equivalent initial effectiveness in the first year of life (1). A study using baboons to model the effects of the acellular and whole cell vaccines showed that the use of acellular vaccines protects from severe disease, however, the effect on infection and transmission were limited (19). Data showed that whole cell vaccines were more effective at protecting against infection and transmission.

Following the routine use of whole-cell vaccines in the 1950's, there was a steady decline in the number of pertussis cases and deaths globally (1,2). More recently, despite widespread use of vaccines globally, trends have shown a resurgence of infection in various regions, including amongst well-vaccinated populations (1). Disease resurgence has been attributed to factors such as pathogen adaptation, waning immunity as a result of the switch to the acellular vaccine (20,21), more sensitive serological markers for identification of infection in adolescents and adults who are commonly asymptomatic carriers of *B. pertussis* or present with atypical symptoms (22), improved awareness by clinicians (due to resurgence and outbreaks of infection) and improved diagnostic techniques (23,24). The burden/resurgence of disease is severe in low- and middle-income countries (LMICs), especially on the African continent amongst young infants (25). This is potentially due to inadequate pertussis surveillance, little to no

awareness of disease by healthcare workers, lack of diagnostic resources, and competing health priorities.

These data highlight that the current methods of control are ineffective and require revision. Additional strategies recommended by the WHO to help control pertussis and prevent infant mortality are as follows: booster doses in adolescents and adults, vaccination of infants, maternal immunization; cocooning (vaccination of infants mother and other household members) and vaccination of healthcare workers (1). During a meeting in 2016, the Global Pertussis Initiative committee recommended the following to control the spread of pertussis on the African continent: continue to use whole cell vaccines (if already part of infant immunization); to improve primary and preschool booster vaccination coverage; maternal immunization in countries with available resources already using the acellular vaccines; followed by, in order of priority, booster doses in older children, adolescents, healthcare workers and adults (25). They also recommended that pertussis surveillance be improved in Africa and other LMIC's so that the data can be used to inform policy.

Maternal immunization has been reported to be the most cost-effective strategy to protect infants too young to be vaccinated (1,26). Maternal immunization was introduced in the USA in 2011, and analysis of the data post-vaccine implementation showed a sustained decline (statistically significant) in pertussis incidence in the targeted infants <2 months when compared to the control 6 – 11-month age group. (27). In 2012, England introduced maternal immunization in the third trimester of pregnancy, following an increase in pertussis hospitalizations and deaths in infants (28). Vaccine

effectiveness (VE) was >90% for infants aged <2 months born to women who had received the vaccine at least 1 week prior to delivery. In 2015, England experienced another peak of pertussis, however, the incidence of disease in infants during this peak was lower when compared to incidence of disease in infants during the 2012 peak (prior to maternal vaccination implementation). The case fatality ratio in infants (born to mothers who were immunized) was also lower compared to the case fatality ratio in infants during the peak of infection in 2012. In 2016, England extended maternal immunization to the second trimester of pregnancy, resulting in an increase in vaccine coverage (29). Cases and hospitalizations stabilized in infants but remained higher in older age groups, and there were no deaths in infants born to mothers who were vaccinated. In 2015, Australia implemented maternal vaccination in women between 28 – 35 weeks of gestation (30). Data from a case control study showed that VE (protecting against infection) was 69% for infants <3 months of age and 94% effective at preventing hospitalizations.

1.5. Epidemiology of pertussis

Pertussis is endemic in most countries with peaks of infection occurring every 2 – 5 years (2,3). Studies have shown that pertussis prevalence/incidence varies by country; as sample populations, diagnostic tests, sample types and vaccination type/status vary between countries and studies. A population-based study in Canada, using data from the Canadian Notifiable Disease Surveillance System between 2005 and 2019, found an average annual incidence of 6.4 per 100 000 population amongst all ages (31). The annual incidence and hospitalization rate was highest in infants <1 year of age. A study in the USA from 2006 to 2015 to determine the epidemiology and burden of *B. pertussis* amongst individuals aged 0 – 64 years found a *B. pertussis* incidence of 15.5 per 100

000 person-years (32). A prospective study conducted in China between 2016 – 2017 to determine the prevalence of *B. pertussis* amongst children aged 1 to 11 years with different cough durations, found 31% of children with laboratory-confirmed *B. pertussis* (33).

In 2013, the WHO estimated that *B. pertussis* was still responsible for 63 000 deaths in children <5 years despite global vaccine coverage of 86% for the third dose of any pertussis-containing vaccine (1). Using data from 2014, the WHO developed a mathematical model to determine the global pertussis estimates and related deaths in children <5 years, and the data generated highlighted an estimated 24.1 million cases and 160 700 deaths from pertussis in children <5 years. (34) The African region contributed the largest proportion of cases and deaths (7.8 million (33%) cases and 92 500 (58%) deaths). A total of 5.1 million (21%) pertussis cases and 85 900 (53%) deaths were in infants younger than 1 year. A USA based study from 2007 – 2019 using mathematical modelling found an annual incidence of diagnosed pertussis of 1 – 5 per 100 000 population (correlated with other data reported in the USA within this time period) (35). Amongst individuals with undiagnosed respiratory illness, an additional 1,053,946 likely pertussis cases were identified by the algorithm which increased the annual pertussis incidence 110-fold (34 – 474 per 100,000 annually).

In Africa, the burden of disease and deaths attributed to *B. pertussis* suggest that pertussis remains a significant cause of morbidity and mortality, especially among infants and young children (36–38). A cross-sectional study conducted in Ethiopia from 2018 – 2019, to determine the epidemiology of pertussis amongst individuals with

clinically compatible illness, found an overall prevalence of 30% amongst individuals of all ages (39). Children aged 6 – 9 years were three times more likely to have *B. pertussis* infection compared to those ≥14 years. A seroprevalence study in Tunisia between 2007 – 2016 amongst children hospitalized with clinical suspicion of pertussis, found a detection rate of 86% (40). Two peaks of infection were observed in 2009 and 2014 and the highest proportion of cases were detected in infants <2 months of age. In Niger, the incidence of *B. pertussis* was 8.2 cases per 1000 population in 2011 amongst children hospitalized with respiratory illness (41). These data highlight ongoing transmission of *B. pertussis* in African communities, which is of importance as these populations may have limited access to vaccination and healthcare services (25,42). A systematic review of the burden of *B. pertussis* in low-to-middle income countries highlighted the increased burden of disease in adolescents compared to other age groups which mimics trends in high-income countries (42).

1.6. Epidemiology of *B. pertussis* of South Africa

In South Africa, pertussis is a category one notifiable medical condition (NMC) which requires notification (for action) to the NMC surveillance system within 24 hours of case diagnosis (43). Apart from the NMC system there was no other surveillance of *B. pertussis* in South Africa (44). In 2012 laboratory testing for *B. pertussis* was initiated in the sentinel, syndromic Pneumonia Surveillance program and the influenza-like illness surveillance program (ILI) (conducted at sentinel sites in five provinces in South Africa). The case definition was expanded to identify individuals with clinical signs and symptoms of pertussis infection (12). Pneumonia Surveillance was initiated in 2009 and is on-going, prospective, active surveillance that includes patients of all ages hospitalized with lower respiratory tract infection irrespective of symptom duration, to

determine the etiology of community-acquired pneumonia with specific focus on *Streptococcus pneumoniae*, influenza virus and respiratory syncytial virus (RSV) (45). ILI surveillance enrolls individuals with milder respiratory illness seeking care at clinics attached to the hospitals enrolling Pneumonia Surveillance cases. Although NMC and the Pneumonia/ILI are strong surveillance systems in the country, both platforms have limitations; in that NMC relies on reporting of diagnosed cases to the system and the Pneumonia/ILI is only conducted at a five surveillance sites which is not representative of South Africa.

The first documented account of *B. pertussis* in South Africa, following the introduction of the whole-cell vaccine in 1950, was a respiratory illness outbreak in the Western Cape amongst children <5 years of age between 1988 – 1989 (46). A total of 239 children were hospitalized with respiratory symptoms and the *B. pertussis* attack rate was 33% despite a vaccine coverage of 95%. Since this outbreak there has been an increasing number of studies/data that has been published describing the burden of disease in various provinces in South Africa (Table 1.1). Most studies use either PCR or a combination of culture and PCR to diagnose infection (17,47,48). Pertussis is endemic in South Africa, with increases in detection rates observed every 3 – 5 years (12,48,49), in 2015, 2018 and 2022 (12,48–51). Our data show that the *B. pertussis* detection rate amongst symptomatic individuals ranged from 1.1% to 14% in different provinces in South Africa (12,17,52–54). In most studies the highest number of cases were in infants <3 months. Infants <1 year of age were at highest risk of severe disease and mortality (17,48,52–55). Data from healthy individuals show a high burden of infection in the adolescent and adult population which follows global trends, highlighting these age

groups as an important source of transmission of *B. pertussis* to infants too young to be vaccinated (49,52).

Table 1.1: Summary of available *B. pertussis* epidemiological data in South Africa.

Location	Time period	Age	Case definition	Detection/Incidence rate	Reference
Bloemfontein	2008 – 2015	Children	Clinical pertussis symptoms	14% (57% of cases in infants <18 weeks)	(52)
Western Cape	2008 – 2011	Adolescents 15 – 18 years	Healthy individuals	74% seropositive to anti-PT IgG	(44)
Soweto (PERCH)	2011 – 2014	Children <5 years	WHO-defined severe pneumonia	2.5% 4% in infants 1 – 5 months	(53)
Soweto (Mat Flu trial)	2011	Infants <6 months	Respiratory illness	5.8 /1000 child-months	(56)
Cape Town	2012 – 2013	Children <13 years	Hospitalized with LRTI	7% 15% in infants <2 months	(54)
Sentinel sites in 5 provinces (Pneumonia Surveillance)	2012 – 2016	All ages	Inpatient and outpatient respiratory illness	1.7% – inpatients 1.1% – outpatients	(12)

Sentinel sites in 5 provinces (Pneumonia Surveillance)	2013 – 2018	All ages	Hospitalized with respiratory illness	17/100 000 population (3.3% of cases in infants <2 months)	(48)
Soweto	2015	Infants <1 year	Hospitalized with respiratory illness	2.3% (86% of cases in infants <3 months)	(17)
North West & Mpumalanga (PHIRST Study)	2016 – 2018	All ages	Healthy individuals	0.21/100 person-weeks	(49)
National (NMC)	2018	All ages	Suspected/probable/confirmed cases	800 cases (end November) (63% in infants <1 year)	(55)
National (NMC)	2022	All ages	Suspected/probable/confirmed cases	Increase from 2021 (N=21) to 147 cases (end September 2022) (79% in infants <3 months)	(50,51)

1.7. *B. pertussis* immunity

There are no established immunological correlates of protection for *B. pertussis*, although the presence of elevated antibodies to PT is believed to play a role in protection against severe disease in infants (1,3,19). Estimates of the duration of protection conferred by whole cell vaccines range between 4 – 14 years whilst protection conferred from acellular vaccines ranges between 5 – 6 years (57). A study using animal models found that vaccination with the whole cell vaccine confers longer lasting immunity similar to natural infection due to the induction of the TH1 and TH17 immune response (19). However, vaccination with the acellular vaccine induces higher TH2 and lower TH1/TH17 immune response which potentially is the reason for immunity waning quicker with this vaccine.

Serology is a useful tool to determine antibody kinetics over time and duration of immunity following natural infection or vaccination, although such studies are limited (3). Following natural infection, anti-PT, PRN, FHA, fim and whole organism antibodies are raised, and increases to anti-PT IgG and anti-FHA IgG are measurable in over 90% of infections (15). The most common serological method used is the enzyme-linked immunosorbent assay (ELISA) using purified PT antigen (most specific antigen for ELISA) and/or FHA, and to a lesser extent, PRN, fim and ACT (8). However, PRN and FHA cross-react with *B. parapertussis* and *B. bronchiseptica*, whilst cross reactions with other bacterial species (non-encapsulated *Haemophilus influenzae*, *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*) can occur with FHA (3). Measuring IgA or IgG to PT is the most specific serological test as PT is unique to *B. pertussis* and decays to below cut-off levels between 4.5 months to a year post-infection (3,58).

PT is present in most acellular vaccines and it is therefore difficult to determine if elevated antibody titers are due to natural infection or prior vaccination (1,3). In addition, the ELISA methodology has several drawbacks, namely, commercial ELISA's are of different antigen compositions and quality, highly purified reference antigens are not readily available, and the lack of standardized antibody level cut-offs, which makes it difficult to compare data between studies (9).

Since there are no immunological correlates of protection for *B. pertussis,* it is important to measure antibody kinetics over time in relation to natural infection or vaccination, to try and better understand *B. pertussis* immunity. In Denmark, between 2006 – 2009, amongst children and adults (either with natural infection or booster vaccination), the median half-life for post-infection antibodies was 221 days and the median half-life for post-vaccination antibodies was 508 days (59). IgG anti-PT antibodies post-infection and post-vaccination decayed in a bi-phasic manner and post-infection antibodies decayed almost two times faster when compared to the decay of antibodies post vaccination. A Swedish study, using sera from young children with laboratory-confirmed pertussis, enrolled into an acellular pertussis vaccine trial found that anti-PT IgG antibodies amongst infected children started to wane 4.5 months after the peak of antibodies were detected (60). Amongst infected children vaccinated with the acellular vaccine, the level of antibodies 12 month's post infection waned significantly faster when compared to the antibody levels of infected children vaccinated with the whole-cell vaccine. A USA based study to determine antibody kinetics from blood samples collected from healthcare practitioners that received a booster of the acellular pertussis vaccine found that once vaccinated, individual

antibody levels waned to below diagnostic cutoff levels (<95 IU/ml) by day 75 (61). A prospective study conducted in Estonia between 2012 – 2014, to determine the dynamics of anti-PT IgG in children and adults with symptomatic pertussis (up to 3 year's post-infection), found that it took a longer time for antibodies to peak in adults when compared to children (397 vs. 292 days); however, the time taken for anti-PT IgG to decay to half-life was shorter in adults when compared to children (62). A mathematical modeling study conducted in the Netherlands (2008 – 2012) to determine the impact of age on long-term serological responses amongst individuals with previous infection found that anti-PT IgG waned fast in all age groups, whilst IgA to PT, FHA, PRN and fim decreased quickly in the young age group but remained high in older individuals, indicating that age plays a role in waning of antibodies (63).

1.8. *B. pertussis* genomics

One of the factors thought to be contributing to the global rise in *B. pertussis* is pathogen adaption, which has increased (predominantly within the genes encoding vaccine antigens) since the introduction of acellular vaccines due to selection pressure from vaccine-induced immunity (64,65). This has resulted in the circulation of strains that have diverged from strains used in the vaccine (66–69). Changes in the *B. pertussis* toxin promoter (*ptxP*) region have been observed, with the majority of isolates now harboring the novel *ptxP*3 allele which replaced the *ptxP*1 allele (65,70). Strains harboring the *ptxP*3 first emerged in the 1980's, following acellular vaccine introduction, and have been shown to result in increased production of pertussis toxin *in vitro* (71). In Europe, from 1998 to 2009, there was an increase in the prevalence of isolates containing *ptxP3* (70). Strains not expressing the vaccine antigens PRN, and FHA have been described in the USA following the switch to acellular vaccines in 1991

(67,72,73). PRN-deficient strains identified in France were shown to be as virulent as the PRN-expressing strains (74). During 2008 – 2012 there was a large outbreak of *B. pertussis* in Australia and genomic analysis of circulating strains revealed that 30% of isolates were PRN deficient with the first deficient isolates emerging in 2008 (66). Circulating strains in the United Kingdom between 2008 – 2012 harbored *ptxP*3 following the switch to the acellular vaccine in early 2000's, whilst strains from earlier years (1920 – 1982) harbored *ptxP*1 (75). Strains lacking vaccine antigens such as PRN now make vaccines containing PRN less useful (76).

B. pertussis was regarded as monomorphic due to lack of observable diversity using available molecular methods and the lack of geographic clustering of circulating isolates (65,77,78). Traditional typing methods (using data from serotyping, real-time PCR or short read sequencing) such as multilocus sequence typing (MLST) of seven housekeeping genes (*adk*, *fumC*, *glyA*, t*ryB*, *icd*, *pepA* and *pgm*) or typing of vaccine antigens (PRN, PT, FHA and fim) provides little discriminatory power as they only consider a small subset of genes within the *B. pertussis* genome, therefore, only resolving isolates into a few types (66,79). An example of the monomorphic nature of *B. pertussis* is the MLST sequence type (ST) 2 that has been dominant globally since the late 1990s (80). Typing of vaccine antigens also provides limited information except when isolates carry mutations within vaccine antigens or completely lack the genes to express the antigens. New Zealand isolates collected between 1982 – 2018 were represented by 2 distinct genotypes, namely, *ptxP1-ptxA1-prn1-fim2-1-fim3-1* (isolates collected prior to 1990) and *ptxP3-ptxA1-prn2-fim2-1-fim3-1* (isolates collected following 1990) (81). A study conducted in the UK, during the 2012 pertussis outbreak

showed that strains circulating in the UK during the outbreak were *ptxP3-ptxA1-ptxB2-prn2-fim3-2* (75).

Traditional typing methods show minimal resolution amongst *B. pertussis* strains. More recently, the use of whole genome sequencing (WGS) has offered more scope to study the evolution and transmission of *B. pertussis* (65). A comparative whole genome analysis of 343 global *B. pertussis* strains collected between 1920 and 2010 showed two different lineages from which the currently circulating strains have evolved and that the highest single nucleotide polymorphism (SNP) density was observed in virulence associated genes and genes associated with transportation/binding functions (66). A study in the UK analyzing isolates from a pertussis outbreak between 2008 – 2012 showed that there was an unusually high SNP density in the genes that encode vaccine antigens (PT, FHA and PRN) when compared to other genes (75). Studies in the USA using WGS data detected novel mutations amongst circulating strains within vaccine antigens PRN and FHA which led to deficiency of the genes encoding these antigens (73,82). The data highlighted that mutations within the *fhaB* gene and in other genes affected the production of FHA. Similarly, using WGS data, additional typing methods have been developed that include parts and/or the entire genome, such as whole genome MLST and core genome MLST have added to the currently available techniques that can be used to classify circulating strains with more resolution (66,79,83). The use of core genome MLST shows promise as a method to study the evolution and transmission of *B. pertussis* with special focus on strains that lack the expression of the key vaccine antigens (83).

The combination of long- and short-read WGS technologies have enabled a better understanding of genomic structural diversity and genome arrangement patterns amongst circulating strains (64,69,81). Studies have shown marked differences amongst *B. pertussis* genome structures despite having an otherwise monomorphic profile (based on other genome characterization methods). Using this methodology, unique genomic structures have been described in the USA (69), New Zealand (81) and India (84). During two state-wide epidemics in the USA in 2010 – 2012, whole genome data were useful in identifying 16 distinct genome structures from 31 isolates that were otherwise monomorphic based on other typing techniques, and all differed from the vaccine strains (67). Data from USA provided additional evidence of diversity amongst genome structures by showing the expansion of 'cluster 01 (same genome structure)' within the USA by analyzing data from pre 2000 to 2016 (85). Similarly, in New Zealand, WGS data showed 19 different genome rearrangements amongst 66 isolates (81). An integrated comparative genomics analysis using isolates collected from Czech Republic between 2008 – 2012 showed that circulating *B. pertussis* strains had substantial variation in genome organization and formed separate phylogenetic clusters based on time of collection (historic isolates vs. currently circulating isolates) (86). When using proteomics, they found that genome rearrangements resulted in changes in gene order, orientation or in large deletions, which affected transcriptomic profiles in *B. pertussis*. Therefore, genome structures/rearrangements could be useful signatures of genome evolution. However, there is little data available that describes the importance of structural variation and more studies are required to fully understand the prevalence of arrangement types, and associations with geographic location, phenotype or strain fitness.

1.9. Problem statement and importance of current study

Pertussis remains a significant public health concern despite widespread use of vaccines, even amongst well-vaccinated populations. There are limited data describing the epidemiology of *B. pertussis* in healthy individuals in the community, and such studies are imperative in quantifying the true burden of disease to improve public health planning. It is important to understand infection in older children and adults as these age groups are likely sources of transmission to infants (20,21). In addition, there are currently no genomic data describing *B. pertussis* lineages in South Africa, and studies from other countries highlight the potential for the emergence of vaccine-escape mutants, following the use of acellular pertussis vaccines. These data emphasize the importance of genomic surveillance for monitoring the evolution of *B. pertussis* in response to immune pressure. Using data from a community cohort study (healthy individuals) and three surveillance populations (individuals with symptomatic infection), we aimed to determine the incidence, transmission dynamics, serological attack rates, antibody dynamics and molecular epidemiology of *B. pertussis* in South Africa.

1.10. Study Aim and Objectives

Aim

To determine the incidence, transmission dynamics, serological attack rates and molecular epidemiology of *B. pertussis* infection (carriage and disease) in South Africa.

Objectives

1. To determine the incidence and duration of *B. pertussis* infection within the community and describe the demographic and epidemiological characteristics associated with infection.
2. To determine the *B. pertussis* seroprevalence, attack rate and antibody responses of infected individuals overtime within the community and describe the demographic and epidemiological characteristics associated with infection.
3. To describe the molecular epidemiology of *B. pertussis* strains causing infection in South Africa

Chapter Two – Materials and Methods

In this chapter the study populations, specimen collection and general laboratory methods used to generate data are described. Additional specific study methods are described in the relevant chapters.

2. Study populations and laboratory methods

2.1. Prospective Household observational cohort study of Influenza, RSV and other respiratory pathogens community burden and Transmission dynamics study (the PHIRST study)

During 2016 – 2018, a prospective household cohort study was conducted in two South African communities – one rural (Agincourt, Mpumalanga Province) and one urban (Klerksdorp, North West Province) – enrolling consenting members of randomly selected households each year (Figure 2.1). The study aimed to estimate the community burden and assess the community transmission dynamics of influenza, RSV, *B. pertussis* and *S. pneumoniae*. A total of 1684 individuals from 327 households were enrolled into the study: 542 individuals in 2016, 577 in 2017, and 565 in 2018 (49). Individuals were followed-up between 6 – 10 months in a year which accounted for a total of 105 783 visits over the study period.

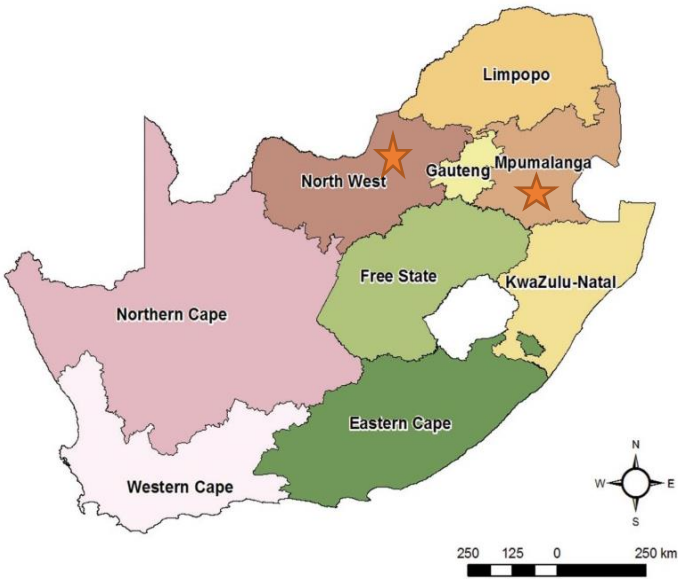


Figure 2.1: Map of the South African provinces (population: 61 million) indicating (marked with an orange star) the two provinces where the PHIRST study was conducted (image: https://www.ncbi.nlm.nih.gov/books/NBK481848).

2.1.1. Household visits

Demographic and baseline health status including human immunodeficiency virus (HIV) status were collected for all participants at enrolment. People were defined as living with HIV if they had one of the following during the follow-up period: two positive rapid HIV tests, evidence of a positive HIV laboratory result or evidence of receiving antiretroviral treatment. Vaccination status was obtained for children aged <5 years using the road-to-health vaccination card (RTHC). Households were visited twice weekly for nasopharyngeal specimen collection and symptom determination by a structured questionnaire. Symptom data included fever (self-reported or measured tympanic temperature ≥38°C), cough, difficulty breathing, sore throat, nasal congestion, chest pain, muscle aches, headache, vomiting, and/or diarrhea. In addition, during 2018, individuals that tested PCR-positive for *B. pertussis* were retrospectively interviewed (interviews were conducted immediately following a positive PCR result) to confirm if

the individual experienced any pertussis-specific symptoms (cough, inspiratory whoop, post-tussive vomiting or apnea) during the infection.

2.1.2. Specimen collection and laboratory testing

2.1.2.1. Nasopharyngeal swabs for identification of *B. pertussis*

At each visit a nasopharyngeal swab was collected and placed in PrimeStore® Molecular Transport Medium (MTM) (Longhorn Vaccines & Diagnostics, San Antonio, Texas, USA) and transported to the laboratory within 48 – 72 hours for testing. Total nucleic acids were extracted using the Roche MagNA Pure 96 instrument (Roche Diagnostics, Mannheim, Germany) and MP96 DNA and Viral NA SV Kit (Roche Diagnostics). Extracts were tested using an internally validated IS*481* and human ribonuclease P (*RNaseP*) duplex real-time PCR assay. Any specimen that tested positive for IS*481* with cycle threshold (C_t) values ≤45 was repeated (from extraction using a fresh aliquot) and re-tested (two replicates) using a second, multi-target real-time PCR assay targeting *B. pertussis*, *B. parapertussis* and *B. holmesii* (11,12). A specimen was considered positive for *B. pertussis* if IS*481* and/or *ptxS1* targets were detected with C_t values ≤45 in at least 2/3 replicates and negative for hIS*1001* (*B. holmesii*) and pIS*1001* (*B. parapertussis*).

