CLINICAL AND IMMUNOLOGICAL EPIDEMIOLOGY OF GROUP B STREPTOCOCCUS (GBS)

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DECLARATION

I, Ziyaad Dangor declare that this thesis is my own work. It is being submitted for the degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

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17th day of October 2015

DEDICATION

I dedicate this work to my loving wife Zaheera, my pillar of strength, my comfort and joy, who has steadfastly supported me throughout my academic endeavours.

To my adorable children, Mahdiyyah (10), Hammaad (8) and Sumayya (4), whom I dearly love and cherish, for generously growing up whilst I undertook this journey.

To my mum, Rookaya, who silently offers her continuous prayers to my success and well-being.

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To my grandmother, Ayesha, a beacon of hope for our entire family.

PUBLICATIONS AND PRESENTATIONS ARISING FROM THIS THESIS

Publications:

1. Dangor, Z., Kwatra, G., Izu, A., Lala, S. G. & Madhi, S. A. 2015. Review on the association of Group B *Streptococcus* capsular antibody and protection against invasive disease in infants. *Expert Rev Vaccines,* 14**,** 135-49. (Appendix 1).

2. Dangor, Z., Lala, S. G., Cutland, C. L., Koen, A., Jose, L., Nakwa, F., Ramdin, T., Fredericks, J., Wadula, J. & Madhi, S. A. 2015. Burden of invasive Group B *Streptococcus* disease and early neurological sequelae in South African infants. *PLoS One,* 10**,** e0123014. (Appendix 2).

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Conference presentations:

1. Immunological correlates of protection against Group B *Streptococcus* (GBS). 8th *World Society for Pediatric Infectious Diseases,* South Africa. November 2013. (Poster presentation, P-290).

2. Burden of invasive Group B *Streptococcus* disease and early neurological sequelae in South African infants. *33th European Society for Pediatric Infectious Diseases in Leipzig, Germany.* May 2015. (Poster presentation, P-318).

3. Association of HIV-1 infection in pregnant women and Group B *Streptococcus* capsular and surface-protein antibody concentrations and transplacental transfer. *33th European Society for Pediatric Infectious Diseases in Leipzig, Germany.* May 2015. (Oral presentation; ESPID-0633).

ABSTRACT

Introduction: Group B *Streptococcus* (GBS) is a leading cause of neonatal sepsis and meningitis. Vaccinating pregnant women against GBS may protect their infants from invasive GBS disease. The licensure of GBS vaccines might be based on immunological parameters should correlates of protection be established. We evaluated the burden of invasive GBS disease, and explored the association between naturally occurring GBS antibody concentrations and invasive GBS disease in South African infants.

Methods: Using a case-control study, we compared maternal and infant GBS serotype-specific capsular and surface-protein IgG antibody concentrations. Neurodevelopmental screening was performed at 3 and 6 months-of-age. Furthermore, we compared the effect of maternal HIV-infection on GBS specific antibody concentrations and transplacental antibody transfer.

Results: The incidence (per 1,000 live births) of invasive GBS disease within 6 days of life was similar between HIV-exposed (1.13) and HIV-unexposed infants (1.46; p=0.487). However, there was a 4.67-fold (95% CI: 2.24-9.74) greater risk of invasive GBS disease at age 7-90 days in HIV-exposed infants (2.27 vs. 0.49; p<0.001). The overall case fatality ratio among cases was 18.0%, and the adjusted odds of developing neurological sequelae at 6 months age was 13.2-fold (95% CI: 1.4-121) greater in cases (13.2%) than controls (0.4%).

Median antibody concentrations (μg/mL) were lower in HIV-infected than HIV-uninfected women for serotypes Ib (p=0.033) and V (p=0.040); and for pilus island (PI)-1 (p=0.016), PI-2a (p=0.015), PI-2b (p=0.015) and fibrinogen-binding protein A (p<0.001). For

serotypes Ia and III, cord to maternal ratios were 37.4% ($p<0.001$) and 32.5% ($p=0.027$) lower in HIV-infected compared to HIV-uninfected mother-newborn dyads.

Using Bayesian modelling, we demonstrated >90% reduction in risk of invasive GBS disease with maternal antibody concentrations ≥6 μg/mL and ≥3 μg/mL for serotype Ia and III, respectively. There was no association between GBS surface-protein antibody concentrations and invasive GBS disease.

Conclusion: The high burden of invasive GBS disease in South Africa is partly due to the high prevalence of maternal HIV-infection (29%), which is associated with lower GBS antibody concentrations and transplacental antibody transfer. We identified putative correlates of protection for GBS serotype-specific capsular antibodies to serotypes Ia and III, which could facilitate vaccine licensure.

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ABBREVIATIONS

aOR	Adjusted odds ratios
ART	Antiretroviral treatment
ATCC	American type culture collection
AZT	Zidovudine
BibA	GBS Immunogenic Bacterial Adhesin
CAMP	Christie Atkinson Munch-Petersen
CDC	Center for Disease Control
CHBAH	Chris Hani Baragwanath Academic Hospital
CLSI	Clinical and Laboratory Standards Institute
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
CPAP	Continuous positive airway pressure
CPS	Capsular polysaccharide
CRP	C-reactive protein
CSF	Cerebrospinal fluid
CT	Computed tomography
DNA	Deoxyribonucleic Acid
DEVANI	Design of a Vaccine Against Neonatal Infections
ELISA	Enzyme Linked Immunosorbent Assay
EOD	Early-onset disease
FbsA	Fibrinogen-binding protein A
FI	Fluorescence intensity
FTC	Emtricitabine
GBS	Group B *Streptococcus*
GMC's	Geometric Mean Concentrations
HIV	Human Immunodeficiency Virus
HREC	Human Research Ethics Committee
IAP	Intra-partum antibiotic prophylaxis
IF	Indirect immunofluorescent
Ig	Immunoglobulins
IQR	Interquartile range
LLD	Lower limits of detection
LLQ	Lower limits of quantification
Lmb	Laminin-binding protein
LMP	Last normal menstrual period
LOD	Late-onset disease
MESH	Medical subject headings
MFI	Median fluorescence intensity
MOU	Midwife obstetric unit
NHLS	National Health Laboratory Service
NVP	Nevirapine
OR	Odds ratio
PCR	Polymerase chain reaction

PI	Pilus Island
PMTCT	Prevention of mother to child transmission
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PROM	Prolonged rupture of membranes
RABA	Radioactive antigen binding assay
RI	Radioimmunoassay
Rib	Resistance to proteases immunity group B
RMMCH	Rahima Moosa Mother and Child Hospital
RMPRU	Respiratory and Meningeal Pathogens Research Unit
RPA	Radiolabelled protein A
SFH	Symphysis fundal height
Sip	Surface immunogenic protein
SSI	Statens Serum Institute
ST	Sequence types
STGG	A medium containing skim milk, tryptone, glucose, and glycerin
TDF	Tenofovir
UK	United Kingdom
USA	United States of America
WCC	White cell count
WHO	World Health Organization
3TC	Lamivudin

PREFACE

This thesis is presented to the reader in the University of the Witwatersrand's recommended "divided block" format. In this format, the thesis consists of two parts: the first part includes the Introduction and Methods chapters that are written in the traditional thesis format, and second part in which the Results and Discussion of the study's objectives are presented as individual chapters.

This thesis deals with the clinical epidemiology of Group B *Streptococcus* (GBS) in a low-middle income setting (Johannesburg, South Africa) with a high burden of disease. In addition, we aimed to identify GBS serotype-specific capsular antibody thresholds that correlate with protection against invasive GBS disease in young infants, which may assist in the licensure of the maternal GBS polysaccharide-protein conjugate vaccine undergoing development.

In the Introduction chapter, I will briefly describe the global epidemiology and clinical significance of invasive GBS disease, and the decline of disease burden observed in some high income countries. I will briefly outline selected microbial characteristics of GBS, and outline the immunological and clinical features of invasive GBS disease in infants. Thereafter, I will discuss the concept of maternal vaccination as an alternative strategy to prevent disease in young infants. In particular, the transfer of serotype-specific capsular antibodies from the mother to the foetus during pregnancy has been associated with protection against invasive disease in young infants, and I will present a systematic review thereof. I will outline the potential use of GBS surface-proteins as alternative vaccine candidates. As my research was undertaken in a high HIV-burden setting, I will also

discuss the potential role of maternal HIV-infection on GBS antibody concentrations and transplacental transfer thereof to their newborns.

In Chapter Two, Materials and Methods are described. In order to achieve the main objectives, two studies were undertaken. The first, a matched case-control study, was conducted to explore the burden of invasive GBS disease in South African infants. This study was also used to establish sero-correlates of protection, against invasive GBS disease in young infants (0-90 days age), using maternal GBS serotype-specific capsular and selected surface-protein antibody levels. The second study, a cross-sectional study, explored the impact on maternal HIV-infection on antibody concentrations and transplacental transfer.

Chapter's 3 to 6 present the results and discussion of my work. In chapter 3, the incidence of invasive disease, risk factors, clinical presentation and outcomes (including short-term neurological sequelae) in infants in our setting is reported. In Chapter 4, the effect of maternal HIV-infection on capsular and surface-protein antibody concentrations and transplacental transfer thereof to newborns is described. In Chapter 5 and 6, the association between naturally occurring maternal IgG capsular and surface-protein antibodies and the risk of invasive GBS disease in young infants is detailed.

The thesis concludes with a summation of the main findings of my research in Chapter 7, which discusses the context of invasive GBS disease in young infants in our setting and the implications thereof more generally.

STUDENT CONTRIBUTIONS

Protocol and ethics: The first draft of the study protocols was prepared by ZD. ZD was responsible for obtaining ethical approval for all the studies.

Recruitment and follow-up: ZD was responsible for all patient enrolment and study staff reported directly to ZD. Cases were identified by ZD through daily surveillance (including weekends and public holidays) of the paediatric wards and microbiology services at the three hospitals over one year. Almost all cases (98%) and approximately 20% of the controls were enrolled by ZD; the remaining controls were enrolled by research assistants and nurses who reported directly to ZD. This included obtaining blood samples and rectal and vaginal swabs. Follow-up visits were conducted by ZD (approximately 30%), research assistants or enrolled nurses. On a regular basis, all data was verified and entered into databases by ZD. Enrolments for the cross-sectional study were done by the nurses and overseen by ZD.

Laboratory component: Culture and serotyping was overseen by ZD and undertaken by a laboratory technician. Assay development and validation was carried out by a previous PhD student (Dr Gaurav Kwatra), whose aim was to measure antibody levels in pregnant women. The antibody assays were conducted by a laboratory technician and overseen by Dr Kwatra and ZD. ZD was familiar with laboratory techniques.

Statistical analysis: All statistical analyses (90%) were performed by ZD, excluding the Bayesian analysis, reverse cumulative plots and interquartile analysis which was performed by Dr Alane Izu.

1.0 Introduction

1.1 Epidemiology

Streptococcus agalactiae is an encapsulated Gram-positive coccus which colonises the human gastrointestinal and genitourinary tracts. This species of *Streptococcus* belongs exclusively in the 'Group B' Lancefield grouping, and thus commonly referred to as Group B *Streptococcus* (GBS) (Lancefield, 1934, Rajagopal, 2009). Invasive GBS disease in adults is uncommon but may occur in immunocompromised patients. The greatest burden of invasive GBS disease, however, is in infants less than three months of age and predominantly occurs through transmission of GBS from mother to infant. By the early 1970's, GBS was recognised to be a significant cause of neonatal sepsis (Reid, 1975, Anthony and Okada, 1977). Currently, GBS is the leading cause of sepsis and meningitis in young infants in the United States of America (USA) (Stoll et al., 2011, Thigpen et al., 2011, Weston et al., 2011), despite successful preventative measures that minimise transmission of the organism. The burden of invasive GBS disease is substantially greater in countries where preventative strategies have been implemented to a limited extent (Edmond et al., 2012); for example, South Africa reports one of the highest incidences (2-3 cases per 1,000 live births) of invasive GBS disease globally, which has remained relatively constant from the 1990s to 2008 (Haffejee et al., 1991, Madhi et al., 2003, Cutland et al., 2015). Globally, the incidence of invasive GBS disease in infants less than 3 months of age has been approximated to be 0.53 cases per 1,000 live births (Edmond et al., 2012).