2.1.2.2. Serum specimens and serological testing

Overall, serum was collected at enrolment, before the influenza season (between Autumn and Spring) and at the end of the intense follow-up period (±5 months between serum collections), however, for the 2016 cohort, serum was only collected before the influenza season and at the end of follow-up. Serum was also collected from the 2016

and 2017 cohorts in subsequent study years (2017 and 2018). In total, the 2016, 2017 and 2018 cohorts had 8, 6 and 3 blood draws, respectively.

Blood specimens were collected in vacutainer tubes and centrifuged, serum aliquoted and stored at -80°C at sites, before being shipped frozen to the reference laboratory at the National Institute for Communicable Diseases in Johannesburg on dry ice. Serological testing for influenza and RSV was prioritized and *B. pertussis* antibodies were tested on residual specimens. Detection of pertussis antibodies was performed using the anti-PT ELISA kit (EUROIMMUN, Lubeck, Germany) (33). The ELISA is a quantitative *in vitro* assay for detection of human IgG antibodies against PT in serum or plasma. The range of the assay was 5 – 200IU/ml.

Results were interpreted as per the kit instructions as follows: anti-PT IgG <40IU/ml: no indication of an acute infection; anti-PT IgG ≥40 – <100IU/ml: indeterminate (to be analyzed in relation to other samples collected from same individual); anti-PT IgG ≥100IU/ml: indication of acute infection or recent vaccination.

2.2. Pneumonia Surveillance

Pneumonia Surveillance was initiated in 2009 and is on-going. Pneumonia Surveillance is prospective, active surveillance that includes patients of all ages hospitalized with lower respiratory tract illness, irrespective of symptom duration. Individuals are enrolled at sentinel sites in the following provinces: Kwa-Zulu Natal (Edendale Hospital), North West (Klerksdorp-Tshepong Hospital Complex), Mpumalanga (Mapuleng-Matikwana Hospital Complex), Gauteng (Helen Joseph Hospital and Rahima Moosa Mother and Child Hospital) and Western Cape (Red Cross Children's Hospital, Groote Schuur Hospital, Mowbray Hospital and Mitchell's Plain Hospital) (45) (Figure 2.2). Testing for *Bordetella* species within the surveillance was initiated in 2012. Enrolled patients had to meet one of the following criteria:

- Infants >2 days to <3 months of age with a diagnosis of suspected sepsis or physician-diagnosed acute lower respiratory tract infection (LRTI), irrespective of symptoms.
- Infants >3 months to <5 months of age with physician-diagnosed acute LRTI (e.g., bronchitis, bronchiolitis, and pneumonia) or pleural effusion.
- Individuals ≥5 years of age with manifestation of acute LRTI with recorded temperature >38 °C or history of fever and cough.

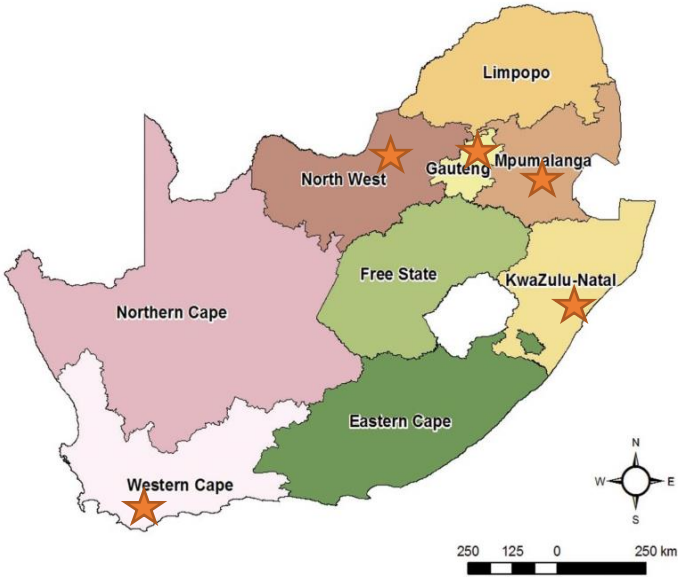


Figure 2.2: Map of the South African provinces (population: 61 million) indicating (marked with an orange star) the 5 provinces where Pneumonia Surveillance is conducted (image: https://www.ncbi.nlm.nih.gov/books/NBK481848).

2.2.1. Demographic and clinical data collection

Demographic and clinical data were collected by surveillance officers through patient or parent/caregiver interviews and hospital record reviews. Data collected included socio-demographic factors, symptoms (including duration), and underlying illnesses, including HIV and tuberculosis. For children aged <5 years, routine immunization history was documented and confirmed from the patients RTHC.

2.2.2. Specimen collection and laboratory testing

Respiratory specimens (nasopharyngeal swabs/aspirates in UTM/VTM and nasopharyngeal swabs in Regan-Lowe) were collected from all enrolled patients and transported on ice packs to the NICD for testing. We cultured *B. pertussis* from all respiratory specimens that were PCR positive for *B. pertussis* with a PCR C_t of <30. Suspected *B. pertussis* colonies on charcoal agar (incubated at 37°C for between 3 – 10

days) (Diagnostic Media Products, Johannesburg, South Africa) were confirmed using matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF, Bruker, Massachusetts, United States) and real-time PCR detection of IS*481* and *ptxS1* genes (positive for both targets with C_t <20) (11,12).

Chapter Three – Incidence and transmission dynamics of *Bordetella pertussis* infection in a rural and urban community in South Africa, 2016 – 2018 (PHIRST)

3. Reference:

Moosa F, Tempia S, Kleynhans J, McMorrow M, Moyes J, du Plessis M, et al. Incidence and Transmission Dynamics of *Bordetella pertussis* Infection in Rural and Urban Communities, South Africa, 2016 – 2018. Emerg Infect Dis. 2023 Feb 1. doi: 10.3201/eid2902.221125.

3.1. Introduction

Data on pertussis epidemiology in low- and middle-income countries, particularly in Africa, are lacking and as a result the epidemiology of the disease is poorly understood (17). In South Africa, between 2013 and 2018, among individuals hospitalized with pneumonia, annual *B. pertussis* incidence was 17 cases per 100,000 population, with the highest incidence of disease and the majority of deaths in children aged <1 year (48). In 2018, clusters of pertussis disease were observed in several provinces in South Africa, with 54% of cases detected in infants <3 months (87). Infants <1 year were at highest risk of severe pertussis disease and mortality. It is important to understand infection in older children and adults as these age groups are likely sources of transmission to infants too young to be vaccinated (20,21). This chapter describes the incidence, factors associated with infection, duration of infection, and transmission dynamics of *B. pertussis* in individuals of all ages in an urban and a rural community in South Africa.

3.2. Materials and Methods

3.2.1. Study population and data collection

Individuals were enrolled into the PHIRST study as described in section 2.1. and 2.1.1.

3.2.2. Specimen collection and laboratory testing

Nasopharyngeal swabs were collected from all enrolled individuals. Total nucleic acids were extracted from all swabs and tested by real-time PCR detecting and differentiating between *B. pertussis*, *B. parapertussis* and *B. holmesii* (see section 2.1.2.1).

3.2.3. Study definitions and data analysis

An episode of *B. pertussis* infection was defined as one or more consecutive visits in which *B. pertussis* was detected. A new episode was defined following a period of at least 12 consecutive visits (i.e. 6 weeks) in which *B. pertussis* was not detected. The incidence of *B. pertussis* was determined by dividing the number of PCR-positive episodes by the person-time under observation, expressed as 100 person-weeks. Factors associated with incidence were assessed using Poisson regression accounting for the person-time under observation. For analysis of incidence, we considered all identified episodes of infections, including multiple episodes in the same individual.

The duration of infection was estimated as the date of the last positive specimen minus the date of the first positive specimen (within the same episode of infection) plus 2.5 days to account for gaps between visits, and was presented as a mean and standard deviation (±SD). Analysis of factors associated with the duration of infection (time-to-event outcome) was performed using accelerated time failure Weibull regression. Analysis of *B. pertussis* bacterial loads were performed using IS*481* C_t as a proxy for

bacterial load. IS*481* bacterial load was characterized as follows: $C_t \leq 34$ (higher bacterial load), C_t 34 – 39 (intermediate bacterial load) and C_t 40 – 44 (lower bacterial load).

An episode of symptomatic infection was defined as an individual with one or more symptoms reported from one visit before to one visit after the *B. pertussis* episode. Symptoms included fever, cough, difficulty breathing, sore throat, nasal congestion, chest pain, muscle aches, headache, vomiting, and diarrhea. In 2018, the pertussis specific symptom data collected were used to determine if positive cases could be classified as pertussis using the South African NMC pertussis case definition [cough lasting ≥14 days (cough of any duration for children <1 year), with one or more of the following symptoms: paroxysms of coughing, inspiratory whoop, post-tussive vomiting and apnea (with or without cyanosis; for infants aged <1 year only)].

A cluster of *B. pertussis* cases included all infections in a single household that occurred within an interval of <7 days. Cluster duration was estimated as the time from the first day of positivity of the first individual in a cluster to the last day of positivity of the last individual. Household cumulative infection risk (HCIR) was defined as the proportion of subsequent infections within a household following the first PCR-positive case. The HCIR was evaluated among all households in which at least one household member had *B. pertussis*. The index case was defined as the first individual testing positive within a cluster. The HCIR was calculated as: number of infected individuals within household (excluding the index case) divided by number of enrolled individuals living within the

household. Households with co-primary cases were excluded from the HCIR analysis. Crowding was defined as >2 individuals sharing a sleeping room within the household.

Logistic regression was used for the analysis of factors associated symptomatic fraction and HCIR. Pertussis vaccine status was calculated based on number of vaccine doses received by age. For all analyses, we accounted for clustering by study site and households within site using hierarchical mixed effect models. For the multivariable models, we assessed all variables that were significant at $p<0.2$ on univariate analysis, and dropped non-significant factors ($p\geq0.05$) with manual backward elimination. Statistical significance was determined at $p<0.05$ for all analyses. All statistical analyses were performed using Stata version 14.1 (Stata Corp LP, College Station, Texas, USA).

3.3. Results

3.3.1. Study population

During 2016 – 2018, 1684 individuals from 327 households were enrolled: 542 individuals in 2016, 577 in 2017, and 565 in 2018 (Table 3.1), as previously described (88). Overall, 16.6% (279/1684) of the participants were aged <5 years, of whom 97.3% (214/220) were fully vaccinated for age with the acellular vaccine. HIV prevalence for the cohort was 15.3% (249/1628).

3.3.2. Specimen level data

There were 122,133 possible individual follow-up visits from which 105,687 (86.5%) nasopharyngeal swabs were collected and tested for *Bordetella* species. Of the 105,687 swabs tested, 276 (0.3%) and 413 (0.4%) swabs were PCR-positive for *B. pertussis* and *B. holmesii*, respectively. *B. parapertussis* was not detected (Appendix B, C and D). Of

the specimen's positive for *B. pertussis*, 20.3% (56/276) were positive for both IS*481* and ptx*S1*, and 79.7% (220/276) were positive for IS*481* only.

3.3.3. Individual level data

3.3.3.1. Incidence and factors associated with incidence

Among 1684 study participants, 118 episodes of *B. pertussis* infection were detected in 107 participants with 11 individuals having two episodes of *B. pertussis* infection. There were no differences observed between individuals with one or two episodes of infection (data not shown). The incidence of *B. pertussis* was 0.21 (95% CI 0.17 – 0.25) per 100 person-weeks (Table 3.2). The highest incidence of *B. pertussis* infection was observed in individuals aged 5 – 14 years [0.27 per 100 person-weeks, (95% CI 0.20 – 0.35)]. Children aged <5 years with incomplete vaccination for age were more likely to be infected with *B. pertussis* compared to vaccinated children [adjusted incidence rate ratio 4.48 (95% CI 1.38 – 14.59)]. Among the 36 enrolled infants, five *B. pertussis* infections were identified in individuals aged 9 – 11 months. Of these, four were fully vaccinated for age and one infant (incomplete vaccination) had symptoms (runny nose) during the infection.

3.3.3.2. Duration of PCR-positive *B. pertussis* infection

The mean duration of *B. pertussis* infection was 12 days (± 19.1). The duration of infection was longer when ≥2 symptoms were reported adjusted Hazard Ratio (aHR) 0.26 (95% CI 0.18 – 0.67) (Table 3.3), when compared to colonized individuals. Duration of infection was longer for participants with higher and intermediate bacterial loads (C_t values ≤34 and 35 – 39) when compared to lower bacterial loads [(C_t 40 – 44) aHR C_t ≤34: 0.16 (95% CI 0.06 – 0.44) and C_t 35 – 39: 0.41 (95% CI 0.22 – 0.74)].

3.3.3.3. Factors associated with symptomatic *B. pertussis* infection

Of the 107 individuals positive for *B. pertussis*, 31.8% (34/107) individuals reported symptoms during any given episode of infection (Table 3.4). Common respiratory symptoms reported during an episode of infection were runny nose (55.9%, 19/34), cough (82.4%, 28/34), fever (29.4%, 10/34), and sore throat (14.7%, 5/34). Other less commonly reported symptoms were muscle aches, difficulty breathing, chest pain and vomiting. In 2018, 47.1% (24/51) of individuals testing positive for *B. pertussis* was retrospectively interviewed (Appendix E). Eighty-three percent (20/24) of these individuals reported at least one symptom consistent with pertussis disease (cough, inspiratory whoop, post-tussive vomiting or apnea) and 60.0% (12/20) of symptomatic individuals met the pertussis clinical case definition. Amongst the symptomatic *B. pertussis* positive individuals aged <5 years, 67.0% (4/6) were fully vaccinated for age. Individuals positive for *B. pertussis* were four times more likely to have symptoms if they were infected for ≥7 days compared to individuals infected for <7 days [aOR 4.12 (95% CI 1.70 – 9.98)] (Table 3.4).

3.4. Household level data – Household cumulative infection risk

The overall household cumulative infection risk was 14.4% (43 of 298 susceptible exposed individuals). Transmission was more likely to occur from male index cases compared to females [aOR 12.20 (95% CI 1.57 – 94.96)] and individuals with ≥7 day's episode duration compared to <7 day's [aOR 24.79 [95% CI 2.74 – 224.30)] (Table 3.5). Within households with confirmed *B. pertussis* transmission, 38.8% (14/36) of index cases transmitting to household contacts were colonized.

Table 3.1: Demographic, clinical and household characteristics of individuals enrolled into the PHIRST study, South Africa, 2016 – 2018 (N=1684).

Characteristic	Overall n/N (%)	Rural n/N (%)	Urban n/N (%)
Year			
2016	542/1684 (32.2)	280/849 (32.9)	262/835 (31.4)
2017	577/1684 (34.3)	289/849 (34.0)	288/835 (34.5)
2018	565/1684 (33.6)	280/849 (32.9)	285/835 (34.1)
Sex			
Male	675/1684 (40.1)	316/849 (37.2)	359/835 (42.9)
Female	1009/1684 (59.9)	533/849 (62.8)	479/835 (57.1)
Age group (years)			
<1	36/1684 (2.1)	15/849 (1.8)	21/835 (2.5)
1 – 4	243/1684 (14.4)	156/849 (18.4)	87/835 (10.4)
5 – 14	547/1684 (32.5)	309/849 (36.4)	238/835 (28.5)
15 – 24	273/1684 (16.2)	124/849 (14.6)	149/835 (17.8)
25 – 44	317/1684 (18.8)	141/849 (16.6)	176/835 (21.1)
45 – 64	195/1684 (11.6)	74/849 (8.7)	121/835 (14.5)
≥65	73/1684 (4.3)	30/849 (3.5)	43/835 (5.2)
HIV status			
Negative	1379/1628 (84.7)	715/832 (85.9)	664/796 (83.4)
Positive	249/1628 (15.3)	117/832 (14.1)	132/796 (16.6)
Nutritional status[a]			
Underweight	131/1676 (7.8)	55/849 (6.5)	76/827 (9.2)
Normal	993/1676 (59.3)	552/849 (65.0)	441/827 (53.3)
Overweight	265/1676 (15.8)	123/849 (14.5)	142/827 (17.2)
Obese	287/1676 (17.1)	119/849 (14.0)	168/827 (20.3)
Underlying illness[b]			
No	1634/1684 (97.0)	844/849 (99.4)	790/835 (94.6)
Yes	50/1684 (3.0)	5/849 (0.6)	45/835 (5.4)
·Pertussis vaccination[c]			
Incomplete vaccination	6/220 (2.7)	3/128 (2.3)	3/92 (3.3)
Fully vaccinated	214/220 (97.3)	125/128 (97.7)	89/92 (96.7)
Crowding[d]			
No	738/1684 (43.8)	361/849 (42.5)	377/835 (45.2)
Yes	946/1684 (56.2)	488/849 (57.5)	458/835 (54.9)

[a] Nutritional status based on individual's body mass index (BMI). We defined BMI categories as follows: underweight - age <18 year's weight for age or BMI <-2 standard deviations of the World Health Organization (WHO) Child Growth Standards, age ≥18 years BMI <18.5kg/m2; overweight - age <18 years BMI >+1 and ≤+2 standard deviations of the WHO growth standards, age ≥18 years BMI ≥25 and <30kg/m2, obese – age <18 years BMI >+2 standard deviations of the WHO growth standards, age ≥18 years BMI ≥30 kg/m2. [b]Underlying illness defined as self-reported history of asthma, lung disease, heart disease, stroke, spinal cord injury, epilepsy, organ transplant, immunosuppressive therapy, organ transplantation, cancer, liver disease, renal disease or diabetes. [c]Only collected for children <5 years of age. [d]Crowding - more than two individuals in a household sleeping in the same room.

Table 3.2: Incidence rate and factors associated with *B. pertussis* incidence, PHIRST study, South Africa, 2016 – 2018 (N=1684).

Characteristic	*B. pertussis* incidence	Univariate analysis		Multivariable analysis[e]	
		Incidence Rate Ratio (95% CI)	p value	Adjusted Incidence Rate Ratio (95% CI)	p value
Overall	0.2 (0.17 – 0.25)				
Year					
2016	0.25 (0.17 – 0.35)	Reference			
2017	0.13 (0.09 – 0.19)	0.53 (0.17 – 1.63)	0.27		
2018	0.26 (0.20 – 0.33)	1.04 (0.44 – 2.49)	0.92		
Site					
Rural	0.17 (0.13 – 0.22)	Reference			
Urban	0.24 (0.19 – 0.31)	1.5 (0.77 – 2.96)	0.23		
Sex					
Male	0.23 (0.17 – 0.30)	Reference			
Female	0.19 (0.15 – 0.24)	0.83 (0.58 – 1.19)	0.31		
Age group (years)					
<5	0.21 (0.14 – 0.33)	1.41 (0.69 – 2.90)	0.37	1.62 (0.56 – 4.65)	0.37
5 – 14	0.27 (0.20 – 0.35)	1.76 (0.96 – 3.21)	0.09	1.73 (0.91 – 3.31)	0.09
15 – 44	0.17 (0.12 – 0.24)	1.12 (0.62 – 2.04)	0.78	1.09 (0.59 – 2.00)	0.78
≥45	0.15 (0.09 – 0.25)	Reference		Reference	
HIV status					
Uninfected	0.22 (0.18 – 0.27)	Reference			
Infected	0.14 (0.08 – 0.25)	0.64 (0.36 – 1.11)	0.11		
Nutritional status[a]					
Underweight	0.20 (0.10 - 0.38)	0.68 (0.28 – 1.66)	0.39		
Normal	0.23 (0.18 – 0.28)	Reference			
Overweight/Obese	0.30 (0.21 – 0.43)	0.76 (0.52 – 1.13)	0.18		

Underlying illness[b]					
No	0.20 (0.17 – 0.24)	Reference			
Yes	0.36 (0.16 – 0.80)	1.78 (0.68 – 4.70)	0.24		
Pertussis vaccination[c]					
Incomplete vaccination	0.91 (0.23 – 3.64)	4.89 (1.47 – 16.02)	0.01	**4.48 (1.38 – 14.59)**	**0.01**
Fully vaccinated	0.19 (0.11 – 0.32)	Reference		Reference	
Unknown	0.20 (0.17 – 0.25)	1.08 (0.61 – 1.91)	0.78	1.32 (0.42 – 4.13)	0.64
Crowding[d]					
No	0.15 (0.11 – 0.21)	Reference		Reference	
Yes	0.25 (0.20 – 0.31)	1.63 (0.86 – 3.08)	0.13	1.53 (0.78 – 2.97)	0.21

[a] Nutritional status based on individual's body mass index (BMI). We defined BMI categories as follows: underweight - age <18 year's weight for age or BMI <-2 standard deviations of the World Health Organization (WHO) Child Growth Standards, age ≥18 years BMI <18.5kg/m2; overweight - age <18 years BMI >+1 and ≤+2 standard deviations of the WHO growth standards, age ≥18 years BMI ≥25 and <30kg/m2, obese – age <18 years BMI >+2 standard deviations of the WHO growth standards, age ≥18 years BMI ≥30 kg/m2. [b]Underlying illness defined as self-reported history of asthma, lung disease, heart disease, stroke, spinal cord injury, epilepsy, organ transplant, immunosuppressive therapy, organ transplantation, cancer, liver disease, renal disease or diabetes. [c]Only collected for children <5 years of age. Vaccine status calculated based on number of doses of vaccine received by age. [d]Crowding - more than two individuals in a household sleeping in the same room. [e]Bold font indicates statistical significance. Variables adjusted for in final model: Age group, pertussis vaccination and crowding.

Table 3.3. Factors associated with PCR-positive *B. pertussis* infection duration, PHIRST study, South Africa, 2016 – 2018 (N=118).

Characteristic	*B. pertussis* mean infection duration in days (±SD)	Univariate analysis		Multivariable analysis[e]	
		Hazard ratio (95% CI)	p value	Adjusted Hazard ratio (95% CI)	p value
Sex					
Male	11.9 (±21.1)	Reference			
Female	14.1 (±18.8)	0.77 (0.45 – 1.31)	0.34		
Age group (years)					
<5	6.3 (±6.2)	1.87 (0.84 – 4.20)	0.13	2.20 (0.87 – 5.58)	0.09
5 – 14	12.3 (±14.5)	1.09 (0.59 – 2.02)	0.78	1.16 (0.58 – 2.33)	0.67
15 – 44	15.4 (±20.8)	1.16 (0.50 – 2.67)	0.73	2.07 (0.81 – 5.27)	0.13
≥45	22.4 (±38.8)	Reference		Reference	
HIV status					
Uninfected	13.8 (±20.7)	Reference			
Infected	9.3 (±10.2)	1.06 (0.50 – 2.26)	0.88		
Nutritional status[a]					
Underweight	4.4 (±2.7)	2.78 (0.98 – 8.00)	0.06		
Normal	13.0 (±18.7)	Reference			
Overweight/Obese	16.2 (±25.3)	0.44 (0.71 – 2.17)	0.44		
Underlying illness[b]					
No	12.4 (±18.7)	Reference			
Yes	28.2 (±34.9)	0.80 (0.27 – 2.33)	0.68		
Pertussis vaccination[c]					
Incomplete vaccination	3.0 (±0.0)	2.44 (0.33 – 17.82)	0.38		
Fully vaccinated	6.7 (±6.6)	Reference			
Unknown	14.4 (±21.1)	0.63 (0.29 – 1.36)	0.24		

Symptoms					
No symptoms	9.3 (±14.1)	Reference		Reference	
1 symptom	22.2 (±20.3)	0.62 (0.19 – 2.04)	0.44	0.39 (0.18 – 1.62)	0.19
≥2 symptoms	11.1 (±10.2)	0.38 (0.16 – 0.93)	0.03	**0.26 (0.18 – 0.67)**	**0.006**
IS*481* C_t category[d]					
≤34	17.0 (±11.6)	0.17 (0.06 – 0.45)	<0.001	**0.16 (0.06 – 0.44)**	**0.000**
35 – 39	13.5 (±20.7)	0.43 (0.24 – 0.76)	0.004	**0.41 (0.22 – 0.74)**	**0.003**
40 – 44	11.1 (±20.7)	Reference		Reference	

[a] Nutritional status based on individual's body mass index (BMI). We defined BMI categories as follows: underweight - age <18 year's weight for age or BMI <-2 standard deviations of the World Health Organization (WHO) Child Growth Standards, age ≥18 years BMI <18.5kg/m2; overweight - age <18 years BMI >+1 and ≤+2 standard deviations of the WHO growth standards, age ≥18 years BMI ≥25 and <30kg/m2, obese – age <18 years BMI >+2 standard deviations of the WHO growth standards, age ≥18 years BMI ≥30 kg/m2. [b]Underlying illness defined as self-reported history of asthma, lung disease, heart disease, stroke, spinal cord injury, epilepsy, organ transplant, immunosuppressive therapy, organ transplantation, cancer, liver disease, renal disease or diabetes. [c]Only collected for children <5 years of age. Vaccine status calculated based on number of doses of vaccine received by age. [d]IS*481* C_t used as a proxy for bacterial load. [e]Bold font indicates statistical significance. Variables adjusted for in final model: Age group, presence/absence of symptoms and IS*481* C_t category.

Table 3.4: Factors associated with symptomatic *B. pertussis* infection, PHIRST study, South Africa, 2016 – 2018 (N=107).