Early-onset disease (EOD) is defined as presenting within the first 6 days of life, although more than 80% of EOD cases present within the first 12 hours of birth (Madhi et al., 2003, Heath et al., 2009). Even though the incidence of EOD in the USA is as low as 0.26 cases

per 1,000 live births in 2010 (Schrag and Verani, 2013), invasive GBS disease contributes to as much as 38-43% of cases of early-onset neonatal sepsis; in contrast disease caused by *Escherichia coli* accounts for 24-29% of cases of early-onset neonatal sepsis (Stoll et al., 2011, Weston et al., 2011). In comparison, the incidence of EOD in South Africa has been reported between 1.5 and 2 cases per 1,000 live births (Haffejee et al., 1991, Madhi et al., 2003, Cutland et al., 2015). Late-onset disease (LOD) is defined as illness presenting from day 7 to 89 of life, with almost half of cases presenting with meningitis (Madhi et al., 2003, Heath et al., 2009). The incidence of LOD, unlike EOD has remained unchanged in high income countries (0.3-0.4 cases per 1,000 live births) (Schrag and Verani, 2013) as well as in South Africa (1.0 cases per 1,000 live births) (Madhi et al., 2003) and may be higher in children exposed to HIV-infection (Epalza et al., 2010, Cutland et al., 2015). The clinical burden attributed to invasive GBS disease in young South African infants has remained significant even though preventative strategies to reduce the incidence of disease are recommended.

The vast majority of epidemiological data regarding invasive GBS disease is reported from the USA, where interventions to reduce the burden of disease have been initially pioneered. With the implementation of intra-partum antibiotic prophylaxis (IAP) and formal screening programs in 1996 in the USA, the incidence of EOD had decreased from 1.7 cases per 1,000 live births (in 1993) to 0.6 cases per 1,000 live births (in 1998) (Schrag et al., 2000), but had subsequently plateaued between 1999 and 2001 at 0.5 cases per 1,000 live births (Verani et al., 2010). This resulted in a revision of the screening programme, in 2002, from a "risk-based" (i.e. the provision of IAP to pregnant women with defined risk factors for GBS transmission) to "universal screening" (i.e. the provision of IAP to all GBS colonized pregnant women irrespective of risk factors); the incidence of invasive

GBS disease had subsequently declined by a further 20-40% (Verani et al., 2010). For EOD, the incidence has decreased to 0.26 cases per 1,000 live births by 2010, but the incidence of LOD has remained largely unchanged over the past 20 years (0.3-0.4 cases per 1,000 live births) (Verani et al., 2010, Schrag and Verani, 2013). Notably, epidemiological data from the USA consistently mentions a higher incidence of invasive GBS disease in black Americans compared to their white counterparts, although reductions in invasive GBS disease incidence have been observed in both groups (Schrag and Verani, 2013). In a recent report by Ferrieri et al., only a modest decline in overall incidence was observed in the USA state of Minnesota between 2000 and 2010, but more concerning was an increase in incidence since 2010 (Ferrieri et al., 2013). It seems that strategies to prevent EOD, such as universal screening and IAP, are limited in contributing to a further decline in incidence of EOD in the USA, and may need to be controlled through other measures (this will discussed in further detail in chapter 1.9). (Schrag and Verani, 2013, Verani et al., 2014).

In high income countries (mostly European) that have continued to favour the risk-based approach, declines in the incidence of invasive GBS disease were noted when IAP was initiated using this strategy, but the incidence of disease has increased in subsequent years. As examples, the incidence of EOD (per 1,000 live births) in the United Kingdom increased from 0.30 in 1991 to 0.41 in 2010 (Lamagni et al., 2013), and increased from 0.11 in 1987 to 0.19 in 2011 in the Netherlands (Bekker et al., 2014). Such surveillance over 20-25 years highlights the limitations in the risk-based strategy for preventing EOD.

The global burden of invasive GBS disease in young infants was recently summarised in a systematic review, which included 56 studies over the period 2000 and 2011; the majority

of which were from European and American countries (Edmond et al., 2012). This review highlighted striking variability in incidence of invasive GBS disease between and within regions. In a meta-analysis of invasive GBS disease in infants less than 90 days of age, the incidence (per 1,000 live births) was reported as 0.57 (range: 0.00-2.60) in Europe, and 0.67 (range: 0.25-2.13) in the Americas; as low as 0.02 (range: 0.00-0.14) in Asia; and highest in Africa as 1.21 (range: 0.24-1.97). The low incidences of invasive GBS disease reported across Asia may be an underestimate because a large number (>70%) of deliveries occur outside the health-care settings, possibly resulting in an ascertainment bias with many of the EOD cases missed at birth (Montagu et al., 2011). For the same reason, it is likely that the incidence in Africa may also be underestimated; poor access to microbiology laboratories to confirm invasive GBS disease may also contribute to underestimating the incidence (Capan et al., 2012, Johri et al., 2013). Furthermore, it should be noted that there were very few studies on invasive GBS disease incidence from low income countries, accounting for 5% of those included in the review (Edmond et al., 2012). In a separate review of studies conducted only in low-middle income countries, high incidences were reported in Africa, with South Africa having the highest reported incidence (3.06 cases per 1,000 live births) (Dagnew et al., 2012).

The incidence of invasive GBS disease in South African infants has been reported in four previous studies (Haffejee et al., 1991, Madhi et al., 2003, Frigati et al., 2014, Cutland et al., 2015). The first study to report on the incidence of invasive GBS disease was conducted between 1986 and 1989 on South African Indians residing in Kwazulu Natal province, which reported an overall incidence of 2.65 (2.09 for EOD and 0.56 for LOD) (Haffejee et al., 1991). Ten years later, Madhi et al. reported a similar incidence of EOD (2.06 cases) in indigenous black South Africans from Soweto but an almost double

incidence for LOD (1.00) (Madhi et al., 2003). Between 2004 and 2008, the incidence of EOD declined marginally in Soweto (1.50; 95% CI: 1.30-1.71), but the incidence of LOD increased (1.22; 95% CI: 1.05-1.42) (Cutland et al., 2015). The increased incidence of LOD might be due to the increase in prevalence of Human Immunodeficiency Virus (HIV) infection among pregnant women from 17.0% in 1997 to 29.3% in 2008 (National Department of Health, 2012). The latter two studies were conducted at the same public hospital in Soweto (i.e. the Chris Hani Baragwanath Academic Hospital); where in excess of 22 000 babies are born each year. This hospital practises a risk-based approach to the prevention against GBS transmission, but the use of IAP has been reported in only 10.2% of vaginal deliveries (Cutland et al., 2012). Other studies from South Africa include a retrospective laboratory-based review over a two year period from the Western Cape province, in which the crude incidence of invasive GBS disease was estimated to be 0.67 cases per 1,000 live births (Frigati et al., 2014).

In settings with a high prevalence of maternal HIV-infection, including South Africa, there is paucity of data on the effect thereof on GBS recto-vaginal colonisation in the women and invasive disease in their infants. Recto-vaginal GBS colonisation has not been found to be higher in HIV-infected women during pregnancy or at birth (El Beitune et al., 2006, Mavenyengwa et al., 2010, Gray et al., 2011, Shah et al., 2011, Cutland et al., 2012). Gray et al. postulated that lower CD4+ T-lymphocyte counts may be associated with alterations in the commensal vaginal flora, with a lower prevalence of GBS colonisation (Gray et al., 2011). Similarly, a lower prevalence of GBS vaginal colonization was reported in HIV-infected compared to HIV-uninfected South African women, with half of those with HIV-infection having CD4+T-lympocyte counts <350 cells/mm3 at the time of sampling (Cutland et al., 2012). Although similar transmission ratios of GBS to the newborns were

reported between HIV-infected and HIV-uninfected mothers, newborns of HIV-infected mothers with low CD4+ T-lymphocyte counts had an increased risk of developing neonatal sepsis (Cutland et al., 2012). Also, newborns of HIV-infected mothers have been reported to have an increased risk of invasive GBS disease compared to those born to HIV-uninfected mothers (Epalza et al., 2010, Cutland et al., 2015). The South African study reported by Cutland et al. in 2015 (for the period 2004-2008) described a 2.25-fold (95% CI: 1.84–2.76) greater incidence of invasive GBS disease in HIV-exposed (4.46) compared to HIV-unexposed infants (1.98). The increased risk in HIV-exposed infants was observed for EOD (2.10 vs. 1.24; risk ratio 1.69, 95% CI: 1.28–2.24) and LOD (2.36 vs. 0.74; risk ratio 3.18, 95% CI: 2.34–4.36) (Cutland et al., 2015). This study was conducted during the early periods of the antiretroviral treatment (ART) treatment program in South Africa. With establishment of the ART program and improvements in the prevention of mother to child transmission (PMTCT) program in South Africa in 2010 (National Department of Health, 2010), pregnant women on ART are expected to be less immunocompromised, which might alter the risk of invasive GBS disease in their neonates.

In summary, the burden of invasive GBS disease in South Africa has generally been higher than studies from other countries, possibly related to poor execution of the risk-based approach for EOD and the increasing burden of maternal HIV-infection. Amongst low-middle income countries, South Africa has a heightened level of access to healthcare, resulting in most deliveries occurring at medical facilities, with invasive GBS cases possibly being more readily identifiable. The past studies from South Africa have, however, not detailed the risk factors for invasive GBS disease in depth and have not evaluated for sequelae related to invasive GBS disease beyond in-facility mortality.

Similarly, such data are lacking from most other studies on invasive GBS disease from low-middle income countries.

1.2 Clinical burden and outcomes of invasive Group B *Streptococcal* disease

Invasive GBS disease in young infants has a high mortality ratio and significant morbidity in young infants. The meta-analysis by Edmond et al. reported a global mortality for invasive GBS disease to be 9.6%, with case fatality ratios as high as 33% in low income countries like Malawi (Edmond et al., 2012). In this meta-analysis, the case fatality ratio was highest in Africa (22%) compared to Europe (7%) and Americas (11%). In a separate review, Dagnew et al. reported case fatality ratios as high as 60% for invasive GBS disease in low-middle income countries (Dagnew et al., 2012). In South Africa, the case fatality ratio has been reported to be as low as 6% in the Western Cape, (Frigati et al., 2014) but consistently higher (16.9%-17.8%) in Soweto (Madhi et al., 2003, Cutland et al., 2015). In addition, GBS meningitis carries a poorer outcome than sepsis alone, with 4-6 fold higher mortality in low-middle income countries compared to high-income countries (Furyk et al., 2011). The higher mortality in low-middle income countries may be attributed to a number of factors including limited access to resources, resource utilization and quality of care. In contrast, reductions in the mortality attributable to invasive GBS disease in high income countries has largely been due to ready access to antibiotics and the successful implementation of preventative strategies (Dermer et al., 2004).

In infants surviving invasive GBS disease, neurological sequelae are thought to result from direct damage to the developing brain from GBS meningitis or from cerebral hypo-perfusion during GBS related septic shock episodes (Law et al., 2005). In addition, GBS precipitates preterm deliveries, which in itself contributes to neurological sequelae. There are limited studies describing the neurological outcomes of infants surviving invasive GBS

disease and GBS meningitis. Of 1037 bacteraemic episodes in neonates with or without meningitis, Chu et al. reported neurological complications in 3.5% of neonates, 41.7% of which were caused by GBS (Chu et al., 2014). A retrospective review of infants with GBS meningitis at hospital discharge between 1998 and 2006 in the USA reported poor neurological outcomes in 11/50 (22%) survivors of EOD and LOD. Infants had clinical signs such as: hypotonia (n=2), hypertonia (n=9), seizures (n=10), clonus (n=2), dysphagia (n=3), ptosis (n=2), cortical blindness (n=1), hearing loss (n=2) and temperature instability (n=1) (Levent et al., 2010). In a multivariate analysis, seizures before or at presentation was found to be the most useful marker of poor neurological outcome at discharge. These findings were similar to a meta-analysis of eight studies in high income countries which showed that 23% (range: 16-38%) of survivors of neonatal meningitis have moderate to severe neurological impairment (Seale et al., 2014).

The drawback of short-term outcome studies, however, is the failure to recognise infants with mild development delay or learning problems. Consequently, a series of studies have addressed the long-term neurological outcomes from GBS meningitis. Edwards et al. has summarised studies (prior to 1985) reporting on GBS meningitis outcomes: an average fatality of 27% was noted and up to one-third of survivors had neurological sequelae measured at different age time points (Edwards et al., 1985). Table 1.1 further summarises studies reporting on long-term neurological sequelae of GBS meningitis survivors from 1985 onwards (Edwards et al., 1985, Wald et al., 1986, Bedford et al., 2001, Libster et al., 2012). Although studies have used different standards and tools for assessing neurological sequelae, all these studies were carried out in well-resourced high-income countries. Overall, 213 children between 3 and 18 years were evaluated in these four studies, with 95

(45%) displaying neurological sequelae, including 19% with severe sequelae which includes cerebral palsy, severe seizures, visual and auditory impairments.

The high morbidity associated with invasive GBS disease is also coupled with a significant economic burden that is almost double in the first two years of life following invasive disease (Platt et al., 1999, Schroeder et al., 2009). Comparing invasive GBS cases to controls, the main cost drivers were a high level of care and duration of hospitalization. Although long term neurological sequelae have not been assessed in low-middle income countries with limited resources, the heightened cost of treating GBS invasive disease may further exacerbate the poorer outcomes seen in these settings.