Characteristic	*B. pertussis* symptomatic n/N (%)	Univariate analysis		Multivariable analysis[e]	
		Odds ratio (95% CI)	p value	Adjusted Odds ratio (95% CI)	p value
Year					
2016	6/33 (18.2)	Reference			
2017	2/29 (31.0)	2.03 (0.62 – 6.62)	0.24		
2018	19/56 (33.9)	2.31 (0.81 – 6.56)	0.12		
Site					
Rural	13/48 (27.1)	Reference			
Urban	21/70 (30.0)	1.15 (0.51 – 2.61)	0.73		
Sex					
Male	10/52 (19.2)	Reference			
Female	24/66 (36.4)	2.40 (1.02 – 5.63)	0.04		
Age group (years)					
<5	4/20 (20.0)	0.25 (0.05 – 1.14)	0.07	0.33 (0.06 – 1.69)	0.18
5 – 14	15/56 (26.8)	0.37 (0.11 – 1.22)	0.10	0.41 (0.11 – 1.54)	0.19
15 – 44	8/28 (28.6)	0.4 (0.11 – 1.51)	0.18	0.28 (0.06 – 1.22)	0.09
≥45	7/14 (50.0)	Reference		Reference	
HIV status					
Uninfected	28/104 (26.9)	Reference		Reference	
Infected	6/12 (50.0)	2.71 (0.81 – 9.11)	0.11	3.37 (0.77 – 14.56)	0.10
Nutritional status[a]					
Underweight	0/9 (0.0)	0.15 (0.01 – 2.65)	0.19		
Normal	20/77 (25.9)	Reference			
Overweight/Obese	13/28 (46.4)	2.44 (1.00 – 5.93)	0.05		
Underlying illness[b]					
No	31/112 (27.7)	Reference			
Yes	3/6 (50.0)	2.61 (0.50 – 13.65)	0.26		
Pertussis vaccination[c]					
Incomplete vaccination	0/2 (0.0)	0.47 (0.02 – 11.81)	0.64		
Fully vaccinated	4/14 (28.6)	Reference			
Unknown	30/102 (29.4)	0.98 (0.30 – 3.20)	0.98		

IS*481* C_t category[d]					
≤34 C_t	6/15 (40.0)	3.05 (0.82 – 11.38)	0.09		
35 – 39 C_t	21/64 (32.8)	2.23 (0.85 – 5.89)	0.11		
40 – 44 C_t	7/39 (17.9)	Reference			
Episode duration					
< 7 days	14/75 (18.7)	Reference		Reference	
≥ 7 days	20/43 (46.5)	3.79 (1.64 – 8.73)	0.002	**4.12 (1.70 – 9.98)**	**0.002**
Smoking (≥15 years)					
No	14/37 (37.8)	Reference			
Yes	5/16 (31.3)	0.75 (0.21 – 2.60)	0.65		

[a]Nutritional status based on individual's body mass index (BMI). We defined BMI categories as follows: underweight - age <18 year's weight for age or BMI <-2 standard deviations of the World Health Organization (WHO) Child Growth Standards, age ≥18 years BMI <18.5kg/m2; overweight - age <18 years BMI >+1 and ≤+2 standard deviations of the WHO growth standards, age ≥18 years BMI ≥25 and <30kg/m2, obese – age <18 years BMI >+2 standard deviations of the WHO growth standards, age ≥18 years BMI ≥30 kg/m2. [b]Underlying illness defined as self-reported history of asthma, lung disease, heart disease, stroke, spinal cord injury, epilepsy, organ transplant, immunosuppressive therapy, organ transplantation, cancer, liver disease, renal disease or diabetes. [c]Only collected for children <5 years of age. Vaccine status calculated based on number of doses of vaccine received by age. [d]IS*481* C_t as a proxy for bacterial load. [e]Bold font indicates statistical significance. Variables adjusted for in final model: Age group, HIV-status and episode duration.

Table 3.5: Factors associated with household cumulative infection risk (HCIR), PHIRST study, South Africa, 2016 – 2018 (N=118).

Characteristic	*B. pertussis* HCIR n/N (%)	Univariate analysis		Multivariable analysis	
		Odds ratio (95% CI)	p value	Odds ratio (95% CI)	p value
Characteristics of the index case					
Sex					
Male	30/143 (20.9)	16.81 (1.43 – 197.69)	0.025	12.20 (1.57 – 94.96)	0.02
Female	6/150 (4.0)	Reference		Reference	
Age group (years)					
<5	2/43 (4.7)	0.05 (0.0003 – 7.40)	0.24		
5 – 14	27/182 (14.8)	0.38 (0.02 – 7.59)	0.53		
15 – 44	1/42 (2.4)	0.03 (0.0001 – 14.52)	0.27		
≥45	6/22 (27.3)	Reference			
IS*481* C_t category[a]					
≤34 C_t	16/57 (28.1)	Reference			
35 – 39 C_t	11/138 (7.9)	0.35 (0.02 – 6.17)	0.47		
40 – 44 C_t	9/98 (9.2)	0.43 (0.02 – 8.87)	0.59		
Episode duration					
<7 days	12/216 (5.7)	Reference		Reference	
≥7 days	24/77 (31.2)	40.84 (3.37 – 495.19)	0.004	24.79 (2.74 – 224.30)	**0.004**
Characteristics of the household contacts					
Sex					
Male	15/110 (13.6)	Reference			
Female	21/183 (11.5)	0.65 (0.22 – 1.93)	0.44		
Age group (years)					
<5	8/50 (16.0)	6.60 (1.22 – 35.64)	0.03		
5 – 14	11/88 (12.5)	2.10 (0.51 – 8.73)	0.31		
15 – 44	13/106 (12.3)	2.32 (0.30 – 17.76)	0.42		
≥45	4/49 (8.2)	Reference			

HIV status			
Negative	31/243 (12.8)	Reference	
Positive	5/44 (11.4)	1.27 (0.32 – 5.14)	0.73
Unknown	0/6 (0.0)	1.00	
Nutritional status[b]			
Underweight	3/23 (13.0)	0.76 (0.08 – 6.95)	0.80
Normal weight	21/160 (13.1)	Reference	
Overweight	11/107 (10.2)	0.76 (0.23 – 2.47)	0.65
Underlying illness[c]			
No	34/285 (11.9)	Reference	
Yes	2/8 (25.0)	16.93 (0.61 – 471.04)	0.09
Crowding[d]			
No	8/100 (8.0)	Reference	
Yes	28/193 (14.5)	1.84 (0.21 – 16.42)	0.59
Sleep in same room as index			
No	24/158 (15.2)	Reference	
Yes	12/134 (8.9)	0.71 (0.22 – 2.26)	0.56

[a]IS*481* C_t as a proxy for bacterial load. [b]Nutritional status based on individual's body mass index (BMI). We defined BMI categories as follows: underweight - age <18 year's weight for age or BMI <-2 standard deviations of the World Health Organization (WHO) Child Growth Standards, age ≥18 years BMI <18.5kg/m2; overweight - age <18 years BMI >+1 and ≤+2 standard deviations of the WHO growth standards, age ≥18 years BMI ≥25 and <30kg/m2, obese – age <18 years BMI >+2 standard deviations of the WHO growth standards, age ≥18 years BMI ≥30 kg/m2. [c]Underlying illness defined as self-reported history of asthma, lung disease, heart disease, stroke, spinal cord injury, epilepsy, organ transplant, immunosuppressive therapy, organ transplantation, cancer, liver disease, renal disease or diabetes. [d]Crowding - more than two individuals in a household sleeping in the same room. [e]Bold font indicates statistical significance. Variables adjusted for in final model: Sex and episode duration.

3.5. Discussion

In this community cohort study of healthy individuals in South Africa, the incidence of *B. pertussis* was 0.21 per 100 person-weeks. Incidence in this study was higher than the mean annual incidence risk of 17 cases per 100 000 population previously reported in South Africa, amongst individuals hospitalized with pneumonia between 2013 – 2018 (48). This difference is likely due to the study conducted by Wolter et al., was a cross-sectional study enrolling hospitalized individuals with respiratory illness, whereas this study is a community cohort study enrolling healthy individuals. It is difficult to compare our incidence with other studies that report incidence data as our study is a community cohort study whereas other studies focus on populations with pertussis or populations with an outbreak of *B. pertussis*. Hence more data describing the incidence of *B. pertussis* infection among healthy individuals over time, and additional longitudinal community data are required for meaningful comparisons to be made. Assessing factors associated with *B. pertussis* incidence, children aged <5 years that were not fully vaccinated for age, were more likely to be infected with *B. pertussis* compared to vaccinated children. Similarly, other studies in South Africa showed that the risk of pertussis disease and the risk of hospitalization due to pertussis disease decreased when individuals were vaccinated (47,48).

Data on the duration of PCR-positive *B. pertussis* infection amongst individuals are limited. In our study, the average duration of naturally-acquired *B. pertussis* infection was 12 days and episode duration was longer for participants with higher bacterial loads, similar to findings for influenza infection in the same cohort (89). A human challenge study, inducing *B. pertussis* colonization in healthy adults, showed that

B. pertussis persisted within the nasopharynx for up to 16 day's post-inoculation in some of the study participants (90). However, it should be noted that in the human challenge study, adults had received vaccination within the previous five years and were treated for colonization from day 14. In our study, vaccination (individuals ≥5) and treatment were missing which could have potentially affected the duration of *B. pertussis* within the nasopharynx of study participants.

Symptoms associated with *B. pertussis* PCR-positive cases vary from mild, non-specific respiratory symptoms to pertussis-specific symptoms. In this study, symptomatic *B. pertussis* infected individuals reported non-specific respiratory symptoms such as runny nose, cough, fever, and/or sore throat. For the 2018 subset of symptomatic individuals that were investigated for *B. pertussis*-specific symptoms, 60% met the NMC pertussis case definition. A Spanish study, determining factors influencing the spread of *B. pertussis* in households, found that participants of all ages experienced pertussis-specific symptoms such as cough lasting >2 weeks, paroxysmal cough, post-tussive vomiting, inspiratory stridor, apnea and fever (91), whereas in South Africa, common symptoms reported in children hospitalized with laboratory pertussis were non-specific cough and fever (54).

There is evidence to suggest that colonization is an important trait of *B. pertussis*. In our study, two-thirds of individuals infected with *B. pertussis* did not report any symptoms. *B. pertussis* incidence data from the USA and UK provided evidence of colonization of *B. pertussis* (92). de Graaf et al. showed that colonization could be induced by the intranasal inoculation of *B. pertussis* in a human challenge model (90). Warfel et al.

found that individuals previously vaccinated with the acellular pertussis vaccine can become colonized with *B. pertussis* (19). There is evidence in the literature supporting colonization of *B. pertussis* in the nasopharynx of individuals, which is also seen in our study. In addition to colonization, our study shows a high proportion of index cases transmitting *B. pertussis* to other household contacts. However, it is difficult to draw any conclusions from this result as additional longitudinal community data are required for meaningful comparisons to be made.

The transmission dynamics of *B. pertussis* within households is poorly understood and requires further research. Within the PHIRST study, the overall HCIR was 14%. In 2001, a pertussis outbreak in New South Wales in Australia showed a 22.3% secondary household attack rate and increased risk of household transmission when the index case commenced antibiotic treatment more than 7 days after the onset of symptoms (93). Transmission in our study was associated with male sex and the index case having a long episode duration (≥7 days). The association between prolonged episode duration and increased transmission is biologically plausible and has been demonstrated for other pathogens such as influenza (89). Previous studies have not documented a difference in transmission between males and females and this needs to be further explored.

This study has limitations that should be acknowledged. The study was only conducted at two sites in South Africa which may not be generalizable to other areas and communities within the country. Over the study period, specimens were collected from each individual twice a week for a period of between 6 – 10 months. Due to this, we presented incidence as per 100-person week as we did not collect samples for a full year

(12 months), and therefore could not accurately estimate the incidence over period of year. Vaccination status was only confirmed for children aged <5 years, therefore analyses regarding vaccination could only be conducted for this age group. Symptom data collection was poor for 2016 but improved in 2017 and 2018. Therefore, the symptom data presented could be an underestimate. The number of *B. pertussis* cases detected in this study is small and therefore we were underpowered in some of our statistical analyses.

The PHIRST study is unique in that it was a community cohort study investigating *B. pertussis* infection amongst healthy individuals. An important strength of the study was the repeated, longitudinal sampling of individuals over time according to a specified schedule, irrespective of symptoms. This study methodology ensured that the data collected over time is robust against sampling bias, and allowed us to identify the large proportion of individuals that were colonized with *B. pertussis*.

In two disparate communities in South Africa, we found a high incidence of *B. pertussis* and two-thirds of cases were colonized. We have demonstrated that individuals that are colonized with *B. pertussis* can transmit to other household members and hence represent a source of transmission to vulnerable age groups.

Chapter Four – *Bordetella pertussis* antibody dynamics in household cohorts in two South African communities, 2016 – 2018

4. Reference

Moosa F, Kleynhans J, Makhathini L, du Plessis M, Tempia S, McMorrow M, et al. *Bordetella pertussis* antibody dynamics over 3 years (2016 – 2018) in household cohorts in two South African communities. – *In preparation*.

4.1. Introduction

There are no established immunological correlates of protection against *B. pertussis* infection (1,3,19). However, serology can be a useful tool to understand antibody kinetics and duration of immunity following natural infection or vaccination, but such studies are limited (3). Following infection/vaccination, pertussis-specific antibodies increase and then wane in a bi-phasic manner (initial rapid decay from peak followed by slower decay lasting years) and post-infection antibodies decay almost two times faster when compared to post-vaccination antibodies (59). Overall, waning generally occurs within 12 months of either natural infection or vaccination (59–63).

There are currently no data describing the sero-epidemiology of *B. pertussis* in South Africa. As described in Chapter three, there was a high incidence of *B. pertussis* within the PHIRST community with 68% of infected persons being asymptomatic. Here we use sera collected from the same cohort to estimate the *B. pertussis* seroprevalence over time, infection attack rates, factors associated with infection and seroconversion, and describe the antibody dynamics of infected individuals over time.

4.2. Materials and Methods

4.2.1. Study population and data collection

Individuals were enrolled into the PHIRST study and data were collected as described in Chapter 2 (see section 2.1. and 2.1.1).

4.2.2. Specimen collection and laboratory testing

Sera were collected from all enrolled individuals and tested using the anti-PT ELISA kit as described in section 2.1.2.2. The blood draws were labelled as blood draw 1 to blood draw 8 and corresponded to the following periods of collection: Blood draw 1: Mar – Apr 2016; Blood draw 2: Oct – Nov 2016; Blood draw 3: Feb – Mar 2017; Blood draw 4: May 2017; Blood draw 5: Oct 2017; Blood draw 6: Jan – Feb 2018; Blood draw 7: May – Jun 2018; Blood draw 8: Oct 2018 (Figure 4.1). For this study, only individuals with at least one serum collected at any point were included

4.2.3. Data analysis

B. pertussis seroprevalence was calculated for each blood draw (8 time points) from 2016 to 2018 as follows: total number of individuals with anti-PT IgG titer ≥100IU/ml /total number of individuals tested × 100. Seroprevalence over time was compared by age group using the chi-squared test. For seroprevalence, individuals that had ≥1 serum collected over the study period were included (N=1618).

Seroconversion in an individual was defined as a ≥4-fold rise in anti-PT IgG titer (94–96) between any two consecutive blood draws and/or if anti-PT IgG reached >100IU/ml (with or without a 4-fold rise), and was considered indicative of recent *B. pertussis* infection/vaccination. When calculating seroconversion, only individuals with ≥2

consecutive sera collected were included (N=1509). The attack rate (no. individuals positive/no. individuals tested) using serology (seroconversion between any 2 consecutive blood draws) and/or PCR (≥1 positive swab) during the intense follow-up period was calculated and compared using chi-squared test. We also performed a sensitivity analysis using a ≥2-fold rise in anti-PT IgG titer for seroconversion and compared the attack rates using the ≥2-fold rise and ≥4-fold rise in antibody titer using chi-squared test.

To determine factors associated with seroconversion among individuals with PCR-confirmed infection, we used logistic regression analysis accounting for clustering by study site and household using hierarchical mixed effects modelling. Individual factors analyzed included: sex, age group, HIV status, nutritional status, underlying medical conditions, pertussis vaccination (for children <5 years), episode duration [defined as date of the last PCR-positive specimen minus the date of the first PCR-positive specimen (within the same episode of infection) plus 2.5 days to account for gaps between visits (49)], household size, household crowding (defined >2 persons in a household sleeping in the same room) and child <5 years living in the household. The factors associated with *B. pertussis* infection (identified by PCR and/or serology) were also determined using hierarchical mixed effects logistic regression. For the multivariable models, we assessed all variables that were significant at $p<0.2$ on univariate analysis, and removed non-significant factors ($p\geq0.05$) with manual backward elimination. Statistical significance was determined at $p<0.05$ for the analyses.

To describe changes in antibody titers over time, for individuals with PCR-confirmed infection that seroconverted (visit date of first PCR-positive specimen considered as day 0 of infection), we described (n/N expressed as percentage) the number of individuals that seroconverted and the time taken (in months as blood draws were ±5 months apart) for anti-PT IgG titers to reach a ≥4-fold rise and to decrease to below 40IU/ml, including stratification by episode duration (<7 days and ≥7 days) and IS*481* C_t value (<35 and ≥35). We also analyzed antibody dynamics by those that were likely to have received the acellular vaccine in the routine infant immunization schedule (cases born from 2009 onwards when acellular vaccine was introduced) and those that were likely to have received the whole cell vaccine (cases born before 2008). This was based on assumption of vaccine received by birth date as vaccination history was only collected for children aged <5 years. Individuals born in 2008 were excluded from these analyses as these cases would likely have received a combination of both vaccine types.

Statistical analyses were performed using Stata version 14.1 (Stata Corp LP, College Station, Texas, USA), GraphPad Instat version 3.1 (Dotmatics, Boston, USA), GraphPad Prism version 10.2 (Dotmatics, Boston, USA) and Microsoft Excel (Microsoft Inc., Washington, USA).

4.3. Results

4.3.1. Study population

During 2016 – 2018, a total of 1684 individuals were enrolled into the PHIRST study. Of these, 96.1% (1618/1684) and 89.6% (1509/1684) of individuals had at least one serum and paired sera collected during the intense follow-up period, respectively. Sera from 66 individuals were not tested due to insufficient volumes. Overall, 13.8%

(223/1618) of participants with ≥1 serum collected were aged <5 years, of whom 97.8% (175/179) were fully vaccinated with the acellular vaccine (only for those with available vaccine status) (Table 4.1). A total of 105,687 NP swabs were collected during the study period and tested for *Bordetella* species by real-time PCR. Of these, 276 (0.3%) were PCR-positive for *B. pertussis* (Figure 4.1).

4.3.2. *B. pertussis* seroprevalence over time

A total of 7170 sera were collected and tested from 1618 (96.1%, 1618/1684) enrolled individuals. At the start of the study period in 2016, the seroprevalence was 3.9% (13/332) and by the end of the study (blood draw 8 at the end of 2018), the seroprevalence was at its highest (5.2%, 71/1363) (Figure 4.2). The lowest seroprevalence was 1.6% at blood draw 6 in Jan – Feb 2018 (22/1419).

Mean anti-PT IgG antibody titers differed by blood draw and age group (Figure 4.3). For all age groups, the mean anti-PT IgG antibody titers were significantly higher for blood draw 8 (Oct 2018) when compared to all blood draws from blood draw 2 to blood draw 7 ($p<0.001$).

4.3.3. *B. pertussis* attack rates (by PCR and serology)

Amongst those tested by PCR with paired sera, the *B. pertussis* PCR attack rate was 6.2% (94/1509) and 3.8% (58/1509) of individuals were identified as infected by PCR only (did not seroconvert following infection) (Table 4.2). The *B. pertussis* serological attack rate (seroconversion between any 2 consecutive serum samples) was 5.8% (87/1509) using data from all 8 blood draws. There were 36 individuals (2.4%, 36/1509) that tested positive by both methods and 145 (9.6%, 145/1509) individuals were identified as infected using either PCR or serology.

When comparing PCR with serology for the identification of *B. pertussis*, we found that the attack rate was similar using both methodologies, [(PCR: 6.2%, 94/1509) vs. serology: 5.8%, 87/1509) vs.; p=0.64] (Table 4.2). The *B. pertussis* PCR attack rate was higher in 2018 when compared to the serological attack rate for the same year [(PCR: 9.3%, 45/484) vs. (serology: 5.4%, 26/484) vs.; p=0.02]. Amongst individuals PCR-positive only, the mean (±standard deviation) episode duration was 11.3 days (±20.8), however, for cases PCR-positive with seroconversion, the mean episode duration was 18.8 days (±20.2), (p=0.09).

When comparing the attack rates by serology using the ≥2-fold rise and ≥4-fold rise in antibody titer, we found that the attack rate using ≥2-fold rise in titer was higher when compared to the ≥4-fold rise [(≥2-fold rise: 128/1509, 8.5%) vs. (≥4-fold rise: 87/1509, 5.8%); p=0.04]. However, when correlating this to PCR-confirmed cases, there was no differences in the number cases PCR-positive with seroconversion (only one additional case detected) when using the ≥2-fold rise in antibody titer [(≥2-fold rise: 37/1509, 2.5%) vs. (≥4-fold rise: 36/1509, 2.4%); p=0.91].

Of the 58 individuals positive by PCR only, 75.9% (44/58) had an episode duration of 3 days (with PCR IS*481* C_t values >35) and 24.1% (14/58) had an episode duration of ≥10 days (IS*481* C_t values ranging from 26 – 41) (Figure 4.4). Of the 14 individuals with longer episode durations, three (21.4%) did not have sera collected at next follow-up visit and two individuals (2018 cohort) only tested PCR-positive at the end of the follow-up period and therefore had no sera after the infection. The remaining nine individuals (64.3%, 9/14) had sera collected between 1.1 – 5.9 months post-positive

PCR. There were eight individuals (57.1%, 8/14) from two households that had family members that were positive by both PCR and serology during the same time period. All eight individuals had IS*481* C_t values >35 and episode duration of <7 days.

Of the 51 individuals positive by serology only, 39 individuals (46.5%, 39/51) seroconverted in subsequent years when no PCR data were available (part of the 2016 and 2017 cohort followed up in subsequent years were only sera were collected). The remaining 12 individuals (23.4%, 12/51) were PCR-negative for *B. pertussis* during the intense follow-up. Eight of these individuals (8/12, 66/7%) were children aged <10 years and all 12 individuals were HIV negative. Of the 12 individuals, 66.7% (8/12), 25.0% (3/12) and 8.3% (1/12) of individuals had between 75 – 80, 51 – 55 and 26 follow-up visits, respectively, over the study period. In addition, all swabs collected from these individuals during the study period had *RNaseP* (sample quality) C_t values of <33.

4.3.4. Factors associated with *B. pertussis* seroconversion

On multivariable analysis, we found that PCR-positive individuals aged 5 – 18 years were 6.84 times [aOR 6.84 (95% CI 1.33 – 35.08)] more likely to seroconvert when compared to individuals aged 19 – 44 years (Table 4.3). In addition, individuals with an episode duration of ≥7 days were 13.31 times more likely to seroconvert when compared to individuals with episode duration of <7 days [aOR 13.31 (95% CI 3.43 – 51.49)].

Exploring PCR-infection detected by any of our methods (PCR or serology), on univariate logistic regression analysis, we found that individuals aged 5 – 18 years were

at an increased risk of *B. pertussis* infection [OR 1.67 (95% CI 1.00 – 2.78)] compared to the 19 – 45 year olds, however, due to small numbers we were underpowered for the multivariable model (Table 4.4).

4.3.5. *B. pertussis* anti-PT IgG antibody titers over time

Individuals PCR-negative with no evidence of seroconversion expressed varying levels of anti-PT IgG antibody titers all below 100IU/ml (Figure 4.5).

Of the 36 individuals PCR-positive with seroconversion, the ≥4-fold rise in titer for all individuals was detected at the next follow-up visit (Figure 4.6 (a) and 4.6 (b)). For these individuals, the mean (range) time taken for sera to be collected following the first positive PCR was 2.9 months (range 3 weeks – 5.9 months). Of the 11 individuals previously reported as having two episodes of infection (49), only two individuals (18.2%) had seroconversion with anti-PT IgG titers ≥40 – <100IU/ml at the end of the second episode of infection. Following seroconversion and at the next follow-up visit, 61.1% (23/36) and 38.9% (14/36) of individuals had antibody titers of >100IU/ml and ≥40 – <100IU/ml, respectively. Thirteen individuals (76.5%, 13/17) with seroconversion still had antibody titers of ≥40 – <100IU/ml 12 month's post testing PCR positive (19 individuals excluded as seroconversion occurred at the last follow-up visit). The remaining four individuals had antibody titers <40IU/ml 12 month's post testing PCR positive.

Amongst infected individuals presumed to have been vaccinated with the whole-cell vaccine, 65.0% (13/20) had titers >100IU/ml following seroconversion and 30.8% (4/13) of these individuals still had antibodies >40IU/ml 12 month's following testing positive by PCR (Figure 4.6 (c)). Although smaller numbers, 40.0% (4/10) of

individuals presumed to have been vaccinated with the acellular vaccine had titers >100IU/ml following seroconversion (Figure 4.6 (d)) and these antibodies waned to < 40IU/ml within 12 month's post testing positive by PCR.