Table 1.1: Summary of studies reporting long-term outcomes amongst survivors of Group B *Streptococcus* meningitis

Author date country	**GBS meningitis; n=**	**Demised; n=**	**Assessed for neurological impairment; n=**	**Ages at follow-up assessment**	**Neurological impairment**		
					normal	**mild-moderate**	**severe**
Edwards 1985 USA[1] (Edwards et al., 1985)	61	13 (21%)	38	3.3-9years (Mean age-6 years)	19 (50%)	8 (21%) Unilateral sensorineural deafness (2); borderline mental retardation (2); spastic or flaccid monoparesis (3); hydrocephalus, arrested (2); seizure disorder, controlled (1); expressive or receptive speech and language delay (2); porenecephalic cyst (1); mild frontal cortical atrophy (1); deficit in visual and auditory memory (1).	11 (29%) Global mental retardation (7); relapse of GBS meningitis (1); uncontrolled seizures (6); cortical blindness (6); microcephalus (3); hydrocephalus (3); spastic or flaccid quadriparesis (3); central diabetes insipidus (1); mild mental retardation (3).
Wald 1986 USA (Wald et al., 1986)	74	20 (27%)	34	3-18years (Mean age-8.6 years)			9 (26%) spastic quadriplegia (4); profound mental retardation (8);hemiparesis (1); deafness (4); cortical blindness (2); seizure disorder (7); hydrocephalus (6).
Bedford 2001 UK[2] (Bedford et al., 2001)	98		98	5 years	50 (51%)	35 (36%) Moderate: disability impaired their functioning but attended mainstream schools; mild neuromotor disabilities; intellectual impairment; moderate sensorineural hearing loss; mild or moderate visual impairment; epilepsy that was controlled with treatment; hydrocephalus without complications.	13 (13%) Unable to attend a mainstream school; severe neuromotor impairment; significant intellectual impairment; severe seizure disorders; severe visual or auditory impairment.
Libster 2012 USA (Libster et al., 2012)	90	5 (6%)	43	3-12 years (Mean age-6.8 years)	43 (56%)	11 (25%) Impairment based on Mullen or WIAT-II score (9); grade retention (3); persistent asymptomatic seizure disorder (3); hydrocephalus with ventriculoperitoneal shunt (1); loss of terminal digit of right thumb and forefinger (1).	8 (19%) Profound global developmental delay (8); hydrocephalus (2); cortical visual impairment (4); bilateral sensorineural deafness (4); cerebral palsy/spasticity (5); persistent symptomatic seizures (4).

[1]USA-United States of America; [2]UK-United Kingdom

1.3 Microbiology of Group B *Streptococcus*

Streptococcus agalactiae is amongst many species belonging to the genus of *Streptococcus*. The *Streptococcus* genus can be classified according to the haemolysis pattern of growth on blood agar; as alpha-haemolytic (for example, *Streptococcus pneumoniae*), beta haemolytic (examples include *Streptococcus pyogenes and Streptococcus agalactiae*) or non-haemolytic (for example, *Streptococcus viridans*). Some species are classified according to the antigenic components of the cell wall, of which *Streptococcus agalactiae* has the B-antigen. *Streptococcus agalactiae* is a membrane bound eukaryotic. They are visualised as Gram-stain positive cocci on microscopy and grow non-fastidiously as 3-4 mm white colonies on blood agar. On a molecular level, the GBS cell surface is comprised of a capsule, peptidoglycan cell wall and a cell membrane (Figure 1.1). The GBS polysaccharide capsule is differentiated into specific serotypes based on the arrangement of the mono- and oligosaccharides, all ending with a sialic-acid residue. The GBS cell surface also comprises of multiple surface-proteins, some of which aid in the adherence of GBS to the epithelial surfaces.

The virulence of GBS is attributed to the capsular and surface-proteins, and extracellular substances produced by the organism. The primary step in the pathogenesis of GBS is attachment to the host, followed by replication and evading host defences. Table 1.2 summarizes few important structural components of the organism that contribute to its virulence.

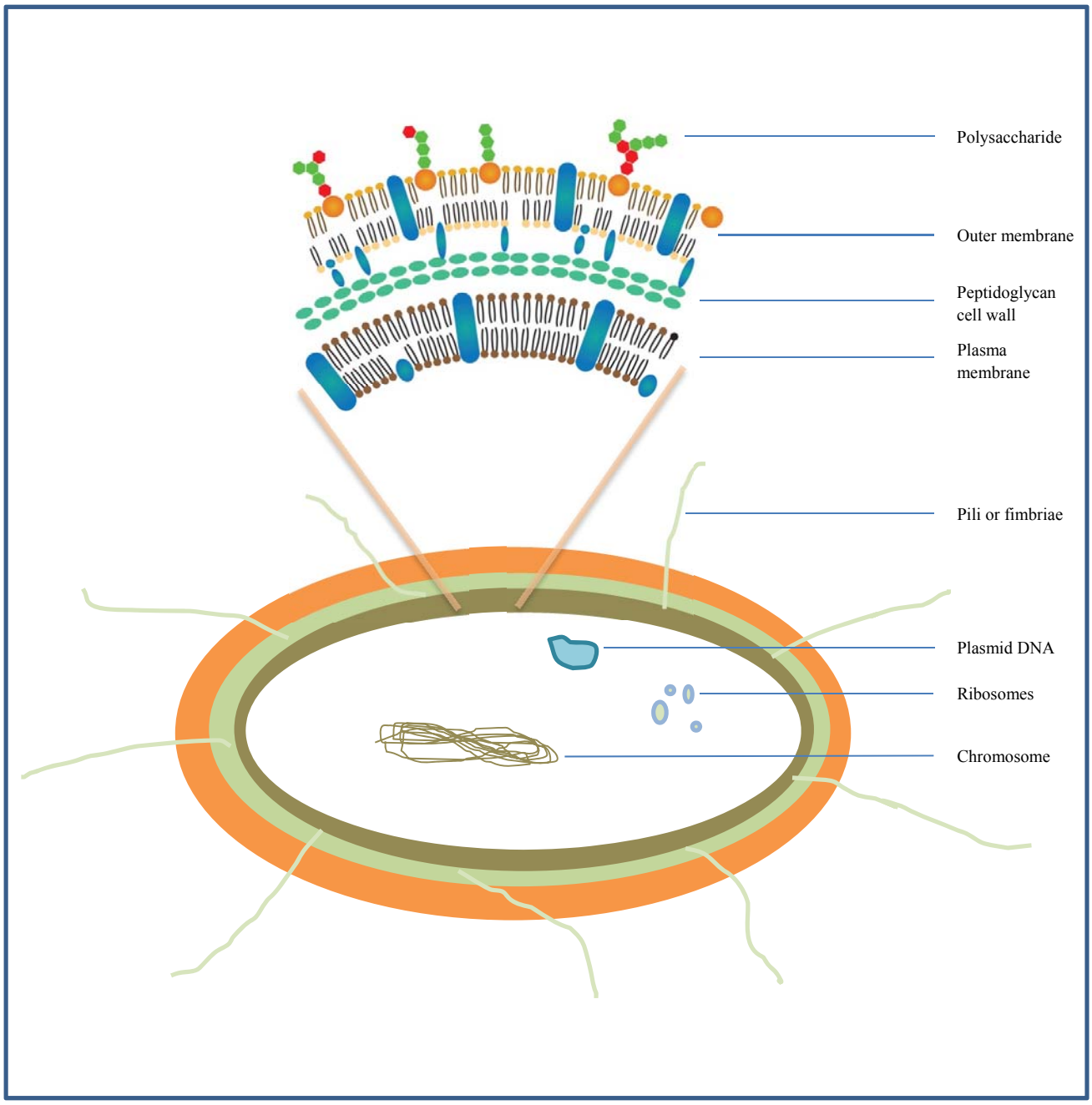


Figure 1.1: Molecular representation of *Streptococcus agalactiae* (Adapted from Figure 4-35a, Brock Biology of Microorganisms, 11th Edition, Pearson Prentice Hall, 2006)

Table 1.2: Structural components of Group B *Streptococcus* (GBS) and its role in organism virulence, Adapted from (Rajagopal, 2009).

Structural component	**Mechanism of action**
Immune evasion	
Capsular polysaccharide	Prevents recognition of GBS through exhibiting similar carbon structures as vertebrate cell
C5a peptidase	Cleaves complement 5a, thus disrupting host cellular recruitment
Superoxide dismutase	Detoxifies oxygen radicals
Toxins	
β-haemolysin	Allows invasion of the host cell and induces apoptosis
CAMP factor	Forms pores in host cell membrane
Adherence	
Pili	Directly adheres to host epithelium
Fibrinogen-binding protein A	Adherence to host epithelium by binding fibrinogen on cell membrane
GBS Immunogenic Bacterial Adhesin	Assists in adherence to host epithelium
Laminin-binding protein	Adherence to host epithelium by binding laminin on cell membrane
C-protein	Assists in adherence to host epithelium
Resistance to antimicrobial peptides	
penicillin-binding protein 1	Affords resistance to antimicrobial peptides

1.3.1 The Group B *Streptococcus* polysaccharide capsule

The sialic acid rich capsular polysaccharide (CPS) is differentiated into 10 serotypes (Types Ia, Ib, II and III–IX) and functions mainly to prevent recognition by the host's immune system (Maisey et al., 2008, Rajagopal, 2009, Melin, 2011). More recently, it has been suggested that the CPS may also be involved in biofilm formation (Xia et al., 2015). Each serotype has its own unique antigenic properties with serotype III being the most virulent and least immunogenic of all serotypes (Davies et al., 2001). Globally, serotype distributions of GBS CPS have been similar for most regions with Ia, Ib, II, III and V making up 94% of the invasive serotypes (Edmond et al., 2012). Serotype Ia and III account for more than two thirds of invasive isolates, with serotype Ia being more prevalent in EOD and III in LOD. There has also been reports of the increased prevalence of serotype V, which is now the dominant serotype in some regions (Le Doare and Heath, 2013). Serotype data from south-east Asia, although scanty, have reported a larger proportion of disease by serotype II (Johri et al., 2013). In South Africa, the serotype distribution is similar to global estimates except that serotype III has been found to be more common for EOD (49-58%) compared to serotype Ia (23-31%) (Madhi et al., 2003, Madzivhandila et al., 2011). Recently, a twenty year surveillance in the United Kingdom demonstrated minor variations in the serotype distributions of invasive isolates (Lamagni et al., 2013); these may mirror serotypes colonizing the pregnant women. Vaginal colonizing serotypes have also been found to be similar in HIV-infected and HIV-uninfected South African women (Madzivhandila et al., 2011). Consequently, a pentavalent (Ia, Ib, II, III and V) CPS vaccine would protect against more than 90% of disease causing serotypes globally, including South Africa.

Multilocus sequence typing (i.e. characterizing organisms using fragments of genes) of CPS serotypes based on the allelic profiles has identified certain sequence types (ST) to be more prevalent (ST1, 17, 19, 23) (Jones et al., 2003). Individual ST's may have different virulence potentials, with ST17 reported as more prevalent in invasive isolates whereas ST19 is more common among colonising isolates. (Davies et al., 2004, Lin et al., 2006, Fluegge et al., 2011). The genotyping of GBS strains, although more costly, is a more accurate assessment of strain identification and may prove to be more useful in future epidemiological studies.

1.3.2 Group B *Streptococcus* surface-proteins

In addition to the virulence properties of the CPS, the GBS bacterium has surface-proteins which also contribute to evading host defences (Lindahl et al., 2005). In this thesis, I will examine the potential role of five surface-proteins [namely pilus island-1, -2a, and -2b, fibrinogen-binding protein A (FbsA) and GBS Immunogenic Bacterial Adhesin (BibA)] as potential epitopes to prevent invasive GBS disease in infants.

Pili or fimbriae are long filamentous strands on the bacterial surface which were first identified through electron microscopy in 2005 (Lauer et al., 2005). The function of the pili are to facilitate adherence and attachment to the cervical and lung epithelium, to resist innate antimicrobial peptides and macrophages, and they play a role in biofilm formation (Maisey et al., 2008, Konto-Ghiorghi et al., 2009, Rajagopal, 2009, Rinaudo et al., 2010, Sheen et al., 2011, Sharma et al., 2013). Pili also facilitate the penetration of GBS through the blood-brain barrier (Maisey et al., 2007, Banerjee et al., 2011, Tazi et al., 2012). Two genomic pilus units have been identified; Pilus Island (PI) 1 and 2, which is further divided into 2a and 2b (Dramsi et al., 2006). Overall, PI distributions among GBS isolates have been reported similarly across

different regions with PI-1 in 70%, PI-2a in 70-80% and PI-2b in 20-30%. PI-1 usually occurs in combination with either PI-2a or PI-2b (Margarit et al., 2009, Madzivhandila et al., 2013, Martins et al., 2013). Importantly, all GBS strains carry at least one PI (Martins et al., 2013). Pilus island distributions have also been found to be associated with CPS serotypes, with most serotype III isolates having the combination of PI-1 and 2b, whilst serotype Ia is commonly associated with PI-2a. The combination of PI-1 and 2a are variably found with serotypes Ib, II, III and V (Madzivhandila et al., 2013, Martins et al., 2013). Each PI has three protein coding genes that code LPXTG (Leu-Pro-X-Thr-Gly) motif-carrying proteins, i.e. the backbone protein (PilB) and two ancillary proteins (PilA and PilC; Figure 1.2), and two sortase genes that code the assembly of the pili. Each backbone and ancillary protein is necessary for the functioning of the pilus island and serve as antigenic targets on the surface of the pili for antibody binding (Dramsi et al., 2006, Rosini et al., 2006, Margarit et al., 2009).

Additional GBS surface-proteins contributing to the virulence of the organism include FbsA and BibA, both of which were discovered recently (Schubert et al., 2004, Santi et al., 2007). Both surface-proteins, like pili, contribute to the adherence of the GBS to the epithelial surfaces and are highly immunogenic (Meinke et al., 2010). The FbsA protein does this by binding to fibrinogen, a glycoprotein which is part of the underlying structure of host epithelial cells (Schubert et al., 2004). Furthermore, the FbsA protein is thought to facilitate penetration of the brain endothelium and cause meningitis (Mu et al., 2014). Although various strains require FbsA to bind, the presence of FbsA is not conserved across all strains (Meinke et al., 2010).

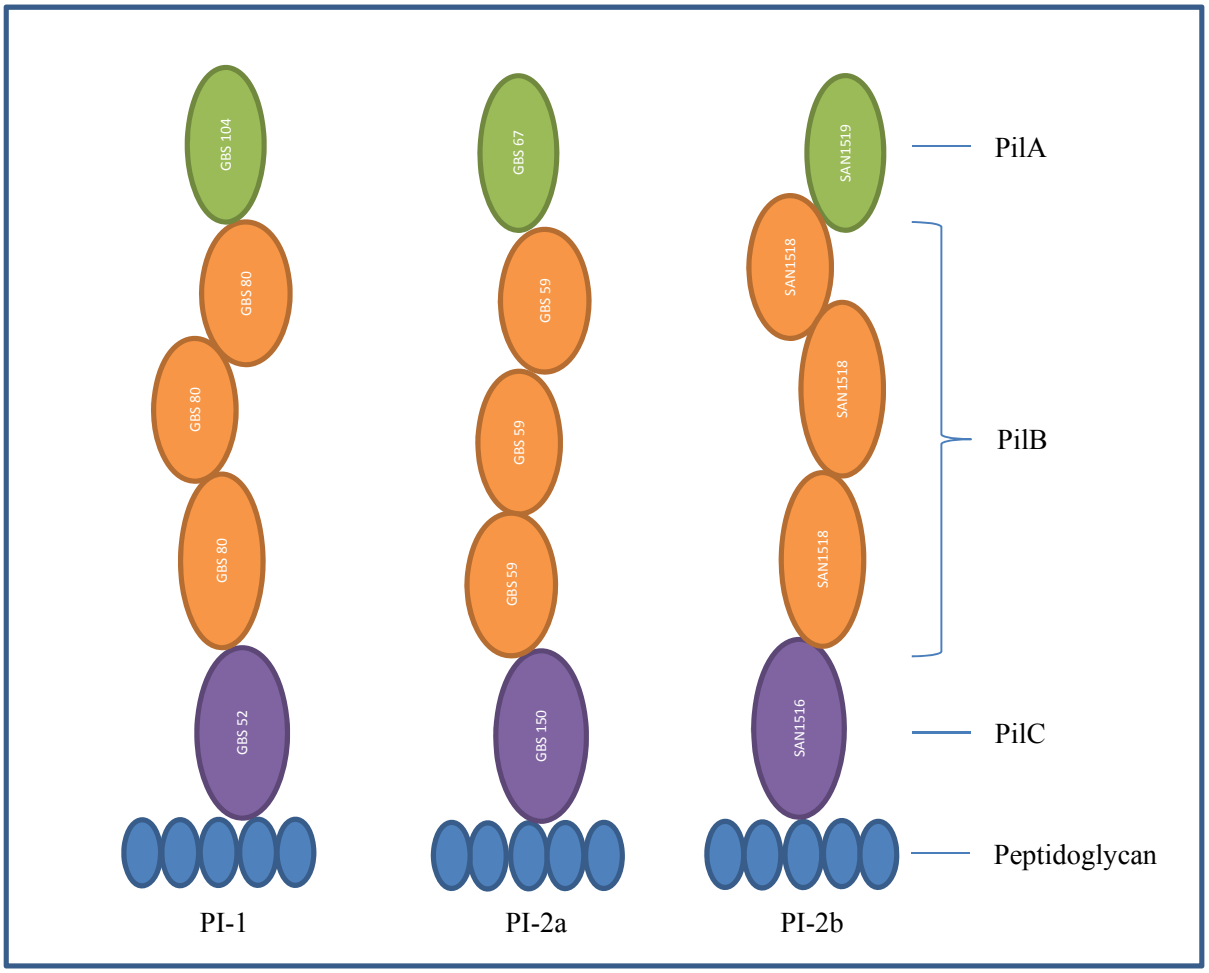


Figure 1.2: Arrangement of pilus island (PI)-1, -2a and -2b proteins. Adapted from (Vengadesan et al., 2011)

Footnote: PilA, PilB and PilC are motif-carrying proteins, i.e. the backbone protein (PilB) and two ancillary proteins (PilA and PilC); *GBS* and *SAN* are encoding genes for the particular proteins.

In addition to facilitating adherence, BibA also plays a role in resisting phagocytosis by blocking complement pathway regulators and is thought to be conserved more widely across strains (Santi et al., 2007, Santi et al., 2009). Overall, surface-proteins have provided a more in-depth understanding of GBS virulence factors and are potential antigenic targets for intervention against invasive disease.

1.4 Pathogenesis of early and late onset Group B *Streptococcus* disease

The pathogenesis of EOD differs from that of LOD. The gastrointestinal tract is the natural habitat for GBS in humans, with GBS being a normal commensal of the gastrointestinal and genitourinary tract in 20-40% of pregnant women, in whom colonisation may be transient, persistent or dynamic (Melin, 2011). Vertical transmission of GBS from colonized mothers to 30-70% of their newborns may occur *in-utero* or during the peri-partum period. Invasive EOD may then follow in approximately 1-3% of colonized newborns (Melin, 2011). After adherence by GBS to the female genital tract, the mediators for this commensal bacterium to cause invasive disease in newborns is unclear. Alterations in gene expression, virulence of the organism and bacterial overgrowth has been proposed as mechanisms to explain this invasive potential (Rajagopal, 2009). Colonizing GBS may ascend and penetrate into the amniotic cavity, regardless of whether the membranes have ruptured or not (Whidbey et al., 2013). Intra-amniotic infection may not necessarily manifest with symptoms in the mother, but aspiration of infected amniotic fluid into the newborns lungs may result in adhesion to lung epithelium, bacterial replication and evasion of the newborn immune system. From the lungs, haematogenous spread may cause septicaemia and/or meningitis (Melin, 2011). Certain

additional virulence factors may facilitate GBS penetration of blood-brain barrier (Magalhaes et al., 2013).

The pathogenesis of LOD, however, is less well understood. Although different modes of transmission have been described, the pre-requisite for invasive disease is thought to be intestinal GBS acquisition, followed by translocation into the mesenteric nodes, rather than the lung being the portal of entry (Filleron et al., 2014). The mother is still thought to be the most likely source; either through colonisation of the infant's gastrointestinal tract at birth from swallowed infected amniotic fluid, or in the days following delivery through direct contact (faecal-oral) or through breast-milk consumption. Studies have demonstrated that the same genotypic colonising strain in the mother at birth was present in a large proportion mother-infant colonised pairs as late as eight weeks of infant age (Berardi et al., 2013a). In addition, almost half of infants with LOD had the same serotype at presentation as the mother did at birth (Dillon et al., 1987). Gastrointestinal colonization of the newborn may result in invasive GBS disease after a period of latency, which could have been precipitated by aberrations in immunity, alteration of virulence potential or bacterial overgrowth.

Breast-milk transmission has also been proposed to contribute to the pathogenesis of LOD, including possibly being responsible for recurrent disease. It is hypothesized that breast-milk is initially contaminated with GBS when the organism is transferred from the throat of a colonised infant. The mother can then subsequently re-infect the infant during breastfeeding, even in the absence of mastitis (Kotiw et al., 2003, Berardi et al., 2013a, Filleron et al., 2014). Alternatively, the breast-milk may become contaminated when enteric-derived GBS is inadvertently transferred to breast-milk via uncleaned hands (Filleron et al., 2014). Lastly,

nosocomial acquisition (<10%), including health worker as a source, might also contribute to GBS transmission to newborns (Berardi et al., 2013a). Further studies are warranted to improve our understanding of the pathogenesis of LOD.

1.5 Immunology of Group B *Streptococcus* colonisation and invasive disease

To further understand the pathogenesis of invasive GBS disease in neonates, an understanding of the immune system and its interaction with GBS is necessary. The normal immune response to a microbial organism is a combination of innate and adaptive immune responses aimed at clearing putative pathogens, as well as establishment of immunological memory. Components of the innate immune system include the mucosal epithelial barrier and its natural antimicrobial peptides, phagocytes (neutrophils and macrophages), natural killer T-lymphocytes, complement factors, and various cytokines and plasma proteins (Abbas and Lichtman, 2006). These components, through various mechanisms, form a non-specific first line of defence against microbes that are identified by specific receptors or pattern recognition molecules (Landwehr-Kenzel and Henneke, 2014). Additionally, some of the components of the innate immune system relay secondary signals to activate the adaptive immune system, which is comprised of cell mediated and humoral immunity, the components of which are T-lymphocyte and B-lymphocytes, respectively (Abbas and Lichtman, 2006). The adaptive immune response is more structured and uses components of the innate system to achieve organism destruction. The host first needs to recognise the GBS organism as ‘foreign’, followed by opsonisation, recruitment of phagocytic cells (neutrophils, macrophages) through releasing chemical mediators (cytokines/chemokines) and finally extra- or intra-cellular killing

by engulfing (phagocytosis) the pathogen and/or releasing toxic metabolites (Abbas and Lichtman, 2006).

As GBS is composed of a CPS and infection is thought to be extracellular, humoral rather than cell mediated immunity dominates in protecting against invasive disease, and thus is the focus of further discussion. Humoral immunity is driven by B-lymphocytes which produce immunoglobulins (Ig) (Abbas and Lichtman, 2006). There are five classes of antibodies, namely; IgM, IgG, IgA, IgE and IgD. The presence of an antigen will stimulate B-lymphocytes to produce antibodies in the lymphoid organs. After primary exposure, some B-lymphocytes differentiate into 'memory' cells which are able to respond more avidly to subsequent attacks by the same organism. The primary immune response to an infectious agent may have a lag time of 5-10 days, is predominantly IgM that is produced in small quantities and not organism specific. In contrast, the secondary response to that organism is with large quantities of IgG that responds more rapidly and more specifically to epitopes of that organism (Abbas and Lichtman, 2006).

IgG antibody functions in many different ways (Abbas and Lichtman, 2006). Firstly, the antibody is able bind to the microbe or its toxins and in doing so block its attachment or destruction to the host cell. In addition to preventing attachment by binding to the microbe, the microbe is now marked (as "foreign") for recognition by phagocytes, a process referred to as opsonisation. Opsonisation of the microbe by antibody is critical in protecting the human host against capsular organism like GBS which are otherwise able to evade phagocytosis. Although uncommon, coating the microbe also results in destruction by natural-killer T-lymphocytes. A further mechanism in which antibody favours microbial destruction is through activation of

the classical pathway complement system. The antibody binds to the microbial surface as well as complement C1 triggering the complement cascade. This, together with innate triggering of the alternate and lectin pathways, leads to deposition of C3b fragments on the bacterial surfaces which are recognised by phagocytes. In addition, complement factors act as triggers for recruitment of inflammatory cells. The complement factors also end in a complex molecule that may directly cause osmotic lysis of the microbial cell membrane (Abbas and Lichtman, 2006).