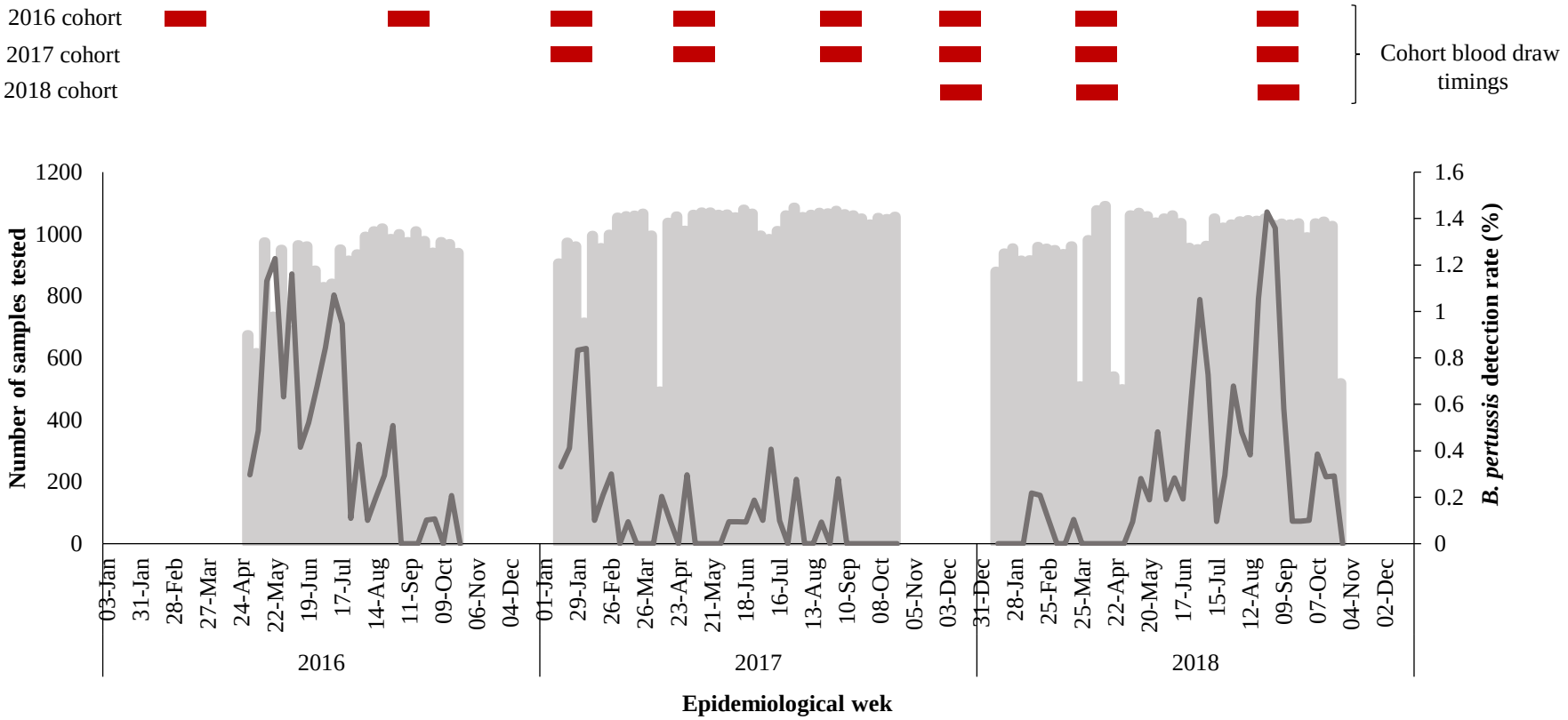


Figure 4.1: Number of nasopharyngeal specimens collected and tested for *B. pertussis* by PCR per week (grey bars), percent testing PCR positive (black line) and timing of blood collection (blood draws 1 – 8, red blocks) for the PHIRST study cohort, South Africa, 2016 – 2018.

2016 cohort = 8 blood draws; 2017 cohort = 6 blood draws; 2018 cohort = 3 blood draws.

Table 4.1: Demographic, clinical and household characteristics of individuals (≥1 serum collected) enrolled into the PHIRST study and tested by anti-PT IgG ELISA, South Africa, 2016 – 2018 (N=1618).

Characteristic	Overall n/N (%)	Rural n/N (%)	Urban n/N (%)
Year			
2016	529/1618 (32.7)	271/812 (33.4)	258/806 (32.0)
2017	553/1618 (34.2)	275/812 (33.9)	278/806 (34.5)
2018	536/1618 (33.1)	266/812 (32.8)	270/806 (33.5)
Sex			
Male	644/1618 (39.8)	298/812 (36.7)	346/806 (42.9)
Female	974/1618 (60.2)	514/812 (63.3)	460/806 (57.1)
Age group (years)			
<1	17/1618 (1.1)	8/812 (1.0)	9/806 (1.1)
1-4	206/1618 (12.7)	132/812 (16.3)	74/806 (9.2)
5-14	540/1618 (33.4)	303/812 (37.3)	237/806 (29.4)
15-24	271/1618 (16.8)	124/812 (15.2)	147/806 (18.2)
25-44	316/1618 (19.5)	141/812 (17.4)	175/806 (21.7)
45-64	195/1618 (12.1)	74/812 (9.1)	121/806 (15.0)
≥65	73/1618 (4.5)	30/812 (3.7)	43/806 (5.3)
HIV status			
Negative	1321/1570 (84.1)	683/800 (85.4)	638/770 (82.9)
Positive	249/1570 (15.9)	117/800 (14.6)	132/770 (17.1)
Nutritional status[a]			
Underweight	127/1611 (7.9)	54/812 (6.7)	73/799 (9.1)
Normal	937/1611 (58.2)	518/812 (63.8)	419/799 (52.4)
Overweight	263/1611 (16.3)	122/812 (15.0)	141/799 (17.7)
Obese	284/1611 (17.6)	11/812 (14.5)	166/799 (20.8)
Underlying illness[b]			
No	1572/1618 (97.2)	808/812 (99.5)	764/806 (94.8)
Yes	46/1618 (2.8)	4/812 (8.7)	42/806 (5.2)
Pertussis vaccination[c] (for age)			
Incomplete vaccination	4/179 (2.2)	2/107 (1.9)	2/72 (2.8)
Fully vaccinated	175/179 (97.8)	105/107 (98.1)	70/72 (97.2)
Number of household members			
≤5	768/1618 (47.5)	362/812 (44.5)	406/806 (50.3)
6-10	728/1618 (45.0)	385/812 (47.4)	343/806 (42.6)
>10	122/1618 (7.5)	65/812 (8.0)	57/806 (7.1)
Children <5 years in household			
No	466/1618 (28.8)	90/812 (11.1)	376/806 (46.7)
Yes	1152/1618 (71.2)	722/812 (88.9)	430/806 (53.4)

Crowding[d]			
No	712/1618 (44.0)	90/812 (11.1)	376/806 (46.7)
Yes	906/1618 (56.0)	722/812 (88.9)	430/806 (53.4)

[a] Nutritional status based on individual's body mass index (BMI). [b]Underlying illness defined as self-reported history of asthma, lung disease, heart disease, stroke, spinal cord injury, epilepsy, organ transplant, immunosuppressive therapy, organ transplantation, cancer, liver disease, renal disease or diabetes. [c]Only collected for children <5 years of age. [d]Crowding - more than two individuals in a household sleeping in the same room.

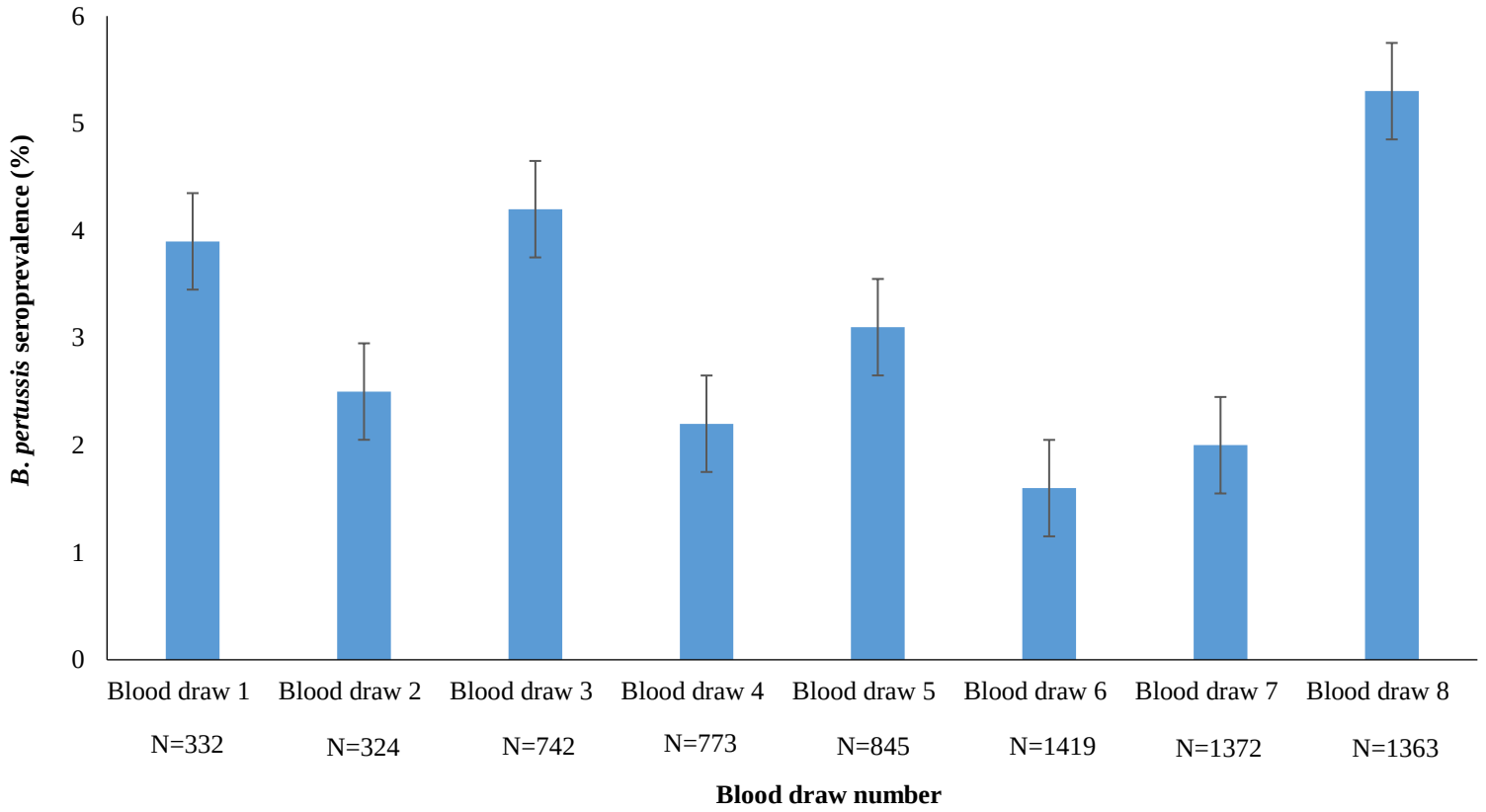
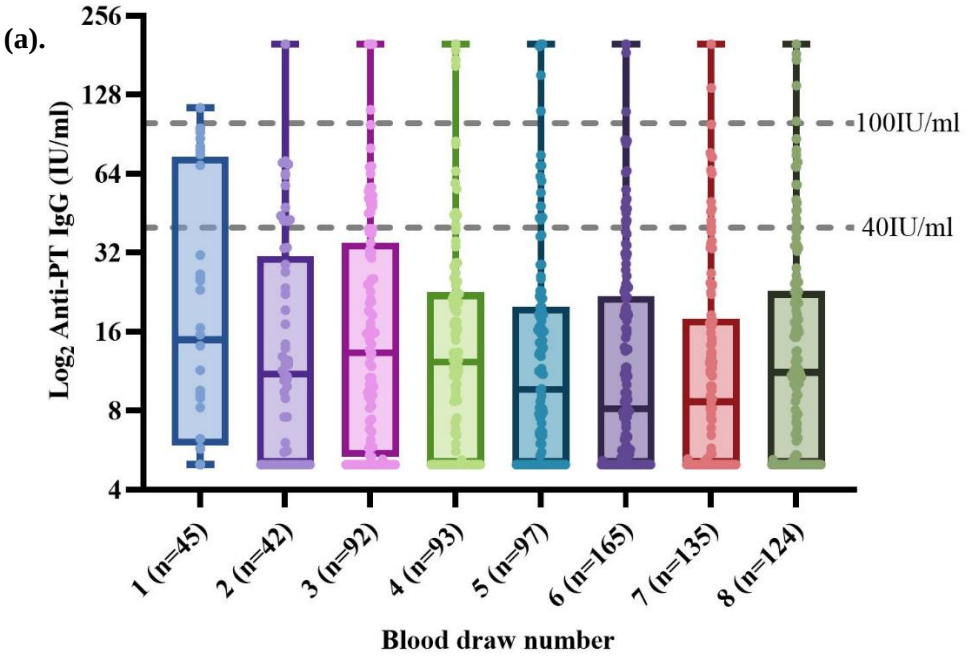
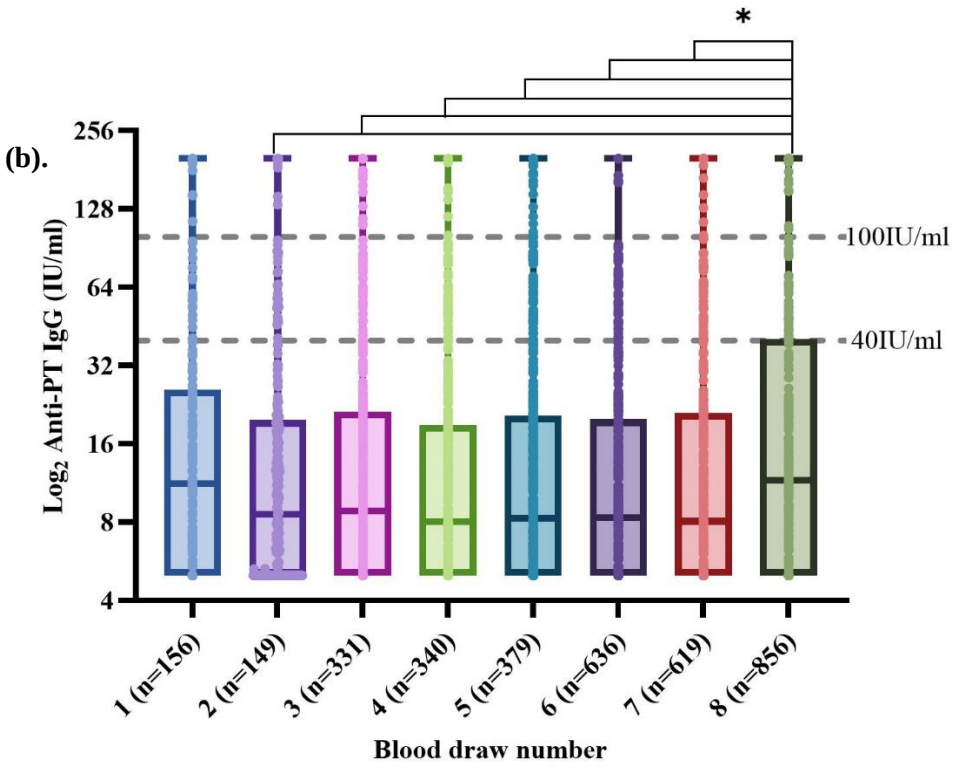


Figure 4.2: Seroprevalence of *B. pertussis* over the study period (per blood draw), PHIRST study, South Africa, 2016 – 2018.

(Blood draw 1: Mar – Apr 16; Blood draw 2: Oct – Nov 16; Blood draw 3: Feb – Mar 17; Blood draw 4: May 17; Blood draw 5: Oct 17; Blood draw 6: Jan – Feb 18; Blood draw 7: May – Jun 18; Blood draw 8: Oct 18). Error bars represent the 95% confidence interval.


(a).
256
128
64
32
16
8
4
Log2 Anti-PT IgG (IU/ml)
100IU/ml
40IU/ml
1 (n=45)
2 (n=42)
3 (n=92)
4 (n=93)
5 (n=97)
6 (n=165)
7 (n=135)
8 (n=124)
Blood draw number


*
(b).
256
128
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Log2 Anti-PT IgG (IU/ml)
100IU/ml
40IU/ml
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2 (n=149)
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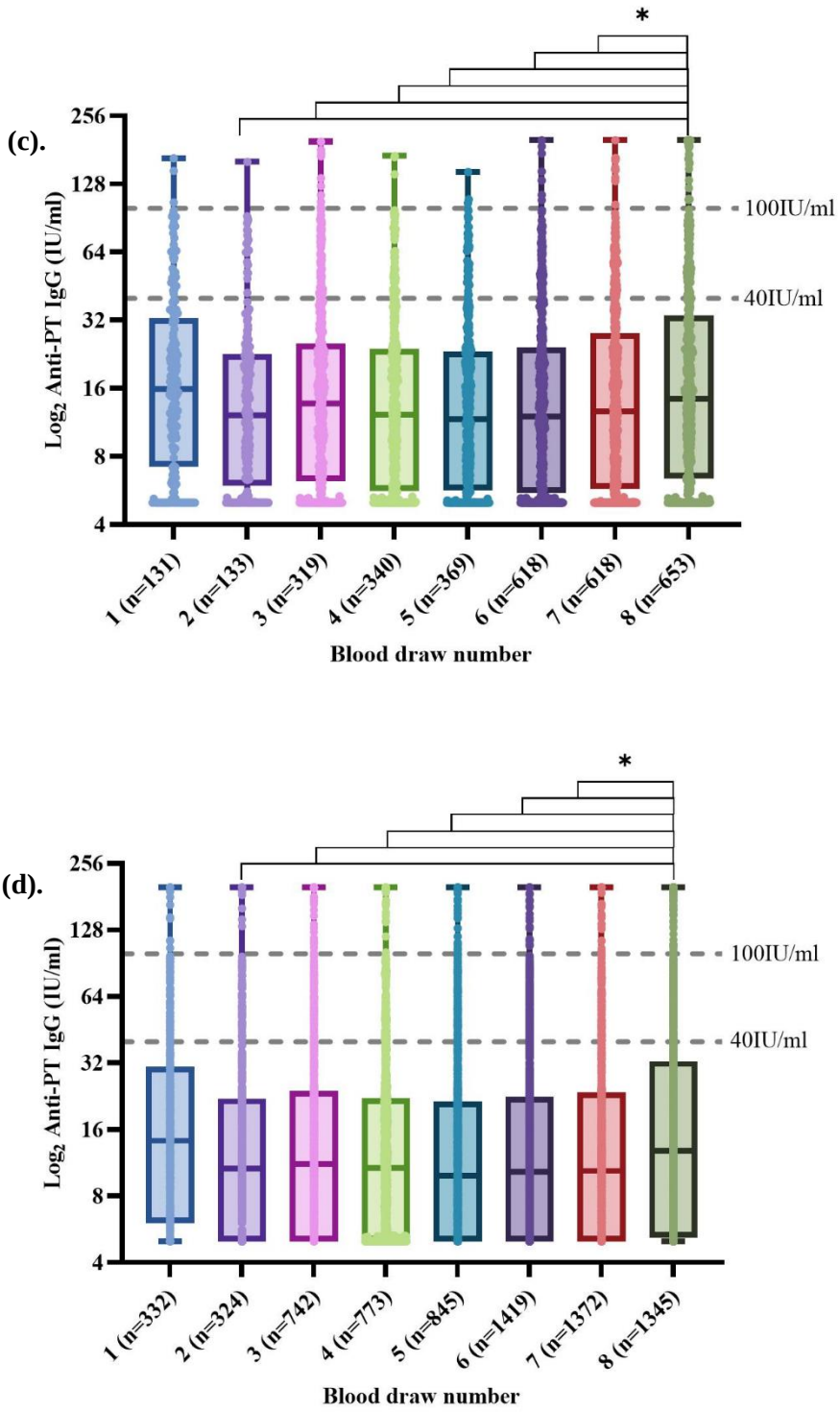
4 (n=340)
5 (n=379)
6 (n=636)
7 (n=619)
8 (n=856)
Blood draw number



4.3: Anti-PT IgG antibody titer by blood draw and age group ((a). <5 years; (b). 5 – 18 years; (c). ≥19 years; (d). overall age groups), PHIRST study, South Africa, 2016 – 2018. * p<0.01.

(Blood draw 1: Mar – Apr 16; Blood draw 2: Oct – Nov 16; Blood draw 3: Feb – Mar 17; Blood draw 4: May 17; Blood draw 5: Oct 17; Blood draw 6: Jan – Feb 18; Blood draw 7: May – Jun 18; Blood draw 8: Oct 18).

Table 4.2: *B. pertussis* attack rate by PCR, serology and a combination of both methods, PHIRST study, South Africa, 2016 – 2018 (N=1509).

Characteristic	Individuals positive by PCR n/N (%)	Individuals positive by serology n/N (%)	p-value[e]	Individuals positive by both PCR and serology n/N (%)	Individuals positive by PCR and/or serology n/N (%)
Attack rate	94/1509 (6.2)	87/1509 (5.8)	0.64	36/1509 (2.4)	145/1509 (9.6)
Year					
2016	26/507 (5.1)	38/507 (7.5)	0.15	8/507 (1.6)	56/507 (11.1)
2017	23/518 (4.4)	23/518 (4.4)	1.00	9/518 (1.7)	37/518 (7.1)
2018	45/484 (9.3)	26/484 (5.4)	**0.02**	19/484 (3.9)	52/484 (10.7)
Male	41/600 (6.8)	34/600 (5.7)	0.47	15/600 (2.5)	60/600 (10.0)
Female	53/909 (5.8)	53/909 (5.8)	1.00	21/909 (2.3)	85/909 (9.4)
Age group (years)					
<5	7/181 (3.9)	8/181 (4.4)	0.79	2/181 (1.1)	13/181 (7.2)
5-18	56/670 (8.4)	46/670 (6.9)	0.35	25/670 (3.7)	77/670 (11.5)
19-44	18/389 (4.6)	17/389 (4.4)	0.86	4/389 (1.0)	31/389 (7.9)
≥45	13/259 (5.0)	15/259 (5.8)	0.84	5/259 (1.9)	23/259 (8.9)
HIV status					
Negative	81/1238 (6.5)	76/1238 (6.1)	0.74	33/1238 (2.7)	124/1238 (10.0)
Positive	12/230 (5.2)	11/230 (4.8)	0.83	3/230 (1.3)	20/230 (8.7)
Nutritional status[a]					
Underweight	8/119 (6.7)	6/119 (5.0)	0.78	1/119 (0.8)	13/119 (10.9)
Normal	58/874 (6.6)	52/874 (5.9)	0.62	24/874 (2.8)	86/874 (9.8)
Overweight	14/244 (5.7)	10/244 (4.1)	0.53	4/244 (1.6)	20/244 (8.2)
Obese	13/270 (4.8)	19/270 (7.0)	0.36	7/270 (2.6)	25/270 (9.3)
Underlying illness[b]					
No	89/1464 (6.1)	83/1464 (5.7)	0.69	34/1464 (2.3)	138/1464 (9.4)
Yes	5/45 (11.1)	4/45 (8.9)	0.72	2/45 (4.4)	7/45 (15.6)
Pertussis vaccination[c]					
Unvaccinated	1/4 (25.0)	0/4 (0.0)	0.28	0/4 (0.0)	1/4 (25.0)
At least 1 dose	5/138 (3.6)	6/138 (4.4)	0.75	2/138 (1.4)	9/138 (6.5)

IS*481* C_t					
<34	20/20 (100.0)	N/A	N/A	15/20 (75.0)	20/20 (100.0)
34-39	54/54 (100)	N/A	N/A	16/54 (29.6)	54/54 (100)
40-45	20/20 (100.0)	N/A	N/A	5/20 (25.0)	20/20 (100.0)
PCR episode duration					
<7 days	55/55 (100.0)	N/A	N/A	11/55 (20.0)	55/55 (100.0)
≥7 days	39/39 (100.0)	N/A	N/A	25/39 (64.10)	39/39 (100.0)
Number of household members					
≤5	35/714 (4.9)	36/714 (5.0)	0.90	12/714 (1.7)	59/714 (8.3)
6-10	50/679 (7.4)	40/679 (5.9)	0.33	17/679 (2.5)	73/679 (10.8)
>10	9/116 (7.8)	11/116 (9.5)	0.81	7/116 (6.0)	13/116 (11.2)
Children <5 years in household					
No	28/437 (6.4)	26/437 (5.9)	0.88	7/437 (1.6)	47/437 (10.8)
Yes	66/1072 (6.2)	61/1072 (6.7)	0.71	29/1072 (2.7)	98/1072 (9.1)
Crowding[d]					
No	32/664 (4.8)	34/664 (5.1)	0.84	10/664 (1.5)	56/664 (8.4)
Yes	62/845 (7.3)	53/845 (6.3)	0.44	26/845 (3.1)	89/845 (10.5)

The *B. pertussis* attack rate was calculated as follows: no. individuals positive / no. individuals tested using serology (seroconversion between any 2 consecutive blood draws) and/or PCR (≥1 positive swab). [a]Nutritional status based on individual's body mass index (BMI). [b]Underlying illness defined as self-reported history of asthma, lung disease, heart disease, stroke, spinal cord injury, epilepsy, organ transplant, immunosuppressive therapy, organ transplantation, cancer, liver disease, renal disease or diabetes. [c]Only collected for children <5 years of age. [d]Crowding - more than two individuals in a household sleeping in the same room. NA – not applicable. C_t – cycle threshold. [e]Chi-squared test comparing cases positive by PCR only to those positive by serology only and bold font indicates statistical significance.