In the neonates, physiological differences of both the innate and adaptive immune system has been proposed to explain their vulnerability to invasive GBS disease, more so in those born prematurely (Wilson, 1986, Kallman et al., 1998). Aberrations in the neonatal immune system may occur at multiple levels. With regard to humoral immunity, neonates without exposure to the organism are dependent on the transplacental transfer of their mother's IgG to protect against invasive GBS disease (Anthony, 1986). Each new acquisition of serotype-specific GBS in the mother results in an increase in the maternal immunoglobulins specific to that serotype. It is the transfer of this serotype-specific IgG from the mother to the foetus in pregnancy, which is dependent on gestational age, that may offer protection against invasive GBS disease in the first few months of infant life (Baker et al., 2003, Amirthalingam et al., 2014, Madhi et al., 2014). Therefore, IgG detected in neonatal serum soon after birth is usually maternal in origin unless there was chronic in-utero infection, which is unlikely with GBS (Niewiesk, 2014, Faucette et al., 2015).

In addition, deficiencies in both the classical and complement pathways have been reported in neonates (Edwards, 1986). Admittedly, controversies have arisen as to the role of the classical

and alternate complement pathways (Edwards, 1986, Wilson, 1986) with some studies reporting antibody-independent killing from activation of the classical pathway (Baker et al., 1982, Baker et al., 1986). It has also been postulated that deficiencies in complement components utilized in the alternate pathway may predispose neonates to developing disease, a deficiency which is thought may be overcome by an increased concentration of antibody (Baker et al., 1986).

Furthermore, the process of recruiting inflammatory cells, as well as the quantity, to the alveolus of the neonatal lung has been shown to be deficient (Wilson, 1986). Additionally, the intra- and extra-cellular killing mechanism by neutrophils have been shown to be deficient in neonates compared to adults (Kenzel and Henneke, 2006). Thus, overall immune system immaturity and the dependence on maternal IgG have left the young infant vulnerable to invasive GBS disease.

1.6 Risk factors for invasive Group B *Streptococcal* disease

Several risk factors for acquiring invasive EOD have been identified (Verani et al., 2010). The only absolute risk for invasive disease in the newborn is maternal genitourinary and/or gastrointestinal GBS colonisation, which is often characterized as heavy colonisation in the presence of GBS bacteriuria (Verani et al., 2010). Certain factors like sexual practises and diet have been suggested as contributors to colonisation but the mechanisms are unclear (Percha et al., 2011). Other maternal risk factors common to most neonatal infections, including GBS are: prolonged rupture of membranes (>18 hours), intra-amniotic infection (chorioamnionitis or endometritis), black race and young maternal age (Verani et al., 2010, Al-Kadri et al., 2013,

Chan et al., 2013, Alam et al., 2014). Infants born to young pregnant women are thought to be at increased risk of invasive GBS disease because their mothers have had less exposure to GBS and thus have lower GBS antibody levels (Anthony et al., 1994, Campbell et al., 2000). Infant risk factors include: a sibling who had GBS during their infancy, prematurity and low birth weight. Prematurity and low birth weight has also been reported as significant contributors to LOD (Berardi et al., 2013d). Premature infants have reduced antibody transfer from the mother, especially if they were less than 34 weeks gestation (Boyer et al., 1984c, Christensen et al., 1984, Lin et al., 2001). Although uncommon, obstetric procedures like internal foetal monitoring and multiple vaginal examinations have been identified as potential risk factors (Verani et al., 2010).

1.7 Clinical features of invasive Group B *Streptococcus* disease in infants

The typical presentation of EOD, usually within 12 hours of birth (75-95%), is respiratory distress (tachypnoea, sternal recessions and/or the need for supplementary oxygen) with an associated bacteraemia in 60-80% of cases (Madhi et al., 2003, Heath et al., 2009, Al-Kadri et al., 2013). Complications include the need for ventilator support or septic shock characterised by hypotension and metabolic acidosis (Madhi et al., 2003, Heath et al., 2009). Newborns with invasive GBS disease at birth may also present with perinatal asphyxia (Heath et al., 2009). In a minority of newborns, bacteraemia without a focus of infection may be present in a relatively healthy newborn, or diagnosed with transient tachypnoea of the newborn (Madhi et al., 2003). Meningitis is the more common manifestation of LOD, with clinical symptoms of irritability, seizures or fever (Heath et al., 2009, Levent et al., 2010). GBS may also manifest as a septic arthritis, osteomyelitis or cellulitis (Heath et al., 2009). Recurrences of invasive

GBS disease have been reported in up to 2% of cases despite antibiotic treatment (Broughton et al., 1976, Heath et al., 2009, Shoda et al., 2012).

1.8 Adverse maternal effects associated with Group B *Streptococcus* colonisation

In addition to the invasive disease manifestations in infants, GBS colonisation has adverse maternal effects during pregnancy that may have bearing on perinatal outcome. Women with intra-amniotic infection may present with fever, uterine tenderness or offensive liquor, but often go undetected in labour. The intra-amniotic infection may contribute significantly towards preterm delivery and stillbirths (Newton and Clark, 1988, Allen et al., 1999, Edwards and Gonik, 2013). Cytokine and chemokines releases, precipitated by *in-utero* GBS infection precipitate labour by weakening amniotic membranes resulting in their premature rupture (Edwards and Gonik, 2013). Thus GBS-colonised parturient women have an increased risk of delivering premature low birth weight babies, placing them at increased risk to the adverse effects of prematurity. Furthermore, maternal GBS infection has been associated with stillbirths with more than half of parturient women with GBS bacteraemia having spontaneous miscarriages or stillbirths (Embleton et al., 1999, Phares et al., 2008, Monari et al., 2013). Although the newborn may not be born with invasive GBS disease, the secondary effect of GBS maternal infection may cause a significant burden on perinatal outcomes.

1.9 Prevention and management of invasive Group B *Streptococcus* disease

Invasive GBS disease, either sepsis and/or meningitis, is managed by administering intravenous antibiotic therapy to the affected infant. Primary prevention of disease in the

newborn is often not possible because the newborn is born with symptoms of invasive disease. Hence, pregnant women have been targeted with interventions, including IAP for colonised mothers, to help reduce the burden of EOD in newborns. In high income countries, the reduction in incidence over the past twenty years has largely been attributable to the successful implementation of IAP to colonised parturient women who have been universally screened between 35 and 37 weeks gestation. The incidence of EOD has declined by more than 80% since the implementation of IAP (Schrag and Verani, 2013). Other high income countries have also successfully adopted these guidelines that were formulated by the Center for Disease Control (CDC) in the USA (Schrag and Verani, 2013). However, these strategies cannot be readily implemented in most low-middle income countries where a large proportion of deliveries occur outside the hospital setting (Montagu et al., 2011). This, coupled with the high cost of screening pregnant women and providing IAP, and creating the necessary infrastructure that enables the administration of intravenous IAP at least 4 hours prior to delivery has hampered preventative efforts in low-middle income countries (Glasgow et al., 2007, Fairlie et al., 2013). Furthermore, other limitations of the CDC guidelines include; false negative cultures in colonised parturient women, failure to institute IAP for sudden deliveries, the risk of resistance to penicillin, and the limited effect of IAP on preventing preterm delivery, stillbirths and LOD (Schrag and Verani, 2013). These limitations, as well as the failure to implement these strategies in low-middle income countries, have led to a paradigm shift regarding the future prevention of invasive GBS disease. In 2012, a symposium of the major international role players in the fight against GBS was held to discuss a move towards a more long-term sustainable global alternative strategy (Rappuoli and Black, 2013). Vaccinating pregnant women against GBS infection may protect infants from invasive GBS disease and may be an attractive and feasible alternative preventative strategy.

1.10 Group B *Streptococcus* prevention through maternal vaccination

Maternal serotype-specific capsular IgG transferred to the foetus has been proposed to be protective against invasive GBS disease in the neonates (Baker and Kasper, 1976). Vaccinating pregnant women in the second trimester of pregnancy could result in an increase in maternal antibody levels which in turn may be transferred to the foetus in the last trimester. This strategy has been successful in contributing to the prevention of neonatal tetanus (Demicheli et al., 2015), the incidence of which has been reduced by >80% since vaccination of pregnant women in low-middle income countries has been widely implemented (Steinhoff, 2013). In the USA, similar vaccination strategies, for pregnant women, have been approved for the prevention of influenza and pertussis in infants (Steinhoff, 2013). Maternal GBS vaccination is potentially cost effective, both in high income (Mohle-Boetani et al., 1993, Oster et al., 2014) and low-middle income countries (Kim et al., 2014). Additionally, there has been growing public awareness about the benefits of maternal vaccination to prevent other diseases in young infants; in addition to also protecting the pregnant women. A recent survey of 1013 women in the United Kingdom reported that at least 72% of women would accept vaccination in pregnancy (McQuaid et al., 2014).

Serotype-specific GBS polysaccharide-protein conjugate vaccines have been in development since the early 1990's (Kasper et al., 1996). Studies have reported that vaccination induces IgG responses which persist for long periods and could protect against LOD (Baker et al., 2003, Edwards et al., 2012). Furthermore, maternal-infant animal studies have demonstrated improved survival in mice pups whose mothers were vaccinated in pregnancy (Paoletti et al., 1994). A trivalent GBS polysaccharide-protein conjugate vaccine (serotypes Ia, Ib and III), has completed phase-II evaluation among pregnant women in Europe, North America and

Africa (Madhi et al., 2013). These serotypes cause 70-80% of all invasive GBS disease in early-infancy. In order to licence this vaccine, a large phase III efficacy trial will be required. This will require a sample size of approximately 60 000 pregnant women and the study will need to be conducted in a setting with a high incidence of invasive GBS disease but where preventative strategies cannot be pragmatically implemented - such a study will incur tremendous logistical challenges. An alternate pathway to license new vaccines is based on using immunologic endpoints for those diseases for which immunological correlates of protection have been established from previous vaccine-studies or through sero-epidemiological studies (Plotkin, 2013). This could then be followed by phase IV studies to establish vaccine effectiveness. This strategy of licensure, i.e. using a correlate of protection, is not novel and has been previously adopted in licensure of meningococcal and influenza vaccines, and more recently new formulations of polysaccharide-protein conjugate pneumococcal vaccines (The European Agency for the Evaluation of Medicinal Products, 1997, Frasch et al., 2009, World Health Organization Immunization Vaccines and Biologicals, 2012). The first step in this process, however, is to determine an antibody concentration associated with a reduced risk of invasive GBS disease; i.e. defined as the sero-correlate of protection.

1.10.1 Sero-correlates of protection to the GBS capsular polysaccharide

A systematic review was undertaken to determine the association between capsular antibodies and invasive GBS disease in young infants, (the published paper is attached as Appendix 1). We searched Pubmed, Medline and Scopus databases using the search terms; “Streptococcus agalactiae” (MESH) OR “Streptococcus agalactiae” OR “Group B Streptococcus” OR “Group B Streptococcal Infection” OR “Group B Strep” AND “Antibody” (MESH) OR “Antibody”

OR “Immunoglobulin” OR “IgG” OR “anti-GBS” OR “immunology” OR “immunity”. The inclusion process of searched articles used The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Figure 1.3).

We identified 18 studies reporting on antibody levels in infants with invasive GBS disease (Baker and Kasper, 1976, Baker et al., 1977, Baker and Kasper, 1977, Wilkinson, 1978, Christensen et al., 1980, Vogel et al., 1980, Baker et al., 1981, Christensen et al., 1982, Klegerman et al., 1983, Gotoff et al., 1984, Gray et al., 1985, Gotoff et al., 1986, Feldman and Ferrante, 1990, Gray et al., 1990, Lin et al., 2001, Matsubara et al., 2002, Lin et al., 2004, Baker et al., 2013). We extracted data on: study region and population, methods to identifying cases and controls/cohorts, clinical presentation of cases, timing of presentation of cases, age range of cases and control/cohorts, serotype distribution, serological assay used and type of antibody determination, the availability of reference serum, quantitative capsular antibody levels in maternal, cord or infant sera and whether a threshold for protection against disease was proposed. Table 1.3 and 1.4 summarises studies addressing correlates of protection against invasive GBS disease and selected animal model studies measuring survival against GBS inoculum after passive immunization with CPS antibody sera, respectively.

The CPS was first reported as immunogenic in mice model studies and antibodies directed against the capsule protected mice from invasive GBS disease (Lancefield and Freimer, 1966, Lancefield et al., 1975). Baker et al. proposed that capsular antibody transferred from mother to the foetus will be protective against invasive GBS disease in the infant and reported a series of case-control studies comparing serotype III capsular antibodies in mothers of infants with GBS to mothers of healthy infant controls (Baker and Kasper, 1976) (Table 1.3).

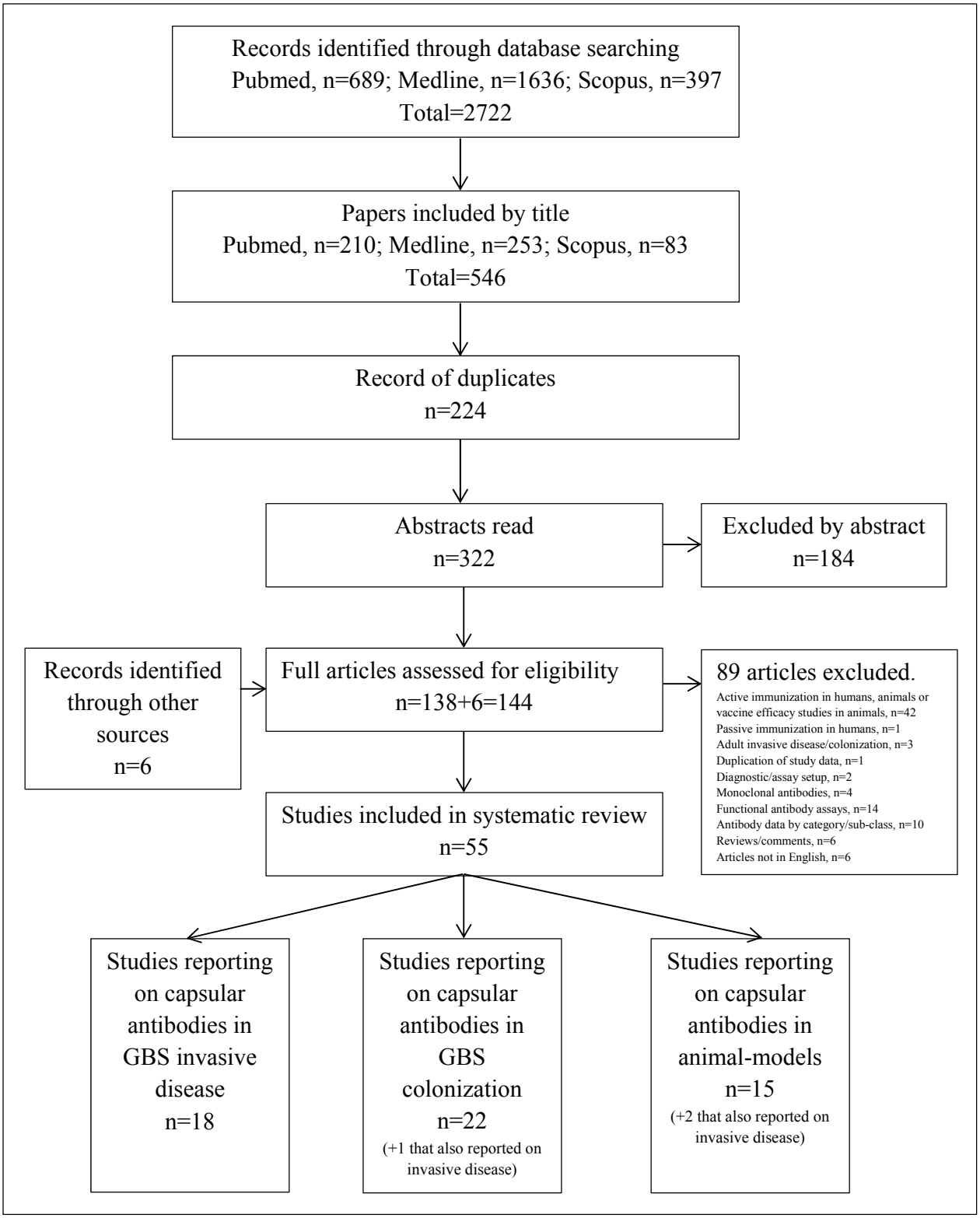


Figure 1.3: Flow diagram of selected studies reporting on capsular Group B *Streptococcus* (GBS) antibodies

Using the radioactive antigen binding assay (RABA) test, which measures total immunoglobulin, median capsular antibody levels to serotype III were lower in cases compared to controls (Baker et al., 1977, Baker et al., 1981). In a comparison of 111 cases and 45 controls, median serotype III capsular antibody levels were significantly lower in both mothers and their infants with invasive GBS disease (0.6 μg/mL and 0.4 μg/mL respectively) than in the control mothers-infant dyads (12.6 μg/mL and 5.8 μg/mL respectively) (Baker et al., 1981). None of the infants with invasive disease had antibody levels >1.7 μg/mL and only 18.6% of mothers of infants with invasive disease had antibody levels above 2 μg/mL as compared to 73% of mothers in the controls. Based on the findings of this study, serotype III capsular antibody levels above 2 μg/mL was postulated as protective against invasive serotype III GBS disease.

Subsequent studies have used the enzyme linked immunosorbent assay (ELISA) method, which is superior to the RABA that was used by the initial studies (Table 1.3). Antibody concentrations measured by ELISA correlate with RABA assays but ELISA is able to specifically measure IgG and detect antibody at lower concentrations (Polin et al., 1982, Guttormsen et al., 1996). During the 1980's, a series of studies were carried out on mice to determine survival at different antibody concentrations. These studies determined that capsular antibody concentration of ≥1 μg/mL for serotype Ia, ≥0.2 μg/mL for serotype Ib and ≥1.3 μg/mL for serotype III protected more than 90% of mice from a lethal challenge with that specific serotype strain (Klegerman et al., 1983, Boyer et al., 1984a, Gotoff et al., 1984, Gotoff et al., 1986) (Table 1.4). The usefulness of these levels, initially obtained from murine studies, was then evaluated as a marker for protection in humans (Table 1.3). Although none of the infants with invasive GBS disease or their mothers had levels above these proposed

concentrations, so too did a large proportion of the colonized mothers of healthy infants. This was also observed in a series of studies conducted by Gray et al., who matched cases to serotype-specific colonised newborns rather than colonised mothers (Gray et al., 1985, Gray et al., 1990). From this, we are able to infer that there may be other mechanisms (for example, microbial virulence factors) that contribute to the risk of invasive GBS disease.

The largest case-control study aimed at establishing a sero-correlate of protection against invasive GBS disease in neonates was undertaken in USA by Lin et al. between 1995 and 1998 (Lin et al., 2001, Lin et al., 2004) (Table 1.3). The samples taken from 138,740 live births in 14 hospitals identified 53 cases with serotype Ia and 29 cases with invasive serotype III GBS disease in infant's ≥34 weeks gestation. These were matched to 336 serotype Ia colonized controls and 330 serotype III colonised controls. Geometric mean antibody concentrations (GMC's) for serotype Ia were 0.32 μg/mL and 0.22 μg/mL in mothers and their newborns with invasive GBS disease, compared to 0.65 μg/mL and 0.52 μg/mL in mothers colonized by the homotypic serotype and their healthy newborns, respectively. This study also estimated the percentage risk reduction for invasive GBS disease at different antibody levels. Using serotype-specific IgG <0.5 μg/mL as reference, the odds ratio (OR) was 0.69 (31% reduction), 0.45 (55% reduction) and 0.12 (88% reduction) with a maternal antibody concentration of ≥0.5 μg/mL, ≥2 μg/mL and ≥5 μg/mL, respectively. For serotype III, GMC's were 2.73 μg/mL and 2.03 μg/mL in mothers and newborns of invasive GBS cases, compared to 4.27 μg/mL and 3.29 μg/mL in serotype III colonized women and their healthy newborns, respectively. Using an IgG concentration <2 μg/mL as reference the OR for invasive GBS disease estimated to be 0.25 (75% reduction), 0.23 (77% reduction) and 0.07 (91% reduction) for maternal antibody concentrations of ≥2 μg/mL, ≥5 μg/mL and ≥10 μg/mL, respectively.

Hence, maternal antibody concentration of ≥5 μg/mL for serotype Ia and ≥10 μg/mL for serotype III was calculated to reduce the risk of invasive GBS disease by approximately 90%. In contrast to the studies by Lin et al., Baker et al. (a re-analysis of samples taken between 1998 and 1999) , using <0.1 μg/mL as reference, reported the relative risk of 0.11 (89% reduction) for serotype Ia, 0.09 (91% reduction) for serotype III and 0.29 (71% reduction) for serotype V if the maternal antibody concentration was ≥0.5 μg/mL (Baker et al., 2013). After Bayesian modelling, the authors suggested that an antibody concentration of ≥1 μg/mL would be protective for Ia and III and probably V against invasive GBS disease. The difference in the proposed correlate of protection between the study by Lin et al. and Baker et al. could have been due to differences in selection of controls and the ELISA assay not being the same between the studies. Importantly, estimating a threshold for protection using odds ratios or relative risk is not statistically sufficient as they measure a relative risk to a given threshold. Thus, using additional statistical methods, including Bayesian modelling as used by Baker et al., is thought to provide a more accurate estimate of a protective antibody threshold.

There have not been any further published studies on correlates of protection against invasive GBS disease since 1999, except for the Design of a Vaccine Against Neonatal Infections (DEVANI) study, which is, a pan-European program that collected sera from mothers of cases and controls. The DEVANI study also reported an association between high antibody concentrations and a reduced risk of invasive GBS disease for serotypes Ia, Ib and III (Melin and Efstratiou, 2013). No studies have been undertaken in low-middle income countries on establishing a correlate for protection against invasive GBS disease.

Table 1.3: Studies describing serotype-specific capsular antibody concentrations in mothers of infants with and without invasive Group B *Streptococcus* (GBS) disease [A summarized version of this table appears in Appendix 1]

Reference Location	Study design	Cases/Infected with GBS n= (# at days after birth & sepsis(S) or meningitis(M) bone/joint(BJ)	Controls/ Cohorts	Matching criteria of controls/cohort	Assay used	GBS Antibodies (Total Ig or IgG)[1]	Ref serum[2]	Antibody levels in cases (in µg/mL) unless otherwise stated	Antibody levels in controls/cohort (in µg/mL) unless otherwise stated	Suggested threshold for protection
Baker 1976 (Baker and Kasper, 1976) USA	Case-control	7 with GBS III *(3 ≤5days, 2S 1M; 4 ≥10days, 2M 2BJ)*	29 Pregnant women vaginally colonized with serotype III	Pregnant women with serotype III vaginal-colonization and infant did not develop GBS	RABA[3] [detectable levels defined as binding capacity of >40% at a dilution of ≥1:2	III (Total Ig)	Not reported	Mothers (n=7): 0 of 7 mothers had detectable levels Infants (n=5): 0 of 5 infants had detectable levels	Mothers (n=29): 22 of 29 (76%) of mothers had detectable levels Infants-cord blood (n=3): 3 of 3 infants had detectable levels	No
Baker 1977 (Baker et al., 1977) USA	Case-control	31 with GBS III *(9 ≤5 days, 4S 5M; 22 >9 days, 4S 11M 7BJ)* 7 of 31 were from 1976 study	43 Pregnant women vaginally colonized with serotype III 12 Pregnant women colonised with other serotypes 16 non-colonized	Pregnant women with serotype III vaginal-colonization and infant did not develop GBS	RABA [the degree of antigen binding was converted to a concentration	III (Total Ig)	Not reported	Mothers (n=29): Median~1 (<1–26) Infants (n=31): Sepsis- Median 0.65(0.34–1.52) Meningitis- Median 0.45(0.32–1.52) Arthritis/Osteomyelitis- Median 1.05(0.33–1.78)	Mothers (n=43): Median~12 (<1–>40) Infants: No data	No

[1]Total Ig or IgG- Total Immunoglobulin or Immunoglobulin G, [2]Reported using standardised reference serum, [3]RABA- Radioactive Antigen Binding Assay, [4]ELISA- Enzyme Linked Immunosorbent Assay, [5]GMC's- Geometric Mean Concentrations; [6]EOD- Early Onset Disease (0-6 days age), [7]LOD- Late Onset Disease (7-89 days age). ~denotes approximation.

Reference Location	Study design	Cases/Infected with GBS n= (# at days after birth & sepsis(S) or meningitis(M) bone/joint(BJ)	Controls/ Cohorts	Matching criteria of controls/cohort	Assay used	GBS Antibodies (Total Ig or IgG)[1]	Ref serum[2]	Antibody levels in cases (in µg/mL) unless otherwise stated	Antibody levels in controls/cohort (in µg/mL) unless otherwise stated	Suggested threshold for protection
Baker 1977 (Baker and Kasper, 1977) USA	Case-Control (Data from 1977 study)	17 with GBS III (8 ≤5 days, 4S 4M 9 >9 days, 4S 5M)	43 Pregnant women vaginally colonized with type III 1 Pregnant women vaginally colonized II 10 non-colonised	Pregnant women with serotype III vaginal-colonization and infant did not develop GBS	RABA[3] [enriched antigen-detectable levels defined as binding capacity of >40% at a dilution of ≥1:2	III (Total Ig)	Not reported	Mothers (n=15): 2 of 15 mothers had detectable levels Infants (n=17): 0 of 17 infants had detectable levels	Mothers (n=43): 31 of 43 (72%) of serotype III colonized mothers had detectable levels 5 of 10 (50%) of non-colonized mothers had detectable levels Infants: No data	No
Wilkinson 1978 (Wilkinson, 1978) USA	Cohort	10 with GBS III (not specified)	4 colonized newborns with serotype III	Healthy newborn with serotype III colonization (ear, cord, gastric)	Radioimmuno assay	III (Total Ig)	Not reported	Mothers (n=4): Mean 31.7 Infants (n=8): Mean 9.3	Mothers (n=2): Mean 12.1 Infants (n=4): Mean 11.0	No

[1]Total Ig or IgG- Total Immunoglobulin or Immunoglobulin G, [2]Reported using standardised reference serum, [3]RABA- Radioactive Antigen Binding Assay, [4]ELISA- Enzyme Linked Immunosorbent Assay, [5]GMC's- Geometric Mean Concentrations; [6]EOD- Early Onset Disease (0-6 days age), [7]LOD- Late Onset Disease (7-89 days age). ˜denotes approximation.