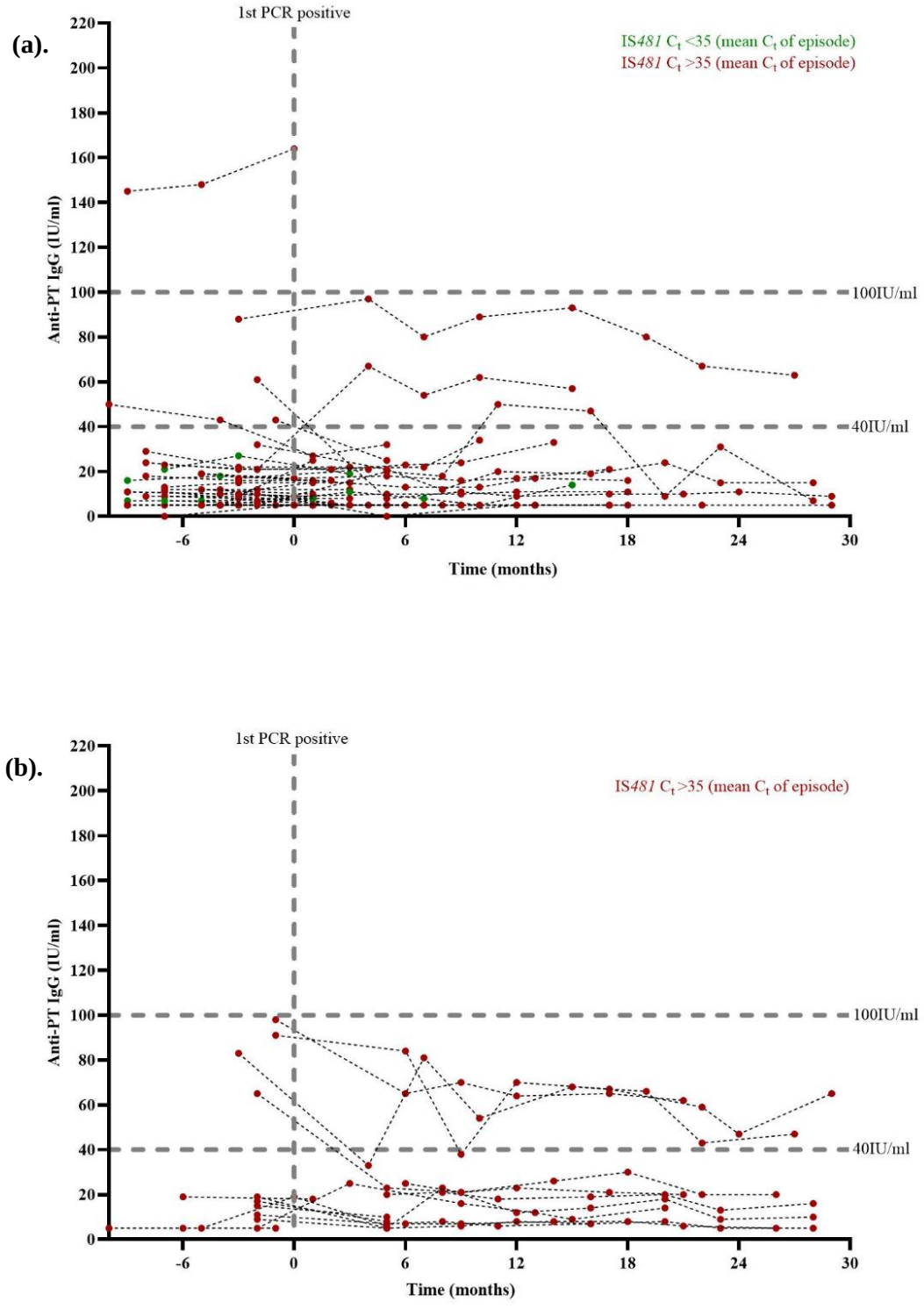


Figure 4.4: Anti-PT IgG antibody titers for *B. pertussis* PCR-positive individuals that did not seroconvert with (a) episode duration <7 days (n=43) and (b) episode duration ≥7 days (n=15), PHIRST study, South Africa, 2016 – 2018.

Table 4.3: Proportion of individuals PCR-positive for *B. pertussis* that seroconverted against anti-PT IgG and factors associated with seroconversion, PHIRST study, South Africa, 2016 – 2018 (N=94).

Characteristic	*B. pertussis* PCR cases with seroconversion n/N (%)	Univariate analysis		Multivariable analysis	
		Odds ratio (95% CI)	p value	Adjusted odds ratio (95% CI)	p value
Overall	36/94 (38.3)	-	-	-	-
Sex					
Male	15/41 (36.6)	Reference			
Female	21/53 (39.6)	1.10 (0.35 – 3.42)	0.86		
Age group (years)					
<5	2/7 (28.6)	1.98 (0.15 – 25.66)	0.60	3.01 (0.22 – 41.06)	0.41
5-18	25/56 (44.6)	4.25 (0.86 – 21.13)	0.07	**6.84 (1.33 – 35.08)**	**0.02**
19-44	4/18 (22.2)	Reference		Reference	
≥45	5/13 (38.5)	4.59 (0.53 – 39.91)	0.17	6.13 (0.74 – 50.45)	0.09
HIV status					
Uninfected	33/81 (40.7)	Reference			
Infected	3/12 (25.0)	0.29 (0.04 – 1.85)	0.19		
Nutritional status[a]					
Underweight	1/8 (12.5)	0.17 (0.01 – 2.40)	0.19		
Normal	24/58 (41.4)	Reference			
Overweight	4/14 (28.6)	0.40 (0.07 – 2.16)	0.29		
Obese	7/13 (5.9)	1.52 (0.32 – 7.28)	0.59		
Underlying illness[b]					
No	34/89 (38.2)	Reference			
Yes	2/5 (40.0)	0.96 (0.09 – 9.69)	0.97		
Pertussis vaccination[c]					
No doses	0/1 (0.0)	Reference			
At least 1 dose	2/5 (40.0)	2.14 (0.05 – 77.53)	0.67		

IS*481* C_t				
<34	15/20 (75.0)	11.72 (2.15 – 64.0)	0.04	
34-39	16/54 (29.6)	1.53 (0.39 – 5.97)	0.53	
40-45	5/20 (25.0)	Reference		
PCR episode duration				
<7 days	11/55 (20.0)	Reference		Reference
≥7 days	25/39 (64.1)	**8.95 (2.79 – 28.73)**	**<0.001**	**13.31 (3.43 – 51.49)**
Number of household members				
≤5	12/35 (34.3)	Reference		
6-10	17/50 (34.0)	0.95 (0.29 – 3.10)	0.93	
>10	7/9 (77.8)	11.01 (0.76 – 158.44)	0.07	
Children <5 years in household				
No	7/28 (25.0)	Reference		
Yes	29/66 (43.9)	2.06 (0.54 – 7.94)	0.29	
Crowding[d]				
No	10/32 (31.3)	Reference		
Yes	26/62 (41.9)	1.74 (0.47 – 6.40)	0.40	

[a] Nutritional status based on individual's body mass index (BMI). [b]Underlying illness defined as self-reported history of asthma, lung disease, heart disease, stroke, spinal cord injury, epilepsy, organ transplant, immunosuppressive therapy, organ transplantation, cancer, liver disease, renal disease or diabetes. [c]Only collected for children <5 years of age. Vaccine status calculated based on number of doses of vaccine received by age. [d]Crowding - more than two individuals in a household sleeping in the same room. Bold font indicates statistical significance. C_t – cycle threshold. Variables adjusted for in final model: Age group and PCR episode duration.

Table 4.4: Factors associated with *B. pertussis* infection (individuals positive on serology and/or PCR), PHIRST study, South Africa, 2016 – 2018 (N=1509).

Characteristic	*B. pertussis* attack rate n/N (%)	Univariate analysis	
		Odds ratio (95% CI)	p-value
Overall	145/1509 (9.6)		
Sex			
Male	60/600 (10.0)	Reference	
Female	85/909 (9.4)	0.92 (0.61 – 1.38)	0.69
Age group (years)			
<5	13/181 (7.2)	1.11 (0.51 – 2.40)	0.78
5-18	77/670 (11.5)	**1.67 (1.00 – 2.78)**	**0.04**
19-44	31/389 (7.9)	Reference	
≥45	23/259 (8.9)	1.23 (0.63 – 2.37)	0.53
HIV status			
Uninfected	124/1238 (10.0)	Reference	
Infected	20/230 (8.7)	0.81 (0.46 – 1.45)	0.49
Nutritional status[a]			
Underweight	13/119 (10.9)	1.13 (0.54 – 2.39)	0.73
Normal	86/874 (9.8)	Reference	
Overweight	20/244 (8.2)	0.67 (0.36 – 1.23)	0.19
Obese	25/270 (9.3)	0.91 (0.53 – 1.57)	0.73
Underlying illness[b]			
No	138/1464 (9.4)	Reference	
Yes	7/45 (15.6)	1.40 (0.48 – 4.06)	0.53
Pertussis vaccination[c]			
Unvaccinated	1/4 (25.0)	5.84 (0.77 – 44.17)	0.09
At least 1 dose	9/138 (6.5)	Reference	
Number of household members			
≤5	59/714 (8.3)	Reference	
6-10	73/679 (10.8)	1.35 (0.77 – 2.38)	0.28
>10	13/116 (11.2)	1.26 (0.36 – 4.33)	0.71
Children <5 years in household			
No	47/437 (10.8)	Reference	
Yes	98/1072 (9.1)	0.78 (0.43 – 1.40)	0.41
Crowding[d]			
No	56/664 (8.4)	Reference	
Yes	89/845 (10.5)	1.19 (0.69 – 2.06)	0.51

[a] Nutritional status based on individual's body mass index (BMI). [b]Underlying illness defined as self-reported history of asthma, lung disease, heart disease, stroke, spinal cord injury, epilepsy, organ transplant, immunosuppressive therapy, organ transplantation, cancer, liver disease, renal disease or diabetes. [c]Only collected for children <5 years of age. Vaccine status calculated based on number of doses of vaccine received by age. [d]Crowding - more than two individuals in a household sleeping in the same room. Bold font indicates statistical significance.

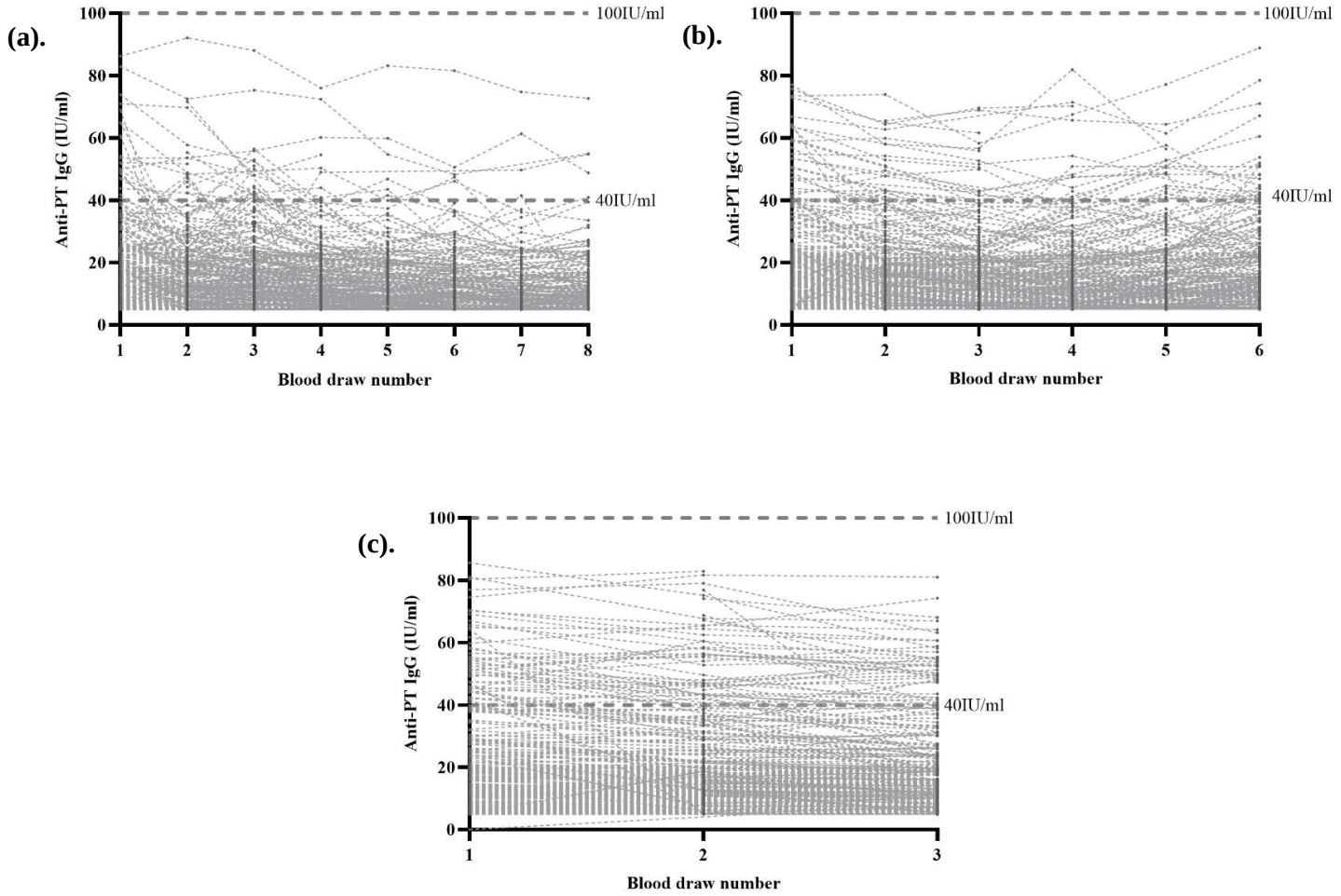
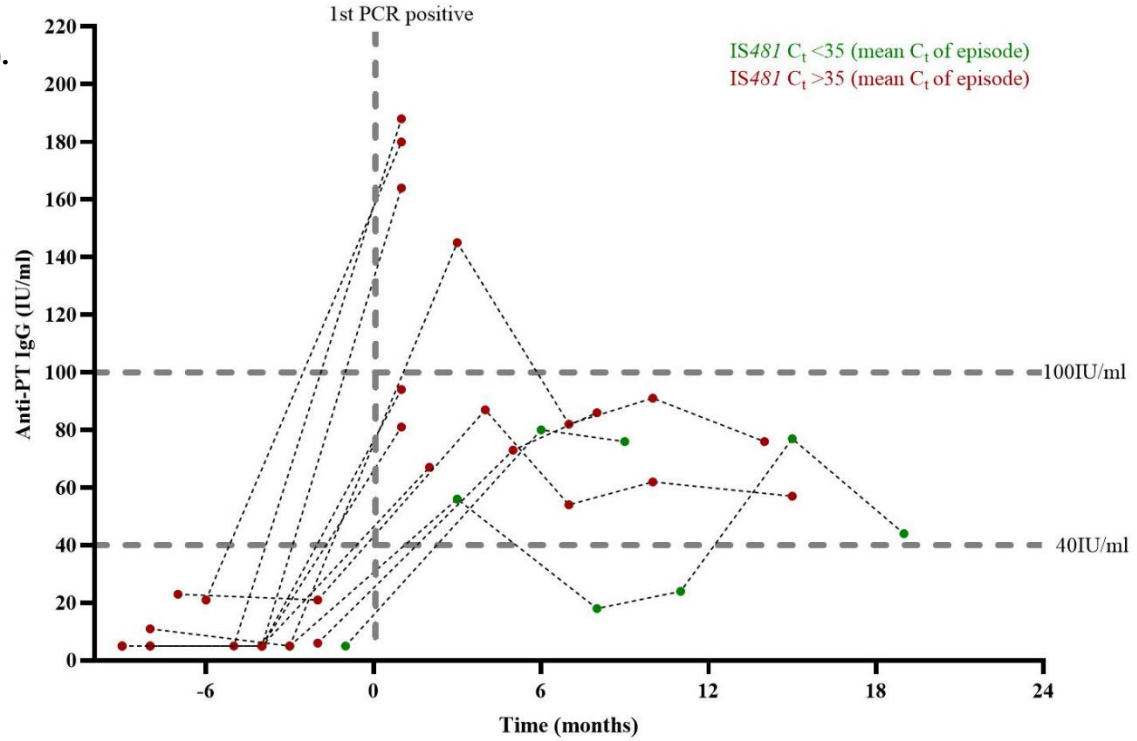


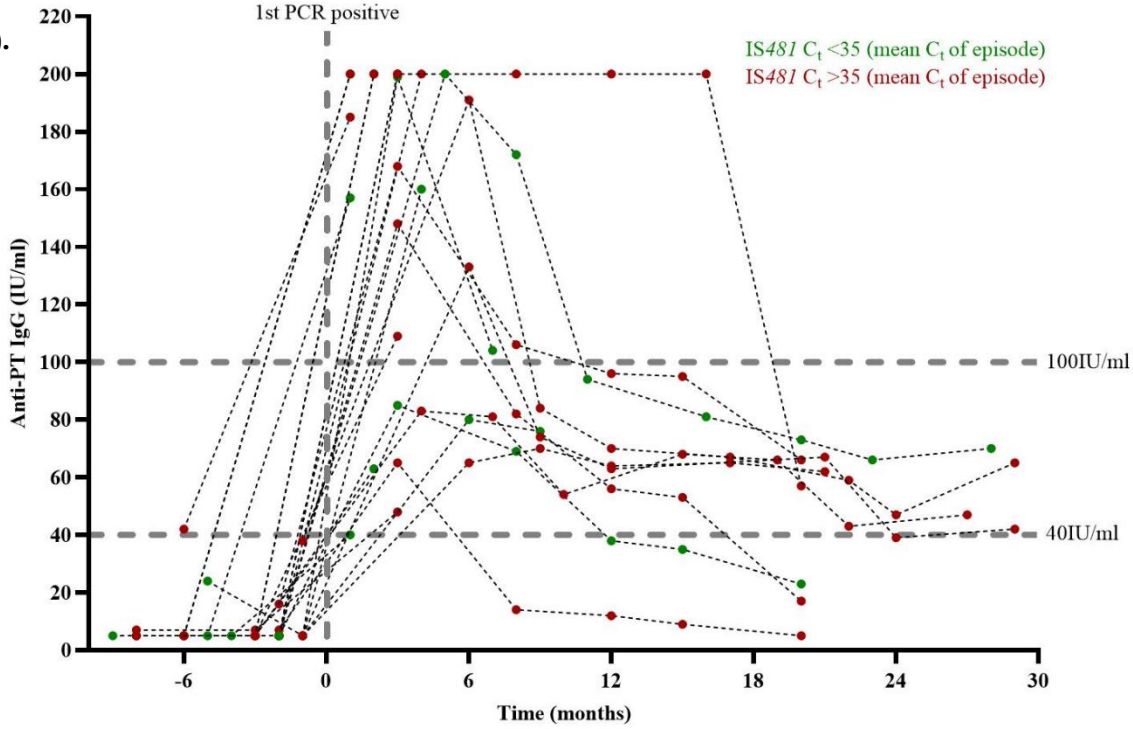
Figure 4.5: Anti-PT IgG titers for individuals negative for *B. pertussis* (by PCR and serology) by blood draw ((a): 2016; (b): 2017 and (c): 2018), PHIRST study, South Africa, 2016 – 2018.

(Blood draw 1: Mar – Apr 16; Blood draw 2: Oct – Nov 16; Blood draw 3: Feb – Mar 17; Blood draw 4: May 17; Blood draw 5: Oct 17; Blood draw 6: Jan – Feb 18; Blood draw 7: May – Jun 18; Blood draw 8: Oct 18)

(a).


1st PCR positive
IS481 Ct <35 (mean Ct of episode)
IS481 Ct >35 (mean Ct of episode)
Anti-PT IgG (IU/ml)
100IU/ml
40IU/ml
Time (months)

(b).


1st PCR positive
IS481 Ct <35 (mean Ct of episode)
IS481 Ct >35 (mean Ct of episode)
Anti-PT IgG (IU/ml)
100IU/ml
40IU/ml
Time (months)

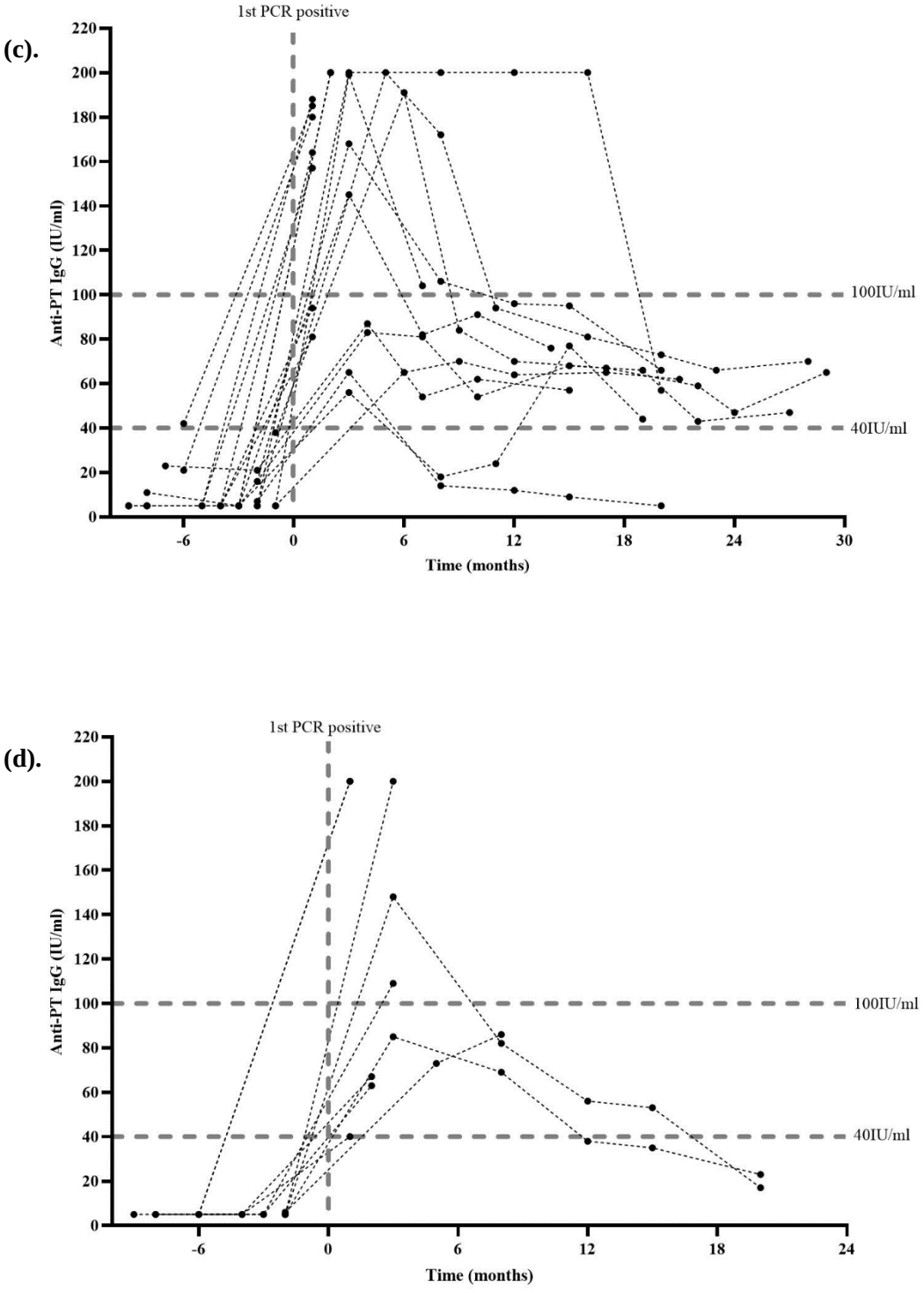


Figure 4.6: Anti-PT IgG antibody titers in individuals PCR-positive for *B. pertussis* with (a) episode duration <7 days (n=11); (b) episode duration ≥7 days (n=25); (c) assumed vaccinated with the whole-cell vaccine (n=20); (d) assumed vaccinated with the acellular vaccine (n=10), PHIRST study, South Africa,2016 – 2018.

4.4. Discussion

Within our study, those aged 5 – 18 years had the highest attack rate, were at increased risk of infection and were more likely to seroconvert following infection. Although different study designs and amongst those with symptomatic infection, a seroprevalence study in Tunisia (population using whole-cell vaccine) in 2018 amongst individuals aged 3 – 18 years of age found that the school aged children 13 – 18 years had the highest serological infection rates (3.1%) compared to the 6 – 12 year olds (1.6%) (40). A Belgian study (population using the acellular vaccine) conducted in 2015 using culture, PCR and serology for the detection of *B. pertussis*, found that 10 – 14 year-olds had the highest detection rate by both PCR (28.7%, 83/300) and serology (43.8%, 110/251) when compared to the other ages (97). Our overall attack rates were lower compared to other studies, however, our study enrolled asymptomatic individuals within the community whilst other studies focus on populations that are symptomatic. The WHO has reported older children/adolescents as an important source of transmission of *B. pertussis* to infants too young to be vaccinated (1) and our data suggests the same. At the start of 2024, the South African infant immunization program was revised to include additional booster doses of the acellular vaccine to children aged 5 and 12 years from 2024. In addition, maternal immunization has also been recommended for pregnant women (16 – 36 weeks of gestation for every pregnancy).

Some studies have shown that the detection rate of *B. pertussis* is higher using serology when compared to PCR, however, in our study the attack rates by both methods were similar with little overlap in results between the two methods. A Belgian study in 2015, using both serology and PCR to diagnose infection amongst symptomatic individuals,

found a higher detection rate using serology (17.3%) when compared to PCR (9.6%) (97). Another study in the Gambia (population using the whole cell vaccine) to determine the prevalence of *B. pertussis* amongst individuals aged three months to 12 years with persistent cough lasting ≥14 days documented a prevalence of 15% using PCR and 20% using serology (36). We are unable to directly compare our data to other studies as we determined serology and PCR attack rates using data from otherwise healthy individuals within the community whilst other studies calculated prevalence based data collected from symptomatic individuals.

Amongst pertussis cases, we were able to show that anti-PT IgG antibodies following infection peak within 2.9 months (3 weeks – 5.9 months) and remain elevated for at least 12 months. Although different study designs and suboptimal timing of sera collection relative to infection in our study, our findings were comparable to other studies. Sera collected from German adults with pertussis (n=11) at regular intervals (1 week after infection, 2 months and then every 6 months for a period of 24 months) showed that antibodies increased 11-fold within the first two months following infection then decreased rapidly although elevated antibodies (>40IU/ml) were detected at the end of follow up (average antibodies between blood draws IU/ml: 22, 242, 104, 81, 46, 45) (98). A study amongst Danish individuals with either natural infection (children vaccinated with acellular vaccine) or booster vaccination (adults that were primed with whole-cell vaccine) found that, following exposure, antibodies reached levels above 75IU/ml at first sampling (median 17 days since symptom onset), and following this, antibodies started to wane within 5 months following infection and within 3 months following vaccination (59).