Reference Location	Study design	Cases/Infected with GBS n= (# at days after birth & sepsis(S) or meningitis(M) bone/joint(BJ)	Controls/ Cohorts	Matching criteria of controls/cohort	Assay used	GBS Antibodies (Total Ig or IgG)[1]	Ref serum[2]	Antibody levels in cases (in µg/mL) unless otherwise stated	Antibody levels in controls/cohort (in µg/mL) unless otherwise stated	Suggested threshold for protection
Christensen 1980 (Christensen et al., 1980) Sweden	Case control	7 with GBS 3 with III 3 with Ib 1 with Ia (4 ≤2 days, 3S 1M; 3 >7 days, 3M) [3 premature: 34-36 weeks]	13 pregnant women colonised (urethra and cervix) with 5 with III 3 with Ia 2 with Ib 3 mix sero	Pregnant women with serotype-specific urethra and cervix colonisation and infant did not develop GBS, all term infants	Radiolabelled protein A	Ia, Ib, III (IgG)	Not reported	Mothers (n=7): 6/7 had lower levels than controls specific to serotype Ia <1cpmX10^4 Ib 3-4cpmX10^4 III <1cpmX10^4 Infants: No data	Mothers (n=13): All had higher antibody levels than cases except 1 case which had similar to controls Infants: No data	No
Vogel 1980 (Vogel et al., 1980) USA	Cohort	54 with GBS 2 with Ia 8 with Ib 4 with II 40 with III (not specified)	108 pregnant women vaginally colonised 22 with Ia 28 with Ib 22 with II 36 with III 129 non-colonised women	Pregnant women with serotype-specific vaginal colonisation and infant did not develop GBS	Indirect immunofluorescent assay	Ia, Ib, II, III (IgG)	Not reported	Mothers (n=54): Serotype-specific antibody detected on 0/2 (0%) with Ia 1/8 (13%) with Ib 0/4 (0%) with II 8/40 (20%) with III [levels were low]	Mothers (n=108): Colonised- Serotype-specific antibody detected on 59% - Ia 57% - Ib 96% - II 50% - III Mothers (n=129): Non-colonised- antibody detected on 26% - Ia 54% - Ib 82% - II 47% - III	No

[1]Total Ig or IgG- Total Immunoglobulin or Immunoglobulin G, [2]Reported using standardised reference serum, [3]RABA- Radioactive Antigen Binding Assay, [4]ELISA- Enzyme Linked Immunosorbent Assay, [5]GMC's- Geometric Mean Concentrations; [6]EOD- Early Onset Disease (0-6 days age), [7]LOD- Late Onset Disease (7-89 days age). ~denotes approximation.

Reference Location	Study design	Cases/Infected with GBS n= (# at days after birth & sepsis(S) or meningitis(M) bone/joint(BJ)	Controls/ Cohorts	Matching criteria of controls/cohort	Assay used	GBS Antibodies (Total Ig or IgG)[1]	Ref serum[2]	Antibody levels in cases (in µg/mL) unless otherwise stated	Antibody levels in controls/cohort (in µg/mL) unless otherwise stated	Suggested threshold for protection
Baker 1981 (Baker et al., 1981) USA	Case-control	111 with GBS III *(32 <5days, 18S 14M; 79 > 7 days, 28S 51M)*	45 Pregnant women vaginally colonized with type III	Pregnant women with serotype III vaginal-colonization and infant did not develop GBS (matched to EOD)	RABA[3]	III (Total Ig)	Not reported	Mothers (n=32): Sepsis- Median 0.6 (0.3–40.3) Meningitis- Median 0.6 (0.3–1.55) 6 of 32 (19%) mothers had level >2 µg/mL Infants EOD (n=32): Sepsis- Median 0.4 (0.3–1.3) Meningitis- Median 0.3 (0.3–1.1) Infants LOD (n=79): Sepsis- Median 0.4 (0.3–1.6) Meningitis- Median 0.4 (0.3–1.2)	Mothers (n=45): Median 12.6 (0.3–40.3) 33 of 45 (73%) mothers had level >2 µg/mL Infants-cord blood (n=45): 5.8 (0.3–40.3) 29 of 45 (64%) infants had levels >2 µg/mL	**>2 µg/mL as protective**
Christensen 1982 (Christensen et al., 1982) Sweden	Case-control	16 with GBS 2 with Ia 4 with Ib 2 with II 8 with III (12 EOD[6], 3LOD[7], 1 death)	29 pregnant women urogenital colonised 10 with Ia 5 with Ib 5 with II 9 with III	Pregnant women with serotype-specific urethra and cervix colonisation and infant did not develop GBS, all term infants (Sera on 5 in 1st trimester)	Radiolabelled protein A	Ia, Ib, II, III (IgG)	Not reported	Mothers (n=16): 14 of 16 had lower serotype-specific antibody than controls	Mothers (n=29): All had higher antibody levels than cases except for 2 cases	No

[1]Total Ig or IgG- Total Immunoglobulin or Immunoglobulin G, [2]Reported using standardised reference serum, [3]RABA- Radioactive Antigen Binding Assay, [4]ELISA- Enzyme Linked Immunosorbent Assay, [5]GMC's- Geometric Mean Concentrations; [6]EOD- Early Onset Disease (0-6 days age), [7]LOD- Late Onset Disease (7-89 days age). ~denotes approximation.

Reference Location	Study design	Cases/Infected with GBS n= (# at days after birth & sepsis(S) or meningitis(M) bone/joint(BJ)	Controls/ Cohorts	Matching criteria of controls/cohort	Assay used	GBS Antibodies (Total Ig or IgG)[1]	Ref serum[2]	Antibody levels in cases (in µg/mL) unless otherwise stated	Antibody levels in controls/cohort (in µg/mL) unless otherwise stated	Suggested threshold for protection
Klegerman 1983 (Klegerman et al., 1983) USA	Cohort	11 with GBS Ia (8 EOD, 7S 1M; 3 LOD, 2S 1M)	25 Pregnant women vaginally or rectally colonised with Ia 50 randomly selected pregnant women	Pregnant women with serotype Ia vaginal or rectal colonisation and infant did not develop GBS	ELISA[4]	Ia (IgG)	Not reported	Mothers and infants/cord (n=11): None of the 11 infected infants had level ≥0.17 µg/mL Median 0.04 (<0.03–0.16)	Mothers colonized (n=25): 36% of colonised had level ≥1 µg/mL Mothers randomly selected (n=50): 12% of randomly selected had level ≥1 µg/mL	No association established between capsular antibody levels in colonised and non-colonised mothers and risk of disease in newborn. Colonised women had higher levels than non-colonised
Gotoff 1984 (Gotoff et al., 1984) USA	Cohort	9 with GBS Ib (5 EOD, 5S; 4 LOD, 2S 2M) [5 premature: 25-35 weeks]	25 Pregnant women vaginally or rectally colonised with Ib 50 randomly selected pregnant women	Pregnant women with serotype Ib vaginal or rectal colonisation and infant did not develop GBS	ELISA	Ib (IgG)	Not reported	Mothers and infants/cord (n=9): Median 0.06 (<0.03–0.09) None of the 9 infected infants had level ≥0.2 µg/mL	Mothers colonized (n=25): Median 0.15 (<0.02–4.7) 44% of colonised had level ≥0.2 µg/mL Mothers randomly selected (n=50): Median 0.03 (<0.02-39.9) 20% of randomly selected had level ≥0.2 µg/mL	No association established between capsular antibody levels in colonised and non-colonised mothers and risk of disease in newborn. Colonised women had higher levels than non-colonised

[1]Total Ig or IgG- Total Immunoglobulin or Immunoglobulin G, [2]Reported using standardised reference serum, [3]RABA- Radioactive Antigen Binding Assay, [4]ELISA- Enzyme Linked Immunosorbent Assay, [5]GMC's- Geometric Mean Concentrations; [6]EOD- Early Onset Disease (0-6 days age), [7]LOD- Late Onset Disease (7-89 days age). ~denotes approximation.

Reference Location	Study design	Cases/Infected with GBS n= (# at days after birth & sepsis(S) or meningitis(M) bone/joint(BJ)	Controls/ Cohorts	Matching criteria of controls/cohort	Assay used	GBS Antibodies (Total Ig or IgG)[1]	Ref serum[2]	Antibody levels in cases (in µg/mL) unless otherwise stated	Antibody levels in controls/cohort (in µg/mL) unless otherwise stated	Suggested threshold for protection
Gray 1985 (Gray et al., 1985) USA	Cohort	15 with GBS II (13 EOD, 12S 1M; 2 LOD, 2M) [9 premature]	347 colonized newborns (4 sites) 94 with Ia/c 69 with Ib 70 with II 99 with III 15 with Ia&III 54 non-colonized	Healthy newborn with serotype colonization at 4 sites-(ear, umbilicus, throat, anus) 95% of infants were cultured, (60% of mothers-de facto)	ELISA[4]	II (IgG)	No (Average was assumed from pooled Ig)	Mothers and infants-cord (n=15): Calculated Mean ~1.77 (0–4.5) 5/15 (33%) with type II disease had levels ≥2 µg/mL	Mothers (n=401): Had GBS II antibody levels >2 µg/mL in 24% of Ia/c 15% of Ib **37% of II (mean4.8)** 17% of III 27% of Ia&III **2% of non-colonized** Infants-cord blood (n=401): Had GBS II antibody levels >2 µg/mL in 26% of Ia/c 16% of Ib **36% of II (mean 4.7)** 14% of III 20% of Ia&III **2% of non-colonized**	They chose >2 µg/mL as an indicative figure for prevalence and not as protective level
Gotoff 1986 (Gotoff et al., 1986) USA	Cohort	42 with GBS III (not specified) [24-41 weeks]	25 Pregnant women vaginally or rectally colonised with III 102 randomly selected pregnant women	Pregnant women with serotype III vaginal or rectal colonisation and infant did not develop GBS	ELISA	III (IgG)	Not reported	Mothers and infants/cord (n=42): Median 0.05 (<0.02-0.3) None of the 42 infected infants had level >0.3 µg/mL	Mothers colonized (n=25): Median 0.78 (0.1-10.7) 9 of 25 (36%) of colonised had level ≥1.3 µg/mL Mothers randomly selected (n=102): Median 0.05 (<0.02-21.7) 13% of randomly selected had level ≥1.3 µg/mL	0.75 µg/mL protected 80% of mice and 1.3 µg/mL protected 97% of mice against lethal challenge

[1]Total Ig or IgG- Total Immunoglobulin or Immunoglobulin G, [2]Reported using standardised reference serum, [3]RABA- Radioactive Antigen Binding Assay, [4]ELISA- Enzyme Linked Immunosorbent Assay, [5]GMC's- Geometric Mean Concentrations; [6]EOD- Early Onset Disease (0-6 days age), [7]LOD- Late Onset Disease (7-89 days age). ~denotes approximation.

Reference Location	Study design	Cases/Infected with GBS n= (# at days after birth & sepsis(S) or meningitis(M) bone/joint(BJ)	Controls/ Cohorts	Matching criteria of controls/cohort	Assay used	GBS Antibodies (Total Ig or IgG)[1]	Ref serum[2]	Antibody levels in cases (in µg/mL) unless otherwise stated	Antibody levels in controls/cohort (in µg/mL) unless otherwise stated	Suggested threshold for protection
Feldman 1990 (Feldman and Ferrante, 1990) UK	Cohort	19 with GBS III (not specified)	10 (2 with III) mothers of colonized newborns 90 randomly selected pregnant women 12 non-pregnant women	Healthy newborn with non-specific serotype colonization	ELISA[4]	III (IgG)	Yes (from C. Baker)	Mothers (n=19): Mean 0.6 (<0.5–4.3) 2 of 19 (11%) mothers had levels ≥2 µg/mL but their infants had levels below	Mothers colonized (n=10): Mean~15 (<0.5–>100) Mothers randomly selected (n=90): Mean~12 (<0.5–>100) Non pregnant(n=12): Mean~11.5 (<0.5–>100)	**>2 µg/mL as protective**
Gray 1990 (Gray et al., 1990) USA	Cohort-1982 abstract	8 with GBS Ia (Not specified)	347 colonized newborns (4 sites) 94 with Ia/c 69 with Ib 70 with II 99 with III 15 with Ia & III 54 non-colonized	Healthy newborn with serotype colonization at 4 sites-(ear, umbilicus, throat, anus) 95% of infants were cultured, (60% of mothers-de facto)	ELISA	Ia (IgG)	No	Mothers and infants-cord (n=8): 2 of 8 (25%) with Ia had antibody levels ≥ 2µg/mL mean~1.05	Mothers (n=401): Mean for Ia (n=94) was 9.1±8.3 Mean for other serotypes (n=253) 7.3±8.2 --- Infants-cord blood (n=401): Mean for Ia (n=94) was 6.5±5.1 Mean for other serotypes (n=253) 6.5±5.7 Had GBS Ia antibody levels >2 µg/mL in 16% of Ia 6% of Ib 14% of II 14% of III and 5% of non-colonized	>2 µg/mL as epidemiological marker and not as protective level.