Within the two communities, the *B. pertussis* seroprevalence varied from 3.9% at the beginning of 2016, dropping to 1.8% in 2017 with an increase to 5.3% by the end of October 2018. These serological data correlate with increases in *B. pertussis* cases reported in South Africa in 2018, detected through the Notifiable Medical Conditions System, Pneumonia Surveillance Program and pertussis PCR data from this PHIRST cohort (12,48,49,55)

Our study had limitations namely; the sera were not collected at optimal and consistent time points relative to the *B. pertussis* infections, which likely resulted in an underestimation of the number of cases with seroconversion. Ideally, the first blood sample should be collected between 3 – 6 weeks following infection and thereafter at regular time intervals. Due to limited data, we defined seroconversion as a ≥4-fold rise in antibody titer between consecutive blood draws, which could have resulted in an underestimation of cases with seroconversion. Although there was a significant increase in the attack rate when using the 2-fold rise in antibody titer, when correlating this to PCR data, only one additional PCR-positive case with seroconversion was identified, hence we chose to err on the side of specificity choosing the 4-fold rise in titer as our definition for seroconversion. We only had vaccine status for children aged <5 years, therefore, when determining antibody dynamics amongst infected individuals (by vaccine type) we used birth date as a proxy for vaccine type. The number of pertussis cases within the study was small so some statistical analyses may have been underpowered.

The strength of this study is the intensive follow-up for all enrolled individuals which included repeated sampling over time regardless of symptoms which allowed us to include individuals with asymptomatic infection contributing to transmission of infection within households. The systematic methodology ensured that the data collected are robust against sampling bias, and allowed us to monitor pertussis infection and antibody kinetics over time, and relative to PCR-confirmed infections.

Within the PHIRST community there was an overall low seroprevalence of *B. pertussis*. Our data showed that older children and individuals with longer episode duration were more likely to seroconvert following infection. Overall, PCR was not able to detect all cases with boost in antibodies and/or seroconversion. The data further highlighted that not all PCR-infected cases (including both symptomatic and asymptomatic individuals) seroconvert, however, amongst those with seroconversion, antibodies wane more rapidly, particularly in those vaccinated with the acellular vaccine.

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Chapter Five – Genomic characterization of *Bordetella pertussis* in South Africa, 2015 – 2019

5. Reference

Moosa F, du Plessis M, Weigand MR, Peng Y, Mogale D, de Gouveia L, et al. Genomic characterization of *Bordetella pertussis* in South Africa, 2015 – 2019. Microb Genomics. 2023 Dec 1;9(12). doi: 10.1099/mgen.0.001162.

5.1. Introduction

B. pertussis can undergo structural changes caused by alterations in their genome either by insertions, deletions or gene rearrangements, resulting in the circulation of strains that have diverged from vaccine strains (67,68,71,78). Strains not expressing the vaccine antigens PRN and FHA have been described in the United States following the use of acellular vaccines (67,72,73). Following acellular vaccine introduction, PRN-deficient strains identified in France were shown to be as virulent as PRN-expressing strains (74). These data highlight the potential for the emergence of other vaccine escape mutants following the use of acellular pertussis vaccines and emphasize the importance of genomic surveillance for monitoring *B. pertussis* strain evolution in response to immune pressure. There are currently no data describing *B. pertussis* lineages in South Africa. Using WGS, we aimed to describe the genotypes and identify new or emerging immune escape mutations amongst circulating *B. pertussis* in South Africa.

5.2. Materials and methods

5.2.1. Surveillance population and data collection

Individuals were enrolled into Pneumonia Surveillance as described in section 2.2 and 2.2.1. We also used individual data and specimens from two additional laboratory platforms, i.e., pertussis pediatric surveillance and NICD diagnostics.

Pediatric pertussis surveillance was conducted at the Chris Hani Baragwanath Academic Hospital (CHBAH) in Soweto, Gauteng Province (17). CHBAH is the third largest hospital in the world and predominantly services the Soweto community where unemployment is high and <10% of community households have private medical insurance. All infants aged <12 months who were hospitalized at CHBAH from January through December 2015 with lower respiratory tract infection or sepsis were approached for enrolment. Nasopharyngeal swabs were collected and tested for *B. pertussis* by culture and real-time PCR (17).

In addition, public sector clinicians in South Africa occasionally send respiratory specimens (nasopharyngeal specimens, sputum, tracheal aspirates) from patients of all ages to the NICD for *B. pertussis* diagnosis.

5.2.2. Specimen collection and laboratory testing

Isolates (n=32) were sourced from national Pneumonia Surveillance (n=14), pediatric pertussis surveillance (n=11) and diagnostic samples (n=7). *B. pertussis* culture was attempted on all respiratory specimens that were PCR-positive for *B. pertussis* as described in section 2.2.2.

5.2.3. DNA extraction, sequencing and genome assembly

Genomic DNA was extracted from cultures using the Gentra Pure Gene Yeast/Bact. kit (QIAGEN, Hilden, Germany) with the QIAGEN protocol (67). DNA was quantified using the Qubit® 2.0 fluorimeter (Invitrogen, Oregon, USA) and Qubit® dsDNA BR assay kit and sequenced using both the Illumina MiSeq (Illumina Inc., California, USA) and the PacBio RSII (Pacific Biosciences, California, USA) sequencing platforms. PacBio sequence data were used to identify genome structural variation. A combination of sequence data from both platforms was used when sequence data were incomplete or when results were not conclusive.

Genomic libraries for Illumina sequencing were prepared (paired-end libraries, 2x 300bp) using the Nextera XT DNA v3 MiSeq sequencing kit. Reads were trimmed at both ends to remove nucleotides with a quality score of less than 45bp using CLC Genomics Workbench (v21.0.3, CLC Bio, Aarhus Denmark). The passed reads were *de novo* assembled using CLC Genomics Workbench. Illumina genomes were assessed for quality using QUAST (http://bioinf.spbau.ru/quast) on genome fraction, duplication ratio, total length, N50 value and number of contigs (78). Acceptable Illumina data included: genome fraction >90%; total length >3.75MB; N50 >5kb and contigs between 300 – 350. Libraries for PacBio sequencing were prepared using the SMRTbell template prep kit (Pacific Biosciences, California, USA) and polymerase binding kit. PacBio sequencing data were *de novo* assembled, polished and reoriented to the common start position (creating a closed genome) using a CDC in-house Perl script as previously described (78). The closed, assembled genomes were assessed on genome fraction, total length, and N50 value (78). Acceptable PacBio data included: genome fraction >90%;

total length >3.75MB; N50 >5kb. The reference genome used was *B. pertussis* E476 (vaccine strain: Tohama I – accession number: CP010964).

5.2.4. Multilocus sequence typing and vaccine antigen gene typing

Assembled Illumina sequences were used to determine the *B. pertussis* MLST profile. Sequence type (ST) was assigned using the BIGSdb platform curated by the Institute Pasteur (https://bigsdb.pasteur.fr/*bordetella*) according to seven housekeeping genes (*adk, fumC, glyA,* t*ryB, icd*, *pepA* and *pgm*).

Detection of the *ptxP, ptxA, ptxB, prn, fimH* genes, and mutations within the *prn* gene was performed using a high stringency Basic Local Alignment Search (BLASTn) alignment (https://blast.ncbi.nlm.nih.gov/Blast) on assembled genomes as previously described (67,78). Assembled PacBio sequences were analyzed in a similar manner to supplement inconclusive results or incomplete Illumina sequence coverage.

5.2.5. Phylogeny of *B. pertussis*

To determine the relationship between South African *B. pertussis* isolates and publicly available genomes of isolates from other countries, a tree was constructed by incorporating genomes from different countries (New Zealand, USA, Netherlands, Sweden, France, Norway, India, Japan, and China) available on NCBI (accessed February 2023) that were representative of circulating strains (different ST's) on each continent between 2012 – 2019, and also represented isolates with varying *ptxP* types (*ptxP1*, *ptxP2* and *ptxP3*). These included the reference genome *B. pertussis* E476 and the additional available vaccine strains from India (84). A maximum-likelihood phylogenetic tree of 99 isolate genomes was constructed based on the concatenated

alignment of the high quality SNP's using CSI phylogeny (v1.4) (99) with additional tree annotation performed using iTOL (v6).

5.2.6. Identification of genome structural variation

Genome structural variation was determined by aligning complete PacBio assemblies from 21 South African *B. pertussis* genomes to each other using progressive Mauve (v20150226) with default parameters (99). The genomes were determined to be collinear if pairwise alignments included no observable inversions or gaps >1500bp. Alignments were manually inspected and grouped into types based on each unique alignment. One representative sequence from each arrangement type (SA001, SA002 and SA003) as well as the singleton sequences (genome structures not shared with any other isolates) were further aligned, using progressiveMauve, to reported structures from the USA (CDC002, CDC010, CDC013, CDC046, CDC082 and CDC237) and New Zealand (NZ004, NZ005, NZ006, NZ007, and NZ008) (78,81).

5.2.7. Data availability

The genome sequences for 32 South Africa *B. pertussis* isolates are available on NCBI, organized under BioProject accession number PRJNA929342.

5.3. Results

5.3.1. *B. pertussis* isolates

A total of 32 *B. pertussis* isolates were available for sequencing. Isolates were obtained from children (n=25) and adults (n=7) from both surveillance as well as clinical diagnostics (Appendix F). All 32 isolates were cultured from sporadic *B. pertussis* cases. There were no known epidemiological links between the *B. pertussis* cases.

From 2015 – 2019, a total of 17,097 hospitalized individuals were enrolled into Pneumonia Surveillance of which 228 (1.3%) tested PCR-positive for *B. pertussis*. We attempted culture on 108 of these PCR-positive samples ($C_t \leq 25$). A total of 14 samples (12.9%, 14/108) yielded a *B. pertussis* culture. Positive cultures were isolated from individuals ranging from <3 months to 64 years of age, and 50.0% (7/14) of cases were people living with HIV.

On the pertussis pediatric surveillance platform, from 1 January to 31 December 2015, *B. pertussis* PCR positivity was 2.3% (42/1839) (17). A *B. pertussis* culture was obtained from 26.2% (11/42) of PCR-positive samples. Cases with positive culture were infants aged <3 months, and the majority were unvaccinated (72.7%, 8/11) and HIV unexposed (63.6%, 7/11).

A total of 770 respiratory samples were received from 2015 – 2019 for *B. pertussis* diagnostic testing, of which 12.1% (93/770) tested positive for *B. pertussis* by PCR. A total of 41 of these positive samples were further cultured and seven samples yielded a *B. pertussis* culture (7/41, 17.1%), all of which were from infants aged <3 months.

5.3.2. Molecular characterization of *B. pertussis*

Using WGS data, all 32 *B. pertussis* isolates were characterized as ST2 (allelic profile: *adk*1, *fumC*1, *glyA*1, *tryB*3, *icd*1, *pepA*1, *pgm*1). Isolates belonged to the *ptxP*3 lineage and all genomes harbored an intact PRN gene with no mutations. The vaccine antigen profile *ptxP*3-*ptxA*1-*ptxB*2-*prn*2-*fimH*2 was identified in 31/32 (96.9%) isolates. Overall, based on molecular characterization, all vaccine antigen genes were wild-type. One isolate (SA12) had a SNP within the *fimH*2 allele (A245G synonymous mutation)

which was further classified as *fimH*2-1. This mutation was confirmed using both the Illumina and PacBio sequence data. The vaccine antigen profile for this isolate was *ptxP*3-*ptxA*1-*ptxB*2-*prn*2-*fimH*2-1.

5.3.3. Phylogeny of *B. pertussis*

All South African isolates clustered within the *ptxP*3 clade and were phylogenetically distinct from *B. pertussis* E476 and other global strains (Figure 5.1). There were two clusters [(Cluster 1: 7 South Africa + 1 New Zealand) and (Cluster 2: 6 South Africa + 4 USA)] where South African isolates clustered with isolates from the USA and New Zealand. Despite having identical ST and vaccine antigen profiles, based on SNP's, the South African isolates were genetically distinct from isolates from other countries (with the exception of the 1 New Zealand and 4 USA strains), clustering closely to each other within the tree. In addition, looking at the South African isolates only, there was no geographical clustering of strains and no temporal association of strains was noted.

5.3.4. *B. pertussis* genome structural variation

Of the 32 isolates sequenced using the PacBio, 21 (65.6%) yielded closed genome sequences that could be analyzed for structural variation. The remaining 11 genomes yielded incomplete assemblies with more than one contig and thus could not be used to assess genome structure. Thirteen isolates (13/21, 62%) could be grouped into three distinct arrangement types [SA001 (n=8), SA002 (n=2) and SA003 (n=3)] based on genome patterns, whilst eight isolates demonstrated unique ('singleton') structures distinct from the other arrangement types (Figure 5.2). Genomic differences between the arrangement types were due to inversions centered around the replication terminus of the *B. pertussis* genome and differed from reference strain E476 (Tahoma I). The inversions were flanked by the *B. pertussis* multi-copy insertion sequence IS*481*.

Comparing the genome structures to isolates from the USA and New Zealand, we found that SA001 was identical in structure to CDC013/NZ005 and SA002 matched structure CDC082/NZ004. In addition, one South African singleton structure (SA20) matched genome structure CDC046/NZ48. There was no concordance between SNP clusters and genome structural clusters amongst South African, USA and New Zealand isolates that shared the same genome structures (Figure 5.1). Amongst the SA001/CDC013/NZ005 isolates, we found <18 SNP differences amongst the South African isolates within the structure and <25 SNP differences amongst South African isolates compared with the USA isolates and the New Zealand isolates. In addition, there were no SNP differences amongst the South African isolates within SA002/CDC082/NZ004 cluster but when the SA isolates were compared to USA and New Zealand isolates, we picked up <18 SNP differences amongst all the isolates.

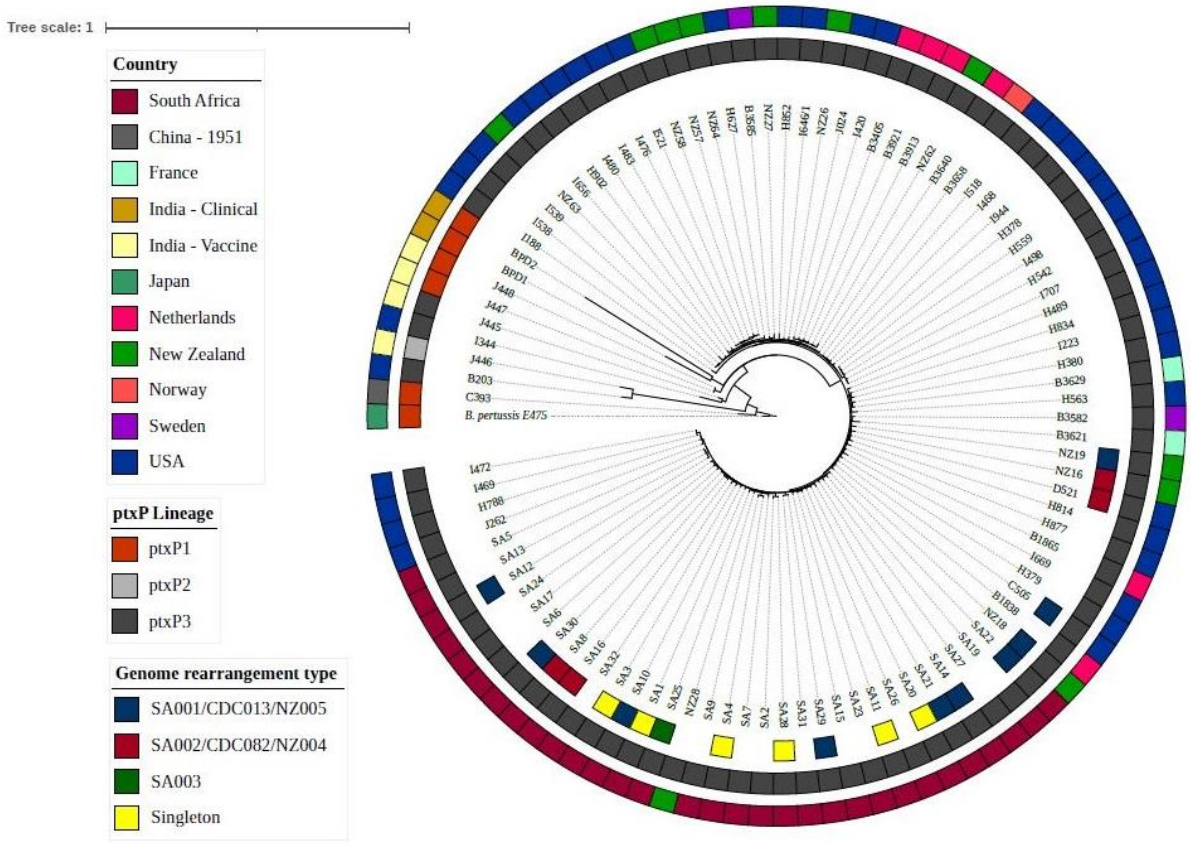


Figure 5.1: Maximum likelihood phylogenetic tree for South African *B. pertussis* isolates and isolates from other geographic locations (2012 – 2019) based on the concatenated alignment of single nucleotide polymorphisms of the whole genome. The tree was rooted using the *B. pertussis* E476 reference (vaccine strain: Tohama I). The inner ring of the figure indicates the genome rearrangement type for South African isolates, the middle ring indicates the *ptxP* lineage and the outer ring indicates the country of isolation.

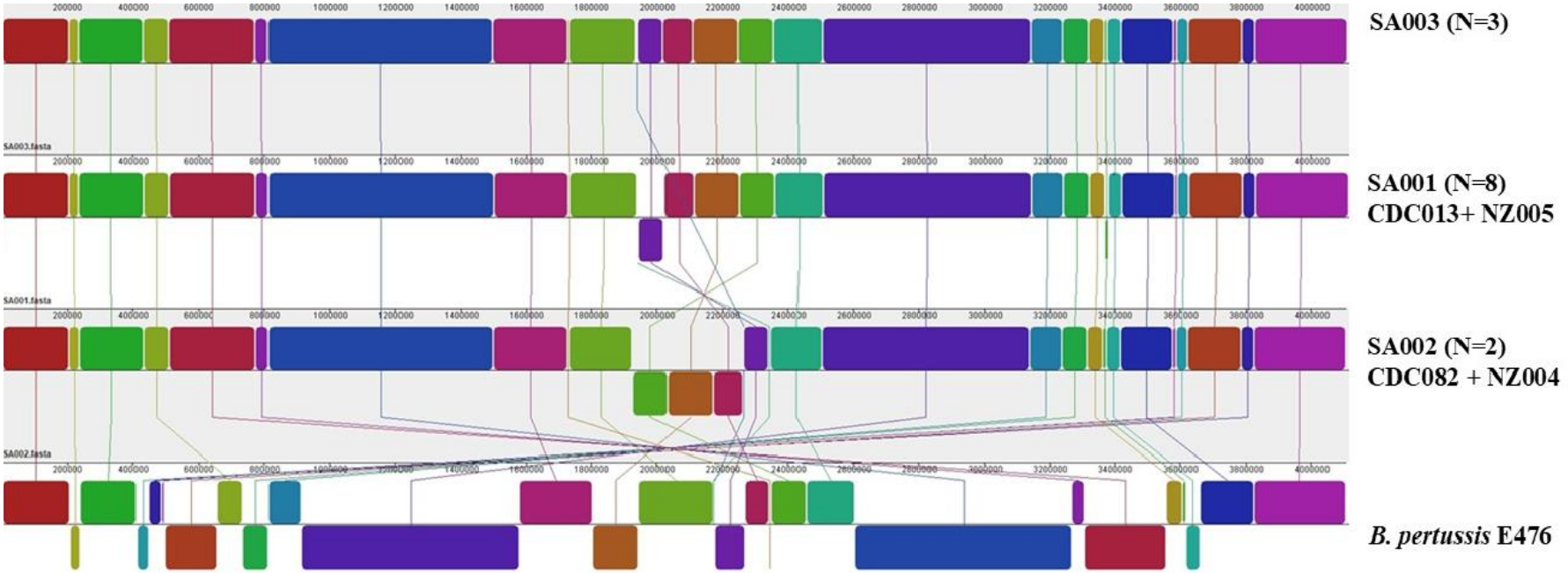


Figure 5.2: Genome organization and re-arrangement patterns amongst South African *B. pertussis* isolates collected in 2015 – 2019 in relation to reference E476 (N=13). Each colored block represents a region of the genome called a localized co-linear block (LCB). A LCB below the central black line indicates an inversion event.

5.4. Discussion

Using MLST, South African *B. pertussis* isolates were the same ST (ST2) that has been dominant globally since the late 1990s (80). In addition, all harbored the same pertussis toxin promoter allele, *ptxP*3. Globally, strains harboring *ptxP*3 first emerged in the 1980s (71). Subsequently, over 90% of globally circulating strains now harbor this allele (100,101).

In other countries, mutations in genes encoding vaccine antigens of *B. pertussis* appear to be more common since the introduction of the acellular vaccine, presumably due to vaccine pressure (64,65,71,102). In our setting, only one isolate contained a SNP within the vaccine antigen *fimH*2 gene. In addition, the South African isolates had an intact PRN gene and no other mutations were identified within the other vaccine antigen genes. In the USA, PRN-containing vaccines were introduced in the 1990's. From 2000 – 2013 in the USA, there was a rapid increase in PRN-deficient *B. pertussis* isolates, resulting in non-expression of the gene (103). In Australia the acellular PRN-containing vaccine was introduced in the 1990's and strains lacking the PRN gene were first detected between 2008 – 2012 (104). Additionally, isolates collected between 2012 – 2017 showed spread of PRN-deficient strains as well as an isolate that was negative for FHA. In Spain, between 1986 – 2018 a total of 342 *B. pertussis* isolates were collected for molecular characterization (105). Of the 342 isolates, 27% (93/342) were PRN-deficient and all of these PRN-deficient isolates were collected after the introduction of the acellular vaccine. Mutations within PRN and other vaccine antigen genes were not detected among South African isolates, likely due to the formulation of the acellular pertussis vaccine (Hexaxim, Sanofi Pasteur), which only contains the PT and FHA

antigens, whilst many other countries are using vaccines containing the PRN antigen as well as two or more other antigens. At the beginning of 2024, maternal immunization has been recommended for use in South Africa. The formulation of the vaccine to be used includes PRN and fim, so ongoing genomic surveillance following the introduction of this vaccine will be important to monitor potential mutations within vaccine antigen genes amongst circulating *B. pertussis* strains in South Africa.

Using the traditional typing method of MLST and vaccine antigen profile data, our isolates represented a single clone. However, both methods rely on a very limited set of genes in the genome, limiting their discriminatory power. Determining the complete genomic structures of *B. pertussis* isolates using long read sequencing technology is relatively new and was first described in 2017 (78). Using this method, WGS provided additional resolution and identified three distinct genome structures amongst 13 isolates. A combination of both MiSeq and PacBio sequence data proved to be advantageous as we used both data sets to create complete genomes from which we were able to define the genome structure. Studies have shown marked differences amongst *B. pertussis* genome structures despite having an otherwise monomorphic profile (based on other genome characterization methods). During two state-wide epidemics in the USA in 2010 – 2012, whole genome data were useful in identifying 16 distinct genome structures from 31 isolates that were otherwise monomorphic based on other typing techniques, and all differed from the vaccine strains (67). In addition, Weigand et al., provides some evidence of phenotypic diversity amongst genome structures by showing the expansion of ‘cluster 01 (same genome structure)’ within the USA by analyzing data from pre 2000 to 2016 (85). Similarly, in New Zealand, WGS data showed 19 different

genome rearrangements amongst 66 isolates (81). An integrated comparative genomics analysis using isolates collected from Czech Republic between 2008 – 2012, showed that circulating *B. pertussis* has substantial variation in genome organization and form separate phylogenetic clusters based on time of collection (historic isolates vs. currently circulating isolates) (86). When using proteomics, they found that genome rearrangements resulted in changes in gene order, orientation or in large deletions, which affected transcriptomic profiles in *B. pertussis*. Results support the authors assumption that genomic rearrangements might affect global expression profiles and phenotypic diversity in *B. pertussis*. Based on the above mentioned data, genome structures/rearrangements could be useful signatures of genome evolution. Within our study, two of the South African genomic structures (SA001 and SA002) identified were shared with structures identified in isolates collected in the USA and New Zealand, showing that these structures are not unique to South Africa. In addition, comparing the SNP differences amongst the South African, USA and New Zealand isolates within shared structures, we see that isolates within the same structures are genetically similar (<25 SNP differences). However, based on this analysis we are unable to determine ancestral relationships amongst the South African isolates and isolates from USA and New Zealand with shared genome structures. Previous studies have demonstrated the low SNP density amongst global *B. pertussis* isolates making it difficult to deduce geographic clustering and/or source attribution (71,106). Similarly, Weigand et al., has also shown that isolates of *B. pertussis* with shared genome structures are not automatically similar based on SNP's (85). There is little data available that describes the importance of structural variation and more studies are required to fully understand

the prevalence of arrangement type, and associations with geographic location, phenotype or strain fitness.

This study has some limitations that need to be considered. To our knowledge, there are no earlier *B. pertussis* genomic data available from South Africa so we are unable to track changes in the genomes over a longer period of time. The absence of earlier data also limits conclusions pertaining to the origin of currently circulating strains. In addition, South Africa changed to the acellular vaccine in 2009, and isolates sequenced in this study were collected between 2015 and 2019. This time period may therefore be too short to observe the effects of the change in vaccine. We had a small sample size of 32 genomes, representing <5% of *B. pertussis* PCR-positive samples. Therefore, these data are not representative of all *B. pertussis* causing disease in the country and we may have missed detecting novel or emerging lineages or vaccine antigen mutations. *B. pertussis* is a fastidious organism that is difficult to culture in the laboratory, requires specialized culture media which has a short shelf life, and long incubation periods of 7 – 10 days, limiting its diagnostic utility. Diagnostic laboratories in South Africa do not routinely perform *B. pertussis* culture and rely predominantly on PCR testing for *B. pertussis* diagnosis. In addition, the low culture yield, incomplete geographical representation of sites and small sample numbers don't allow for confidence regarding potential temporal and geographic conclusions. The Pneumonia Surveillance program focuses on respiratory viral detection and nasopharyngeal swabs are transported in viral transport medium which is not conducive to *B. pertussis* culture. Ideally, WGS of *B. pertussis* directly from clinical specimens needs to be optimized in our setting for future molecular characterization studies (65).

Although limited in number and spanning a short time period of 5 years, we show that circulating *B. pertussis* strains in South Africa do not yet harbor mutations in vaccine antigen genes, as has been observed in other countries. WGS provided additional discriminatory power and identified three distinct genome structures.

Chapter Six - Conclusion

Data on pertussis epidemiology in low- and middle-income countries, particularly in Africa, are lacking, and as a result the epidemiology of the disease is poorly understood.

Within our community cohort, there was a high incidence of *B. pertussis* and the majority of infected individuals were asymptomatic. We detected 118 episodes of *B. pertussis* in 107 participants, with 11 participants having two episodes of *B. pertussis* infection. The overall household cumulative infection risk was 14%, and 39% of index cases in the household were asymptomatic. The mean duration of infection was 12 days. Individuals positive for *B. pertussis* were more likely to report symptoms if they were infected for ≥7 days. Transmission was more likely to occur from male index cases and individuals with longer infection duration. Our data showed that asymptomatic individuals that were infected with *B. pertussis* transmitted to other household members and hence represent a source of transmission to vulnerable age groups that are too young to be vaccinated. Overall, the higher incidence of *B. pertussis* infection in children with incomplete vaccination underscores the need for robust vaccination programs.

Despite a high incidence of cases within the community, detected by PCR, there was low seroprevalence of *B. pertussis* that ranged from 3.9% at the beginning of 2016, dropping to 1.6% (beginning of 2018) and then increasing to 5.2% by the end of the study period. The increase in seroprevalence at the end of 2018 correlated with increases in *B. pertussis* cases reported in South Africa in 2018, detected through the Notifiable Medical Conditions System, Pneumonia Surveillance Program and pertussis

PCR data from this PHIRST cohort. The attack rate using serology was 5.8%, which was similar to the PCR attack rate (6.2%). Among individuals with PCR-confirmed infection, 38.3% had seroconversion confirmed in our study design, with individuals aged 5 – 18 years and with longer episode duration more likely to seroconvert. For all individuals PCR-positive with seroconversion, the ≥4-fold rise in anti-PT IgG titer was detected by the next blood draw [mean of 2.9 months (range 3 weeks – 5.9 months) following infection], and 76% of these individuals maintained antibody titers of >40IU/ml 12 month's post infection. Our data highlight that not all individuals with PCR-confirmed infection could have seroconversion detected or confirmed in our study. These data underscore the need for further studies to understand and define the correlates of protection to *B. pertussis* infection. The resurgence of pertussis in our community appears to be linked to the waning of antibodies amongst vaccinated/infected individuals. These data emphasize that current acellular vaccine schedules should be revised to include booster doses in age groups with lower immunity, to prevent spread to vulnerable age groups, those too young to be vaccinated.

There are currently limited *B. pertussis* genomic data from Africa and no previously published data from South Africa. Although limited in number and spanning a short time period of five years, we show that all circulating *B. pertussis* strains in South Africa were the globally-disseminated sequence type 2. The dominant vaccine antigen genotype in all isolates except one was *ptxP3-ptxA1-ptxB2-prn2-fimH*2 and there was no genotypic evidence of mutations within the genes encoding vaccine antigens, as has been observed in other countries using the acellular containing vaccines. In addition, WGS provided additional discriminatory power and identified three distinct genome

structures circulating in South Africa that were identical to structures observed in the USA and New Zealand. These findings now provide baseline genomic data for future studies within our country and in the global context. Maintaining rigorous genomic surveillance for *B. pertussis* is important to detect any future changes in the pathogen's genome that could affect vaccine effectiveness. However, due to the complications associated with WGS from *B. pertussis* culture, WGS of *B. pertussis* directly from clinical specimens needs to be optimized in our setting for future molecular characterization studies.

In conclusion, this study highlights the persistent challenge posed by *B. pertussis*, even in the context of widespread vaccination. Our data have implications to help guide recommendations for improved vaccination strategies in low- and middle-income countries including South Africa. By identifying specific risk factors and transmission dynamics, this research offers a roadmap for targeted interventions to aid in better management of pertussis in South Africa. The lack of significant genetic variation in our circulating strains suggest that the resurgence in cases in South Africa are not likely due to bacterial evolution and points towards waning immunity due to the switch to the acellular vaccine (which also does not protect against *B. pertussis* colonization) and/or population immunity gaps.

At the start of 2024, the South African infant immunization program was revised to include additional booster doses of the acellular vaccine (vaccine formulation contains pertactin and fimbriae) to children aged 5 and 12 years (https://www.amayeza-info.co.za/vaccine-info/ - date accessed: March 2024). In addition, maternal

immunization has also been recommended for pregnant women (16 – 36 weeks of gestation for every pregnancy). Therefore, ongoing surveillance (including genomic surveillance) is imperative following the introduction of these booster doses to monitor VE in the population, pertussis epidemiology, as well as to determine potential genomic evolution amongst circulating *B. pertussis* strains, with emphasis on monitoring changes in genes encoding vaccine antigens.

6. References

1. Pertussis vaccines: WHO position paper – August 2015. Available from: https://www.who.int/wer/2015.
2. S WVH. *Bordetella pertussis*. In: R MGBJD, editor. Mandell, Douglas, and Benett's Principals and Practice of Infectious Diseases. 7th ed. 2011. p. 2955–64.
3. Crowcroft NS, Pebody RG. Recent developments in pertussis. Lancet. 2006;367(9526):1926-36.
4. Preston A. *Bordetella pertussis*: The intersection of genomics and pathobiology. CMAJ. 2005;173(1):55-62.
5. Gregory DS, Family L, Residency M, Gregory DS, Family L, Residency M. Pertussis : A disease affecting all ages. Am Fam Physician. 2006 ;74(3):420-6.
6. Mattoo S, Cherry JD. Molecular pathogenesis, epidemiology, and clinical manifestations of respiratory infections due to *Bordetella pertussis* and other *Bordetella* subspecies. Clin Microbiol Rev. 2005;18(2):326–82.
7. Riffelmann M, Ko CHW Von, Caro V. Nucleic acid amplification tests for diagnosis of *Bordetella* infections. 2005;43(10):4925–9.
8. Guiso N, Berbers G, Fry N, He Q, Riffelmann M, Wirsing Von König CH, et al. What to do and what not to do in serological diagnosis of pertussis: Recommendations from EU reference laboratories. Eur J Clin Microbiol Infect Dis. 2011;30(3):307–12.
9. Barkoff AM, Gröndahl-Yli-Hannuksela K, He Q. Seroprevalence studies of pertussis: what have we learned from different immunized populations. Pathog Dis. 2015;73(7):50.
10. Tatti KM, Wu K, Tondella ML, Cassiday PK, Cortese MM, Wilkins PP, et al.

Development and evaluation of dual-target real-time polymerase chain reaction assays to detect *Bordetella* spp. Diagn Microbiol Infect Dis. 2008;61(3):264-72.

11. Tatti KM, Sparks KN, Boney KO, Tondella ML, Tatti KM, Sparks KN, et al. Novel multitarget real-time PCR assay for rapid detection of *Bordetella* Species in clinical specimens. J Clin Microbiol. 2011;49(12):4059-66.
12. Moosa F, du Plessis M, Wolter N, Carrim M, Cohen C, Von Mollendorf C, et al. Challenges and clinical relevance of molecular detection of *Bordetella pertussis* in South Africa. BMC Infect Dis. 2019;19(1):1–11.
13. Tatti KM, Martin SW, Boney KO, Brown K, Clark TA, Tondella ML. Qualitative assessment of pertussis diagnostics in United States laboratories. Pediatr Infect Dis J. 2013;32(9):942–5.
14. Muyldermans G, Soetens O, Antoine M, Bruisten S, Vincart B, Doucet-Populaire F, et al. External quality assessment for molecular detection of *Bordetella pertussis* in European laboratories. J Clin Microbiol. 2005;43(1):30–5.
15. Mandal S, Tatti KM, Woods-stout D, Cassiday PK, Faulkner E, Griffith MM, et al. Pertussis pseudo-outbreak linked to specimens contaminated by *Bordetella pertussis* DNA from clinic surfaces. Pediatrics. 2012 Feb;129(2):e424-30.
16. Howson CP, Fineberg H V. Adverse events following pertussis and rubella vaccines: Summary of a Report of the Institute of Medicine. JAMA J Am Med Assoc. 1992;267(3):392–6.
17. Soofie N, Nunes MC, Kgagudi P, Van Niekerk N, Makgobo T, Agosti Y, et al. The burden of pertussis hospitalization in HIV-exposed and HIV-unexposed south african infants. Clin Infect Dis. 2016;63(Suppl 4):S165–73.
18. WHO and UNICEF estimates of immunization coverage: 2019 revision. 2020.

[cited 2020 Apr 24]. Available from: https://www.who.int/immunization/monitoring surveillance/data/zaf.pdf.

19. Warfel JM, Zimmerman LI, Merkel TJ. Acellular pertussis vaccines protect against disease but fail to prevent infection and transmission in a nonhuman primate model. Proc Natl Acad Sci. 2014;111(2):787–92.
20. Baxter R, Bartlett J, Rowhani-rahbar A, Fireman B, Klein NP. Effectiveness of pertussis vaccines for adolescents and adults : case-control study. BMJ. 2013; 17;347:f4249.
21. Witt MA, Katz PH, Witt DJ. Unexpectedly limited durability of immunity following acellular pertussis vaccination in preadolescents in a north American outbreak. Clin Infect Dis. 2012;54(12):1730–5.
22. Dalby T, Harboe ZB, Krogfelt KA, Dalby T, Harboe ZB, Krogfelt KA. Seroprevalence of pertussis among danish patients with cough of unknown etiology. Clin Vaccine Immunol. 2010;17(12):2016-23.
23. Zouari A, Smaoui H, Brun D, Njamkepo E, Sghaier S, Guiso N, et al. Prevalence of *Bordetella pertussis* and *Bordetella parapertussis* infections in Tunisian hospitalized infants: results of a 4-year. Diagn Microbiol Infect Dis. 2012 ;72(4):303-17.
24. Fisman DN, Tang P, Hauck T, Richardson S, Drews SJ, Low DE, et al. Pertussis resurgence in Toronto , Canada : a population-based study including test-incidence feedback modeling. BMC Public Health. 2011;11(1):694.
25. Muloiwa R, Wolter N, Mupere E, Tan T, Chitkara AJ, Forsyth KD, et al. Pertussis in Africa: Findings and recommendations of the Global Pertussis Initiative (GPI). Vaccine. 2018;36(18):2385–93.

26. Abu-Raya B, Forsyth K, Halperin SA, Maertens K, Jones CE, Heininger U, et al. Vaccination in pregnancy against pertussis: A consensus statement on behalf of the Global Pertussis Initiative. Vaccines. 2022;10(12):1990.

27. Skoff TH, Deng L, Bozio CH, Hariri S. US infant pertussis incidence trends before and after implementation of the maternal tetanus, diphtheria, and pertussis vaccine. JAMA Pediatr. 2023;177(4):395-400.

28. Amirthalingam G, Campbell H, Ribeiro S, Fry NK, Ramsay M, Miller E, et al. Sustained effectiveness of the maternal pertussis immunization program in England 3 years following introduction. Clin Infect Dis. 2016;63(suppl 4):S236–43.

29. Amirthalingam G, Campbell H, Ribeiro S, Stowe J, Tessier E, Litt D, et al. Optimization of timing of maternal pertussis immunization from 6 years of postimplementation surveillance data in England. Clin Infect Dis. 2023; 76(3):e1129–39.

30. Saul N, Wang K, Bag S, Baldwin H, Alexander K, Chandra M, et al. Effectiveness of maternal pertussis vaccination in preventing infection and disease in infants: The NSW Public Health Network case-control study. Vaccine. 2018;36(14):1887–92.

31. Bhagat D, Saboui M, Huang G, Domingo FR, Squires SG, Salvadori MI, et al. Literature Surveillance on COVID-19: Pertussis epidemiology in Canada, 2005–2019. Can Commun Dis Rep. 2023;49(1):21-28.

32. Tong J, Buikema A, Horstman T. Epidemiology and disease burden of pertussis in the United States among individuals aged 0–64 over a 10-year period (2006–2015). Curr Med Res Opin. 2020;36(1):127–37.

33. Wu DX, Chen Q, Yao KH, Li L, Shi W, Ke JW, et al. Pertussis detection in children with cough of any duration. BMC Pediatr. 2019;19(1):1–9.

34. Yeung KHT, Duclos P, Nelson EAS, Hutubessy RCW. An update of the global burden of pertussis in children younger than 5 years: a modelling study. Lancet Infect Dis. 2017;17(9):974–80.

35. Macina D, Mathur S, Dvaretskaya M, Ekhtiari S, Hayat P, Montmerle M, et al. Estimating the pertussis burden in adolescents and adults in the United States between 2007 and 2019. Hum Vaccin Immunother. 2023;19(1):2208514.

36. Kayina V, Kyobe S, Katabazi FA, Kigozi E, Okee M, Odongkara B, et al. Pertussis prevalence and Its determinants among children with persistent cough in urban Uganda. PLoS One. 2015;10(4):e0123240.

37. Razafimahatratra SL, Wesolowski A, Rafetrarivony L, Heraud JM, Jones FK, Cauchemez S, et al. Seroprevalence of pertussis in Madagascar and implications for vaccination. Epidemiol Infect. 2020;148:e283.

38. Scott S, Van Der Sande M, Faye-Joof T, Mendy M, Sanneh B, Barry Jallow F, et al. Seroprevalence of pertussis in the Gambia: Eevidence for continued circulation of *Bordetella pertussis* despite high vaccination rates. Pediatr Infect Dis J. 2015;34(4):333–8.

39. Taye S, Tigabu Z, Damtie D, Yismaw G, Moodley C, Nicol MP, et al. Pertussis among patients with clinically compatible illness in the Amhara Regional State, Ethiopia. Int J Infect Dis. 2021;106:421–8.

40. Fraj I Ben, Zghal M, Hsairi M, Kechrid A, Smaoui H. Seroprevalence of *Bordetella pertussis* toxin antibodies in children and adolescents in Tunis, Tunisia. Epidemiol Infect. 2019 Jan;147:e199.

41. Jusot V, Aberrane S, Alé F, Laouali B, Moussa I, Alio SA, et al. Prevalence of *Bordetella* infection in a hospital setting in Niamey, Niger. J Trop Pediatr. 2014;60(3):223–30.

42. Muloiwa R, Kagina BM, Engel ME, Hussey GD. The burden of laboratory-confirmed pertussis in low-and middle-income countries since the inception of the Expanded Programme on Immunisation (EPI) in 1974: A systematic review and meta-analysis. BMC Med. 2020 Aug 28;18(1):233.

43. Category 1 Notifiable Medical Conditions. Available from: www.nicd.ac.za/Disease-list_2018-per-category1.pdf: date accessed: December 2023.

44. Rensburg MA, Esser M, de Beer C. The seroprevalence of *Bordetella pertussis* antibodies in adolescents in the Western Cape . South African J Epidemiol Infect. 2013;28(4):202–6.

45. Cohen C, Moyes J, Tempia S, Groom M, Walaza S, Pretorius M, et al. Severe influenza-associated respiratory infection in high HIV prevalence setting, South Africa, 2009-2011. Emerg Infect Dis. 2013;19(11):1766–74.

46. Strebel P, Hussey G, Metcalf C, Smith D, Hanslo D, Simpson J. An outbreak of whooping cough in a highly vaccinated urban community. J Trop Pediatr. 1991;37(2):71–6.

47. MuloiwaID R, Dube FS, Nicol MP, Hussey GD, Zar HJ. Risk factors for *Bordetella pertussis* disease in hospitalized children. PLoS One. 2020;15(10):e0240717.

48. Wolter N, Cohen C, Tempia S, Walaza S, Moosa F, du Plessis M, et al. Epidemiology of pertussis in individuals of all ages hospitalized with Respiratory

illness in South Africa, January 2013—December 2018. Clin Infect Dis. 2021;73(3):e745-e753.

49. Moosa F, Tempia S, Kleynhans J, McMorrow M, Moyes J, du Plessis M, et al. Incidence and transmission dynamics of *Bordetella pertussis* infection in rural and urban communities, South Africa, 2016–2018. Emerg Infect Dis. 2023;29(2):294–303.

50. Increase in Pertussis cases in South Africa (21 Sept 2022) - NICD. Available from: https://www.nicd.ac.za/increase-in-pertussis-cases-in-south-africa-21-sept-2022/: date accessed: December 2023.

51. An increase in pertussis cases (13 Dec 2022) - NICD. Available from: https://www.nicd.ac.za/an-increase-in-pertussis-cases-13-dec-2022/: date accessed: December 2023.

52. Hallbauer UM, Joubert G, Goosen Y. Pertussis in children in Bloemfontein, South Africa: A 7-year retrospective review. S Afr Med J. 2016 Sep 8;106(10):1042-1046.

53. Barger-Kamate B, Knoll MD, Kagucia EW, Prosperi C, Baggett HC, Brooks WA, et al. Pertussis-associated pneumonia in infants and children from low-and middle-income countries participating in the perch study. Clin Infect Dis. 2016;63(Suppl 4):S187–96.

54. Muloiwa R, Dube FS, Nicol MP, Zar HJ, Hussey GD. Incidence and diagnosis of pertussis in South African children hospitalized with lower respiratory tract infection. Pediatr Infect Dis J. 2016 Jun;35(6):611–6.

55. Update on increase in pertussis cases in outh Africa, November. 2018. Available from: www.nicd.ac.za/up: date accessed: December 2023.

56. Nunes MC, Downs S, Jones S, van Niekerk N, Cutland CL, Madhi SA. *Bordetella pertussis* infection in South African HIV-infected and HIV-uninfected mother-infant dyads: A longitudinal cohort study. Clin Infect Dis. 2016;63(suppl 4):S174–80.

57. Wendelboe AM, Van Rie A, Salmaso S, Englund JA. Duration of immunity against pertussis after natural infection or vaccination. Pediatr Infect Dis J. 2005 May;24(5 Suppl):S58-61.

58. Ward JI, Cherry JD, Chang SJ, Partridge S, Keitel W, Edwards K, et al. *Bordetella pertussis* infections in vaccinated and unvaccinated adolescents and adults, as assessed in a national prospective randomized acellular pertussis vaccine trial (APERT). Clin Infect Dis. 2006;43(2):151–7.

59. Dalby T, Petersen JW, Harboe ZB, Krogfelt KA, Dalby T. Antibody responses to pertussis toxin display different kinetics after clinical *Bordetella pertussis* infection than after vaccination with an acellular pertussis vaccine. J Med Microbiol. 2010;59(9):1029-1036.

60. Hallander HO, Ljungman M, Storsaeter J, Gustafsson L. Kinetics and sensitivity of ELISA IgG pertussis antitoxin after infection and vaccination with *Bordetella pertussis* in young children. APMIS. 2009;117(11):797–807.

61. Pawloski LC, Kirkland KB, Baughman AL, Martin MD, Talbot EA, Messonnier NE, et al. Does tetanus-diphtheria-acellular pertussis vaccination interfere with serodiagnosis of pertussis infection. Clin Vaccine Immunol. 2012;19(6):875–80.

62. Jõgi P, Soeorg H, Oona M, Kaart T, Toompere K, Maskina T, et al. Dynamics of pertussis toxin IgG after symptomatic pertussis in children and adults. Vaccine. 2020;38(16):3196–200.

63. Van Twillert I, Marinović AAB, Kuipers B, Van Gaans-Van Den Brink JAM, Sanders EAM, Van Els CACM. Impact of age and vaccination history on long-term serological responses after symptomatic *B. pertussis* infection, a high dimensional data analysis. Sci Rep. 2017;7:40328.

64. Ring N, Abrahams JS, Bagby S, Preston A, MacArthur I. How Genomics is changing what we know about the evolution and genome of *Bordetella pertussis*. Adv Exp Med Biol. 2019;1183:1–17.

65. Barkoff AM, He Q. Molecular Epidemiology of *Bordetella pertussis*. Adv Exp Med Biol. 2019;1183:19–33.

66. Bart MJ, Harris SR, Advani A, Arakawa Y, Bottero D, Bouchez V, et al. Global population structure and evolution of *Bordetella pertussis* and their relationship with vaccination. mBio. 2014 Apr 22;5(2):e01074.

67. Bowden KE, Weigand MR, Peng Y, Cassiday PK, Sammons S, Knipe K, et al. Genome structural diversity among 31 *Bordetella pertussis* isolates from two recent U.S. whooping cough statewide epidemics. mSphere. 2016;1(3):e00036-16.

68. Van Gent M, Bart MJ, Van Der Heide HGJ, Heuvelman KJ, Mooi FR. Small mutations in *Bordetella pertussis* are associated with selective sweeps. PLoS One. 2012;7(9):46407.

69. Weigand MR, Peng Y, Loparev V, Batra D, Bowden KE, Burroughs M, et al. The history of *Bordetella pertussis* genome evolution includes structural rearrangement. J Bacteriol. 2017;199:806–22.

70. Advani A, Hallander HO, Dalby T, Krogfelt KA, Guiso N, Njamkepo E, et al. Pulsed-field gel electrophoresis analysis of *Bordetella pertussis* isolates

circulating in Europe from 1998 to 2009 from nine countries with distinct vaccination programs. J Clin Microbiol. 2013;51(2):422-8.

71. Bart MJ, Harris SR, Advani A, Arakawa Y, Bottero D, Bouchez V, et al. Global population structure and evolution of *Bordetella pertussis* and their relationship with vaccination. MBio. 2014;5(2):1–13.

72. Pawloski LC, Queenan AM, Cassiday PK, Lynch AS, Harrison MJ, Shang W, et al. Prevalence and molecular characterization of pertactin-deficient *Bordetella pertussis* in the United States. Clin Vaccine Immunol. 2014;21(2):119-25.

73. Weigand MR, Pawloski LC, Peng Y, Ju H, Burroughs M, Cassiday PK, et al. Screening and genomic characterization of filamentous hemagglutinin-deficient *Bordetella pertussis*. Infect Immun. 2018;86(4):e00869-17.

74. Evolution of French *Bordetella pertussis* and *Bordetella parapertussis* isolates: increase of Bordetellae not expressing pertactin. Clin Microbiol Infect. 2012;18(9):e340-6.

75. Sealey KL, Harris SR, Fry NK, Hurst LD, Gorringe AR, Parkhill J, et al. Genomic analysis of isolates from the United Kingdom 2012 pertussis outbreak reveals that vaccine antigen genes are unusually fast evolving. J Infect Dis. 2015;212(2):294-301.

76. Plotkin SA. The pertussis problem. Clin Infect Dis. 2014;58(6):830–3.

77. Sealey KL, Belcher T, Preston A. *Bordetella pertussis* epidemiology and evolution in the light of pertussis resurgence. Infect Genet Evol. 2016;40:136–43.

78. Weigand MR, Peng Y, Loparev V, Batra D, Bowden KE, Burroughs M, et al. The history of *Bordetella pertussis* genome evolution includes structural rearrangement. J Bacteriol. 2017;199(8):e00806-16.

79. Weigand MR, Peng Y, Pouseele H, Kania D, Bowden KE, Williams M, et al. Genomic surveillance and improved molecular typing of *Bordetella pertussis* using wgMLST. J Clin Microbiol. 2021;59(5):e02726-20.

80. Kim S-H, Lee J, Sung HY, Yu JY, Kim SH, Park MS, et al. Recent trends of antigenic variation in *Bordetella pertussis* isolates in Korea. J Korean Med Sci. 2014;29:328–33.

81. Ring N, Davies H, Morgan J, Sundaresan M, Tiong A, Preston A, et al. Comparative genomics of *Bordetella pertussis* isolates from New Zealand, a country with an uncommonly high incidence of whooping cough. Microb Genom. 2022;8(1):000756.

82. Weigand MR, Peng Y, Cassiday PK, Loparev VN, Johnson T, Juieng P, et al. Complete genome sequences of *Bordetella pertussis* isolates with novel pertactin-deficient deletions. Genome Announc. 2017;5(37):e00973-17.

83. Bouchez V, Guglielmini J, Dazas M, Landier A, Toubiana J, Guillot S, et al. Genomic sequencing of *Bordetella pertussis* for epidemiology and global surveillance of whooping cough. Emerg Infect Dis. 2018;24(6):988-994.

84. Alai S, Ghattargi VC, Gautam M, Patel K, Pawar SP, Dhotre DP, et al. Comparative genomics of whole-cell pertussis vaccine strains from India. BMC Genomics. 2020;21(1):345.

85. Weigand MR, Peng Y, Batra D, Burroughs M, Davis JK, Knipe K, et al. Conserved patterns of symmetric inversion in the genome evolution of *Bordetella* respiratory pathogens. mSystems. 2019;4(6):e00702-19.

86. Dienstbier A, Pouchnik D, Wildung M, Amman F, Hofacker IL, Parkhill J, et al. Comparative genomics of Czech vaccine strains of *Bordetella pertussis*. Pathog

Dis. 2018;76(7).

87. Increase in pertussis cases in South Africa. Available from: https://www.nhls.ac.za/increase-in-pertussis-cases-in-south-africa/: date accessed: December 2023.

88. Cohen C; McMorrow, M.L.; Martinson, N; Kahn, K.; Treurnicht, F.K.; Moyes, J.; et al. Cohort profile: A prospective household cohort study of influenza, respiratory syncytial virus and other respiratory pathogens community burden and transmission dynamics in South Africa, 2016-2018. Influenza Other Respir Viruses. 2021;15(6):789-803.

89. Cohen C, Kleynhans J, Moyes J, Mcmorrow ML, Treurnicht FK, Hellferscee O, et al. Asymptomatic transmission and high community burden of seasonal influenza in an urban and a rural community in South Africa, 2017-18 (PHIRST): a population cohort study. Lancet Glob Health. 2021;9(6):e863-e874.

90. de Graaf H, Ibrahim M, Hill AR, Gbesemete D, Vaughan AT, Gorringe A, et al. Controlled human infection With *Bordetella pertussis* induces asymptomatic, immunizing colonization. Clin Infect Dis. 2020 Jul;71(2):403–11.

91. Godoy P, Toledo D, Carmona G, Caylà JA, Alsedà M, Àlvarez J, et al. Factors influencing the spread of pertussis in households: a prospective study, Catalonia and Navarre, Spain, 2012 to 2013. Euro Surveill. 2016 Nov 10;21(45):30393.

92. Althouse BM, Scarpino S V. Asymptomatic transmission and the resurgence of *Bordetella pertussis*. BMC Med. 2015;13:146.

93. Terry JB, Flatley CJ, Berg DJ Van Den, Morgan GG, Trent M, Turahui JA, et al. A field study of household attack rates and the effectiveness of macrolide antibiotics in reducing household transmission of pertussis. Commun Dis Intell Q

Rep. 2015;39(1):e27-33.

94. De Melker HE, Versteegh FGA, Conyn-Van Spaendonck MAE, Elvers LH, Berbers GAM, Van Der Zee A, et al. Specificity and sensitivity of high levels of immunoglobulin G antibodies against pertussis toxin in a single serum sample for diagnosis of infection with *Bordetella pertussis*. J Clin Microbiol. 2000;38(2):800–6.

95. Beynon KA, Young SA, Laing RTR, Harrison TG, Anderson TP, Murdoch DR. *Bordetella pertussis* in adult pneumonia patients. Emerg Infect Dis. 2005;11(4):639.

96. Voysey M, Kandasamy R, Yu LM, Baudin M, Sadorge C, Thomas S, et al. The predicted persistence and kinetics of antibody decline 9 years after pre-school booster vaccination in UK children. Vaccine. 2016;34(35):4221–8.

97. Martini H, Rodeghiero C, Van Den Poel C, Vincent M, Pierard D, Huygen K. Pertussis diagnosis in Belgium: results of the National Reference Centre for *Bordetella* anno 2015. Epidemiol Infect. 2017;145(11):2366.

98. Heininger U, Cherry JD, Stehr K. Serologic Response and Antibody-Titer Decay in Adults with Pertussis. Clin Infect Dis. 2004;38(4):591–4.

99. Darling AE, Mau B, Perna NT. Progressivemauve: Multiple genome alignment with gene gain, loss and rearrangement. PLoS One. 2010;5(6).

100. Lam C, Octavia S, Bahrame Z, Sintchenko V, Gilbert GL, Lan R. Selection and emergence of pertussis toxin promoter *ptxP*3 allele in the evolution of *Bordetella pertussis*. Infect Genet Evol. 2012;12(2):492–5.

101. Fraj I Ben, Kechrid A, Guillot S, Bouchez V, Brisse S, Guiso N, et al. Pertussis epidemiology in Tunisian infants and children and characterization of *Bordetella*

pertussis isolates: results of a 9-year surveillance study, 2007 to 2016. J Med Microbiol. 2018;68(2):241–7.

102. Carriquiriborde F, Regidor V, Aispuro PM, Magali G, Bartel E, Bottero D, et al. Rare detection of *Bordetella pertussis* pertactin-deficient strains in Argentina. Emerg Infect Dis. 2019;25(11):2048–54.

103. States U, Weigand MR, Williams MM, Peng Y, Kania D, Pawloski LC, et al. Genomic survey of *Bordetella pertussis* diversity, United States, 2000-2013. Emerg Infect Dis. 2019;25(4).

104. Xu Z, Octavia S, Luu LDW, Payne M, Timms V, Tay CY, et al. Pertactin-negative and filamentous hemagglutinin-negative *Bordetella pertussis*. Emerg Infect Dis. 2019;25(6):1196–9.

105. Mir-Cros A, Moreno-Mingorance A, Martín-Gómez MT, Abad R, Bloise I, Campins M, et al. Pertactin-deficient *Bordetella pertussis* with unusual mechanism of pertactin disruption, Spain, 1986–2018. Emerg Infect Dis. 2022;28(5):967–76.

106. Lefrancq N, Bouchez V, Fernandes N, Barkoff AM, Bosch T, Dalby T, et al. Global spatial dynamics and vaccine-induced fitness changes of *Bordetella pertussis*. Sci Transl Med. 2022;14(642):3253.

7. Appendices

Appendix A

Ethics clearance certificate

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R49 Ms F Moosa and Professor C Cohen

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M210676

NAME: (Principal Investigator)	Ms F Moosa and Professor C Cohen
DEPARTMENT:	School of Pathology Dept of Clinical Microbiology & Infectious Diseases Medical School University
PROJECT TITLE:	*Prevalence and molecular epidemiology of Bordetella pertussis infection in South Africa*
DATE CONSIDERED:	Ad hoc
DECISION:	Approved unconditionally
CONDITIONS:	Sub-study under M140824/150808/180832
SUPERVISOR:	Drs N Wolter, M du Plessis and A von Gottberg
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2021/06/30

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

DECLARATION OF INVESTIGATORS

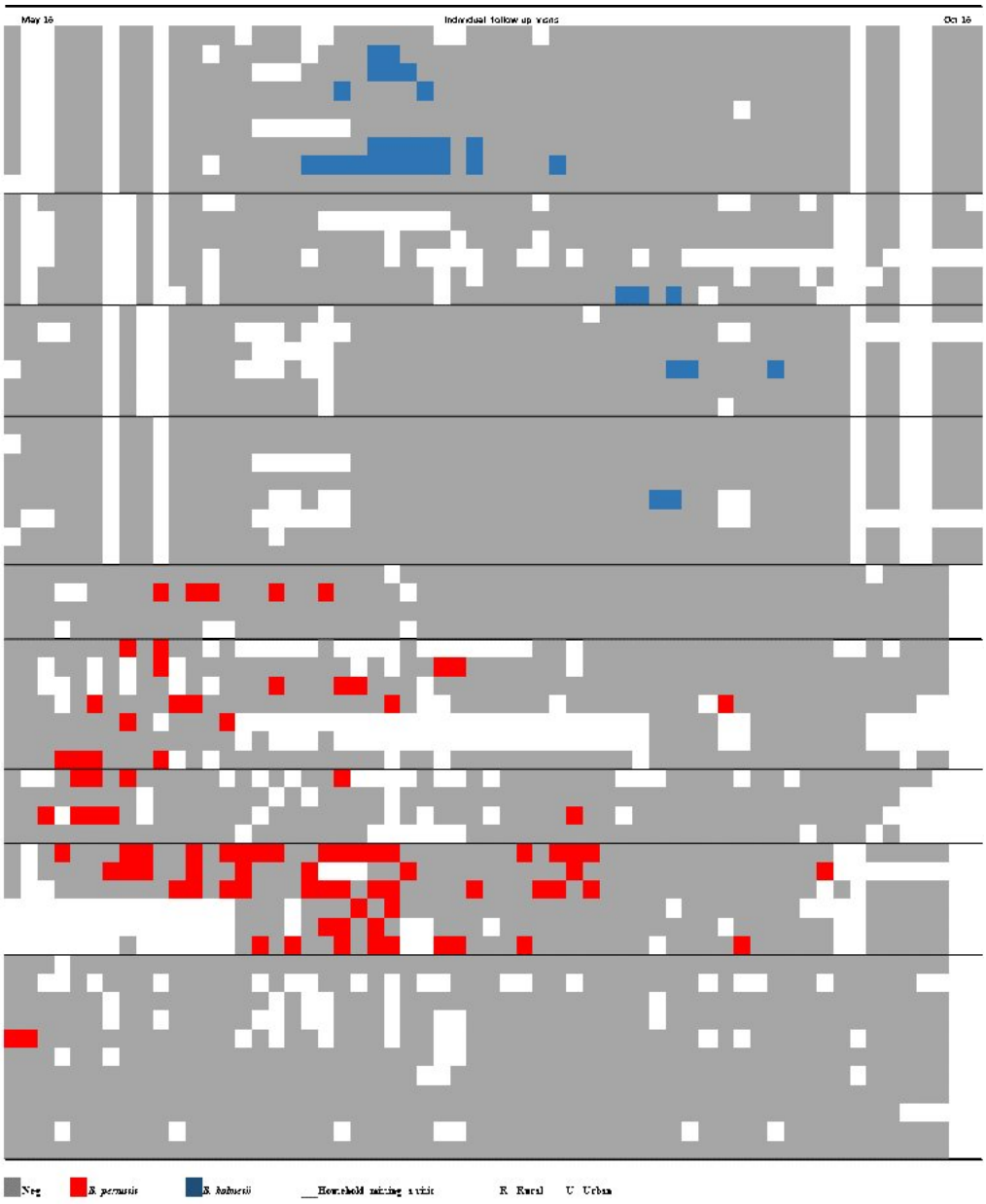
To be completed in duplicate and **ONE COPY** returned to the Research Office secretariat on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

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 submit details to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **June** and therefore reports and re-certification will be due in the month of **June** each year. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).

Signature of Principal Investigator

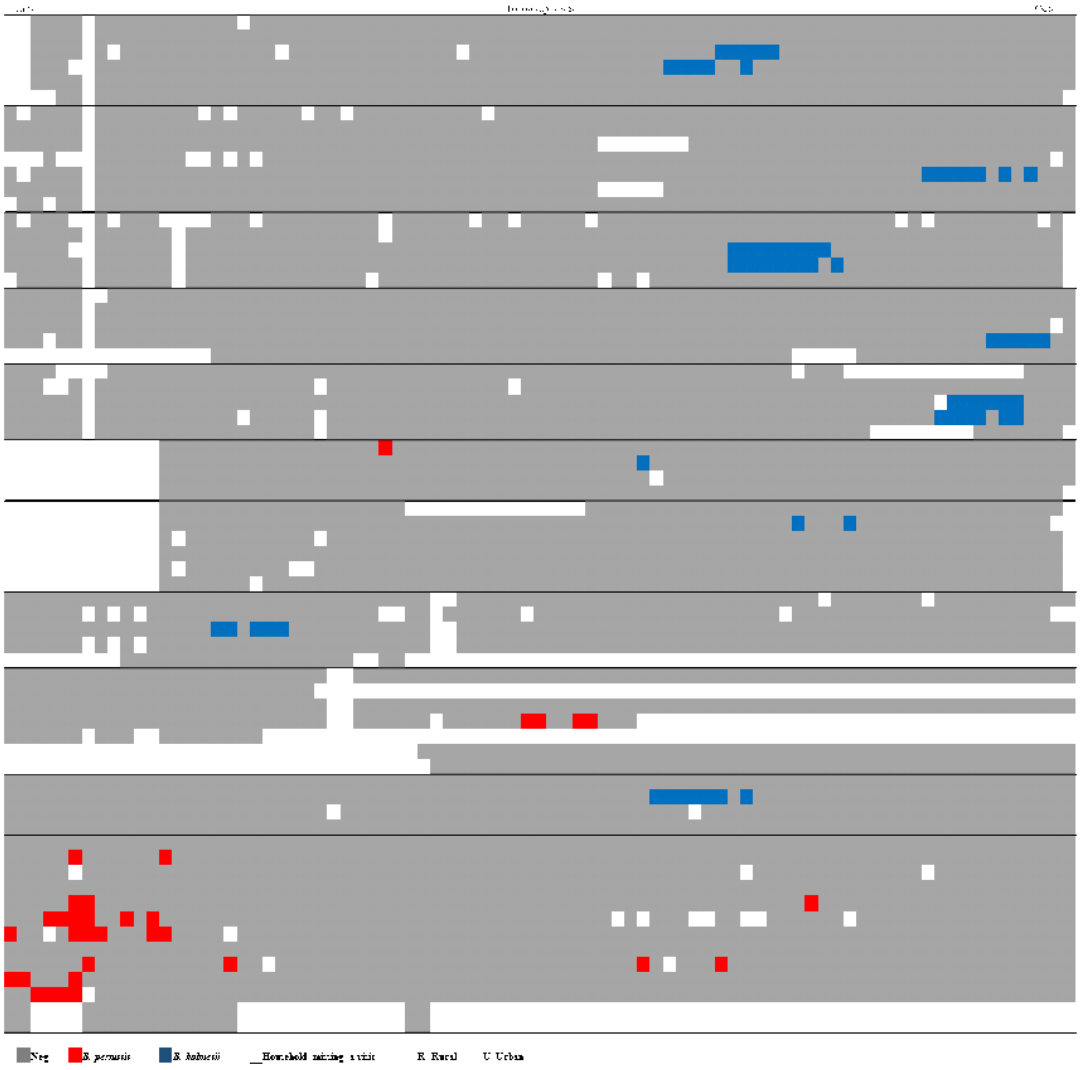
2021-06-30
Date

Appendix B



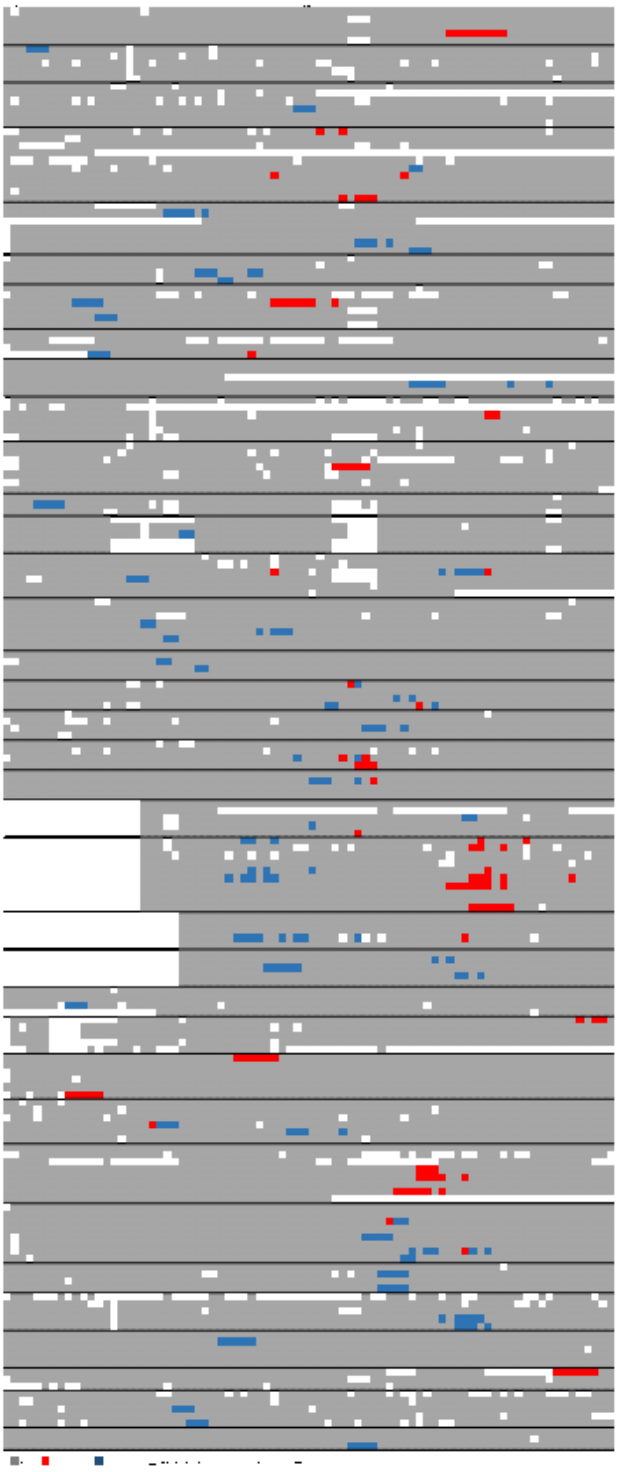
Community *Bordetella pertussis* and *Bordetella holmesii* infections among study participants (households with cases only). Columns are individual follow-up visits and rows are individual study participants, PHIRST study, South Africa, 2016.

Appendix C



Community *Bordetella pertussis* and *Bordetella holmesii* infections among study participants (households with cases only). Columns are individual follow-up visits and rows are individual study participants, PHIRST study, South Africa, 2017.

Appendix D



Community *Bordetella pertussis* and *Bordetella holmesii* infections among study participants (households with cases only). Columns are individual follow-up visits and rows are individual study participants, PHIRST study, South Africa, 2018.

Appendix E

Characteristics of individuals PCR-positive for *B. pertussis* that were retrospectively interviewed, PHIRST study, South Africa, 2018, N=24.

Case	Age	HIV status	[a]Vaccine status	Cough	Paroxysmal cough	Inspiratory whoop	Post-tussive vomiting	[b]Apnea	[c]Pertussis case definition
1	3	Negative	Incomplete	No	No	No	No	N/A	No
2	3	Negative	Full coverage	Yes	Yes	No	No	N/A	Yes
3	3	Negative	Incomplete	Yes	Yes	Yes	No	N/A	Yes
4	5	Negative	Full coverage	Yes	Yes	Yes	Yes	N/A	Yes
5	5	Negative	Full coverage	Yes	No	No	No	N/A	No
6	5	Negative	Full coverage	Yes	No	No	No	N/A	No
7	8	Negative	N/A	Yes	Yes	No	No	N/A	Yes
8	8	Negative	N/A	Yes	Yes	Yes	No	N/A	Yes
9	9	Negative	N/A	Yes	No	No	No	N/A	No
10	9	Negative	N/A	Yes	Yes	Yes	Yes	N/A	Yes
11	9	Negative	N/A	Yes	No	No	No	N/A	No
12	9	Negative	N/A	No	No	No	No	N/A	No
13	10	Unknown	N/A	Yes	No	No	No	N/A	No
14	12	Negative	N/A	Yes	No	No	No	N/A	No
15	12	Negative	N/A	Yes	Yes	Yes	No	N/A	Yes
16	12	Negative	N/A	Yes	No	No	No	N/A	No
17	12	Negative	N/A	No	No	No	No	N/A	No
18	15	Negative	N/A	Yes	No	Yes	No	N/A	Yes
19	36	Positive	N/A	Yes	No	No	No	N/A	No

20	37	Positive	N/A	Yes	Yes	Yes	No	N/A	Yes
21	38	Negative	N/A	Yes	Yes	No	No	N/A	Yes
22	38	Negative	N/A	No	No	No	No	N/A	No
23	40	Negative	N/A	Yes	Yes	Yes	Yes	N/A	Yes
24	59	Positive	N/A	Yes	Yes	No	No	N/A	Yes

[a]Only for children <5 years of age. Vaccine status calculated based on number of doses of vaccine received by age; [b] Apnea in infants <1 year; [c]Clinical case definition = cough with one of the following: paroxysmal cough, inspiratory whoop, post-tussive vomiting and apnea. N/A not applicable. A total of 51 individuals tested positive for *B. pertussis* in 2018.

Appendix F

Clinical and epidemiological characteristics of cases culture positive and PCR-positive for *B. pertussis* in South Africa, 2015-2019 (N=32).

Identifier	Study	Year	Individual level data								
			Site*	Specimen	Age**	Sex	Vaccine status##	Pertussis symptoms	Fever	HIV	Underlying medical conditions
SA1	Pneumonia Surveillance	2015	KZN	Nasopharyngeal Aspirate	≤3 m	M	Too young to be vaccinated	Cough	Yes	Exposed	No
SA2	Pneumonia Surveillance	2015	KZN	Nasopharyngeal Aspirate	≤3 m	F	Full coverage	Cough, Post-tussive vomiting	No	Exposed uninfected	No
SA3	Pneumonia Surveillance	2015	GP	Nasopharyngeal Aspirate	≤3 m	F	Too young to be vaccinated	No symptoms	No	Exposed	No
SA4	Pneumonia Surveillance	2015	NW	Nasopharyngeal swab	25-44 y	F	N/A	Cough	No	Positive	No
SA5	Pneumonia Surveillance	2015	NW	Nasopharyngeal swab	25-44 y	F	N/A	Cough	Yes	Positive	No
SA6	Pneumonia Surveillance	2016	GP	Nasopharyngeal Aspirate	1-4 y	F	Full coverage	Cough	Yes	Unexposed	No
SA7	Pneumonia Surveillance	2015	WC	Nasopharyngeal Aspirate	≤3 m	M	Full coverage	Cough	No	Negative	No
SA8	Pneumonia Surveillance	2017	NW	Nasopharyngeal swab	25-44 y	F	N/A	Cough	Yes	Positive	No
SA9	Pneumonia Surveillance	2018	WC	Nasopharyngeal Aspirate	≤3 m	F	Too young to be vaccinated	Cough, paroxysmal cough	No	Exposed uninfected	No
SA10	Pneumonia surveillance	2018	NW	Sputum	15-24 y	F	N/A	Cough	Yes	Positive	No
SA11	Pneumonia Surveillance	2015	NW	Sputum	45-64 y	F	N/A	Cough	No	Positive	No
SA12	Pneumonia Surveillance	2015	NW	Sputum	25-44 y	F	N/A	Cough	No	Positive	No
SA13	Pneumonia Surveillance	2015	NW	Sputum	25-44 y	F	N/A	Cough	Yes	Positive	No

SA14	Pneumonia Surveillance	2016	WC	Aspirate	≤3 m	M	Too young to be vaccinated	Cough, paroxysmal cough	No	Unexposed	Unknown
SA15	NICD diagnostic	2015	WC	Sputum	≤3 m	M	Too young to be vaccinated	Unknown	Unknown	Unknown	Unknown
SA16	NICD diagnostic	2018	EC	Nasopharyngeal Aspirate	≤3 m	M	Too young to be vaccinated	Cough, paroxysmal cough, apnea	Unknown	Unknown	No
SA17	NICD diagnostic	2019	EC	Nasopharyngeal Aspirate	1-4 y	M	Unknown	Cough, paroxysmal cough, Post-tussive vomiting	Unknown	Unknown	No
SA18	NICD diagnostic	2019	EC	Nasopharyngeal Aspirate	1-4 y	F	Not vaccinated	Cough, paroxysmal cough, inspiratory whoop, apnea	Unknown	Unknown	No
SA19	NICD diagnostic	2019	KZN	Nasopharyngeal swab	≤3 m	M	Too young to be vaccinated	Unknown	Unknown	Unknown	Unknown
SA20	NICD diagnostic	2019	EC	Nasopharyngeal Aspirate	≤3 m	F	Too young to be vaccinated	Cough, Post-tussive vomiting	Unknown	Unknown	No
SA21	NICD diagnostic	2016	EC	Nasopharyngeal Aspirate	1-4 y	F	Unknown	Unknown	Unknown	Unknown	Unknown
SA22	Pediatric surveillance	2015	GP	Nasopharyngeal swab	≤3 m	M	Unvaccinated	Cough	No	Unexposed	No
SA23	Pediatric surveillance	2015	GP	Nasopharyngeal swab	≤3 m	M	Full coverage	Cough	No	Exposed	No
SA24	Pediatric surveillance	2015	GP	Nasopharyngeal swab	≤3 m	F	Unvaccinated	Cough	No	Unexposed	No
SA25	Pediatric surveillance	2015	GP	Nasopharyngeal swab	≤3 m	F	Unvaccinated	Cough	No	Unexposed	No
SA26	Pediatric surveillance	2015	GP	Nasopharyngeal swab	≤3 m	F	Unvaccinated	Cough	No	Exposed	No
SA27	Pediatric surveillance	2015	GP	Nasopharyngeal swab	≤3 m	F	Full coverage	Cough	No	Unexposed	No
SA28	Pediatric surveillance	2015	GP	Nasopharyngeal swab	≤3 m	F	Unvaccinated	Cough, Post-tussive vomiting	No	Exposed	No

SA29	Pediatric surveillance	2015	GP	Nasopharyngeal swab	≤3 m	F	Unvaccinated	Cough	No	Unexposed	No
SA30	Pediatric surveillance	2015	GP	Nasopharyngeal swab	≤3 m	M	Unvaccinated	Cough	No	Unexposed	No
SA31	Pediatric surveillance	2015	GP	Nasopharyngeal swab	≤3 m	F	Unvaccinated	Cough	No	Unexposed	No
SA32	Pediatric surveillance	2015	GP	Nasopharyngeal swab	≤3 m	F	Full coverage	Cough	No	Exposed	No

*KZN: KwaZulu-Natal, GP: Gauteng, NW: North West, WC: Western Cape, EC: Eastern Cape. **m: Months, y: years. [$]M: Male, F: Female. ##Full coverage: fully vaccinated for age. Only patient 2262 dies, all other patients recovered and were discharged.

Appendix G

Turnitin report

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20 March 2024
University of the Witwatersrand
Faculty of Health Sciences, Postgraduate Office
Parktown, 2193
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To whom it may concern

RE: Turnitin report for Fahima Moosa (702185) PhD Thesis

Ms Moosa has submitted her PhD thesis through the Turnitin software and received a 36% similarity index.

We have looked through the report with Ms. Moosa and we are confident that this 36% score is not due to plagiarism of someone else's work but rather due to the system picking up Ms Moosa's own work from published papers arising from this thesis, as well as her previously published data on *Bordetella pertussis*.

- **Moosa F**, Tempia S, Kleynhans J, McMorrow M, Moyes J, du Plessis M, et al. Incidence and Transmission Dynamics of *Bordetella pertussis* Infection in Rural and Urban Communities, South Africa, 2016–2018. Emerg Infect Dis. 2023 Feb 1. – **This work is included in Chapter 3 of the PhD thesis – 8%, 7% and 9% from Turnitin**.

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Fahima Moosa, Stefano Tempia, Jackie Kleynhans, Meredith McMorrow et al. "Incidence and Transmission Dynamics of Infection in Rural and Urban Communities, South Africa, 2016–2018", Emerging Infectious Diseases

- **Moosa F**, du Plessis M, Weigand MR, Peng Y, Mogale D, de Gouveia L, et al. Genomic characterization of *Bordetella pertussis* in South Africa, 2015-2019. Microb Genomics. 2023 Dec 1;9 (12). – **This work is included in Chapter 5 of the PhD thesis –3% from Turnitin.**

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The below 2% plagiarism is attributed to Ms Moosa using some of her own work from her MSc (dissertation that she submitted to the university in 2015), as the pathogen of interest in both her MSc and PhD is the same – *Bordetella pertussis.*

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The below 2% plagiarism is attributed to Ms Moosa using data published from the Center for Respiratory diseases and Meningitis (where she is employed) to describe the PHIRST study population which is included in her thesis. Ms. Moosa was involved in the PHIRST study and used data from this study to conduct her PhD.

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2

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