[1]Total Ig or IgG- Total Immunoglobulin or Immunoglobulin G, [2]Reported using standardised reference serum, [3]RABA- Radioactive Antigen Binding Assay, [4]ELISA- Enzyme Linked Immunosorbent Assay, [5]GMC's- Geometric Mean Concentrations; [6]EOD- Early Onset Disease (0-6 days age), [7]LOD- Late Onset Disease (7-89 days age). ~denotes approximation.

Reference Location	Study design	Cases/Infected with GBS n= (# at days after birth & sepsis(S) or meningitis(M) bone/joint(BJ)	Controls/ Cohorts	Matching criteria of controls/cohort	Assay used	GBS Antibodies (Total Ig or IgG)[1]	Ref serum[2]	Antibody levels in cases (in µg/mL) unless otherwise stated	Antibody levels in controls/cohort (in µg/mL) unless otherwise stated	Suggested threshold for protection
Lin 2001 (Lin et al., 2001) USA	Case-control	53 with GBS Ia *(All <7days, 47S 5SM 1M)* [4 premature: <34 weeks]	336 colonised newborns with Ia	Healthy newborn with serotype Ia colonization at 4 sites-(ear, umbilicus, throat, anus)	ELISA[4]	Ia (IgG)	Yes (serum-20-Nabi Pharm)	Mothers (n=49): GMC[5] 0.32 65% had level <0.5 µg/mL 2 of 49(4%) had level >5 µg/mL --- Infants-cord blood (n=49): GMC 0.22 1 of 49 (2%) had level ≥4 µg/mL	Mothers (n=326): GMC 0.65 52% had level <0.5 µg/mL --- Infants-cord blood (n=323): GMC 0.52 53 of 323 (16%) had level ≥4 µg/mL	On 45 cases and 319 controls pairs Using <0.5 as reference (Odds=1) Mother: OR 0.69 if ≥0.5 (31% reduction OR 0.45 if ≥2 (55% reduction) OR 0.12 if ≥5 (88% reduction) Cord: OR 0.58 if ≥0.5 (42% reduction OR 0.09 if ≥4 (91% reduction) **Suggested ≥5 µg/mL as protective**
Matsubara 2002 (Matsubara et al., 2002) Japan	Cohort	4 with GBS VIII *(All <7 days 4S)*	13 Pregnant women vaginally colonised with VIII and 538 non colonised at 28 weeks	Pregnant women vaginally colonised with VIII	ELISA	VIII (IgG)	yes	Mother (n=4): GMC 0.41±0.07 (0.26-0.57) --- Infant (n=4): GMC 0.49±0.12(0.35-0.84)	Mother: GMC Colonised with VIII (n=13) 5.53±2.79 (0.47-33.85) Non colonised (n=535) 1.53±0.32 (0.07-104.97)	**Suggested >1 µg/mL as protective**

[1]Total Ig or IgG- Total Immunoglobulin or Immunoglobulin G, [2]Reported using standardised reference serum, [3]RABA- Radioactive Antigen Binding Assay, [4]ELISA- Enzyme Linked Immunosorbent Assay, [5]GMC's- Geometric Mean Concentrations; [6]EOD- Early Onset Disease (0-6 days age), [7]LOD- Late Onset Disease (7-89 days age). ~denotes approximation.

Reference Location	Study design	Cases/Infected with GBS n= (# at days after birth & sepsis(S) or meningitis(M) bone/joint(BJ)	Controls/ Cohorts	Matching criteria of controls/cohort	Assay used	GBS Antibodies (Total Ig or IgG)[1]	Ref serum[2]	Antibody levels in cases (in µg/mL) unless otherwise stated	Antibody levels in controls/cohort (in µg/mL) unless otherwise stated	Suggested threshold for protection
Lin 2004 (Lin et al., 2004) USA	Case-control	29 with GBS III (All<7 days 25S 4SM)	330 colonised newborns (4 sites) with III	Healthy newborn with serotype III colonization at 4 sites (ear, umbilicus, throat, anus)	ELISA[4]	III (IgG)	Yes (Serum-19-Nabi Pharm)	Mothers (n=28): GMC[5] 2.73 11 of 28 (41%) had level <2 µg/mL Infants-cord blood (n=27): GMC 2.03	Mothers (n=306): GMC 4.27 27 of 306 (9%) had level <2 µg/mL Infants-cord blood (n=312): GMC 3.29	On 26 cases and 143 controls pairs Using <2 as reference (Odds=1) Mother: OR 0.25 if ≥2 (75% reduction OR 0.23 if ≥5 (77% reduction) OR 0.09 if ≥10 (91% reduction) Cord: OR 0.31 if ≥2 (69% reduction OR 0.26 if ≥5 (91% reduction) OR 0.15 if ≥6 (85% reduction) **Suggested ≥10 µg/mL as protective**
Baker 2013 (Baker et al., 2013) USA	Case-control	33 GBS 17 with Ia 9 with III 7 with V (all<7days 29S 4SM)	99 Pregnant women vaginally or rectally colonised matched for ethnicity, age and colonising serotype	Pregnant women with serotype Ia, III or V vaginal or rectal colonisation and infant did not develop GBS	ELISA	Ia, III, V (IgG)	Yes	Mothers: Median (IQ range) Ia(n=17)-0.20(0.06-1.68) III(n=9)-0.06(0.02-0.12) V(n=7)-0.09(0.04-0.80)	Mothers: Median (IQ range) Ia(n=51)-1.83(0.20-5.54) III(n=27)-1.64(0.14-5.51 V(n=21)-0.53(0.07-1.0)	Using <0.1 as reference, Relative risk Mother: For Ia-RR 0.11 if ≥0.5 (89% reduction) For III-RR 0.09 if ≥0.5 (91% reduction) For V-RR 0.29 if ≥0.5 (71% reduction) **Suggested ≥1 µg/mL as protective for Ia and III and probably V**

[1]Total Ig or IgG- Total Immunoglobulin or Immunoglobulin G, [2]Reported using standardised reference serum, [3]RABA- Radioactive Antigen Binding Assay, [4]ELISA- Enzyme Linked Immunosorbent Assay, [5]GMC's- Geometric Mean Concentrations; [6]EOD- Early Onset Disease (0-6 days age), [7]LOD- Late Onset Disease (7-89 days age). ~denotes approximation.

Table 1.4: Studies reporting on capsular antibody and animal survival against Group B *Streptococcus* challenges

Author and Year of publication	Animal model	Anti-sera	Inoculum [Lethal dose LD]	Assay used	GBS Antibodies (Total Ig or IgG)[1]	Standardised reference serum	Comments
Stewardson-Krieger 1977 (Stewardson-Krieger et al., 1977)	Mice	-Human sera -Gamma-globulin	2 stock strain injected intra-peritoneal at the same time as sera [LD90]	Lancefield method	Ia (Total Ig)	Not reported	-14/56 sera protected ≥75% of mice challenged with GBS Ia -Pooled gamma globulin protected 100% of mice challenged with GBS Ia
Baltimore 1981 (Baltimore et al., 1981)	Mice	-Commercial human immune globulin -Laboratory volunteers -Adults immunised with GBS-III vaccine	1 clinical strain injected intra-peritoneal [LD90]	RABA[2]	III (Total Ig)	Not reported	-None of the groups of mice given the commercial human immune globulin or laboratory volunteer sera demonstrated ≥50% mice survival. -Groups of mice given the vaccinated adult sera demonstrated ≥50% mice survival
Fleming 1982 (Fleming, 1982)	Mice	-Pooled human immune globulin -Rabbit antisera -Adults immunised with GBS-III vaccine.	2 stock strains injected intra-peritoneal [LD50]	RABA	III (Total Ig)	Not reported	-100% of mice given the commercial human immune globulin and rabbit antisera demonstrated survival. -52% of mice litters given the vaccinated adult sera demonstrated survival
De Cueninck 1982 (De Cueninck et al., 1982)	Rats	-Human sera (pre and post vaccination with GBS-III vaccine).	2 stock strain injected subcutaneously [LD50]	ELISA[3]	III (IgG)	Yes	-Pre-vaccinated sera did not protect rats. 0.8 µg/mL of subject 1 sera protected 100% of rats 0.6 µg/mL of subject 2 sera protected 90% of rats 0.2 µg/mL of 6 subjects sera protected 50% of rats
Larsen 1983 (Larsen et al., 1983)	Monkey	-5 received IVIG 24 hours prior to inoculum -5 received IVIG 24 hours prior to inoculum as well as their babies at birth -17 did not have any IVIG	Intra-amniotic inoculum24 hours before delivery.	RABA	III (Total Ig)	Not reported	60% mortality in IVIG group 71% mortality in no IVIG group Similar mean antibody levels
Klegerman 1983 (Klegerman et al., 1983)	Mice	-Human donor sera with antibodies to GBS Ia.	1 stock strain and 4 clinical strains injected intra-peritoneal 24 hours later [LD90]	ELISA	Ia (IgG)	Not reported	0.50 µg/mL protected 100% of mice (stock strain) 0.25-1 µg/mL protected 100% of mice dependant on strength of inoculum of clinical strains **Therefore ≥1.0 µg/mL suggested as protective**
Boyer 1984 (Boyer et al., 1984a)	Mice	-Human donor sera with antibodies to GBS Ib.	1 stock strain and 4 clinical strains injected intra-peritoneal 24 hours later [LD90]	ELISA	Ib (IgG)	Not reported	0.038-0.175 µg/mL protected 100% of mice dependant on strength of inoculum **Therefore ≥0.2 µg/mL suggested as protective**
Gotoff 1986 (Gotoff et al., 1986)	Mice	-Human donor sera with antibodies to GBS III.	Inoculum from 4 clinical strains injected intra-peritoneal 24 hours later [LD90]	ELISA	III (IgG)	Not reported	0.50 µg/mL protected 50% of mice 0.75 µg/mL protected 80% of mice 1.3 µg/mL protected 97% of mice **Therefore ≥1.3 µg/mL suggested as protective**

[1]Total Ig or IgG- Total Immunoglobulin or Immunoglobulin G, [2]RABA- Radioactive Antigen Binding Assay, [3]ELISA- Enzyme Linked Immunosorbent Assay

In a meta-analysis, we selected studies reporting the proportion of invasive GBS disease cases and controls with an antibody concentration ≥2 μg/mL to serotypes Ia and III (Figures 1.4 and 1.5). An antibody concentration of ≥2 μg/mL was chosen as this was initially suggested by Baker et al. to protect against invasive serotype III GBS disease (Baker et al., 1981). Although these studies are not directly comparable due to differences in methodology and absence of standardized immunological assay, the proportion of invasive GBS disease cases with a serotype-specific capsular antibody ≥2 μg/mL was generally lower than in controls. The odds of invasive GBS disease was 2.38 (95% CI: 1.20-4.70) and 6.56 (95% CI: 2.10-20.55) times greater in infants whose mothers had antibody levels <2 μg/mL for serotype Ia and III, respectively (Figure 1.4 and 1.5).

Over the past four decades, quantitative serotype-specific capsular antibody concentrations have been reported to be lower in infants with invasive GBS disease compared to levels in mothers of healthy infants or colonized but healthy infant controls. This systematic review, however, highlighted that differences in study-design, age-range of invasive GBS cases, antibody assay methods and lack of standardized reference serum (available from Dr Carol Baker and Nabi Biopharmaceuticals) between tests have limited the comparability of studies as well as the interpretation of the serologic outputs proposed as putative measures of protection. As such, these studies have been unsuccessful in determining an antibody level that could be used as a sero-correlate of protection. In addition, studies have not independently explored the association between antibody levels and LOD, and most studies mainly focused on disease caused by serotypes Ia and III.

Study	Cases	Controls	OR (95% CI)	pval
Klegerman, 1983	0/8 (0%)	5/25 (20%)	2.45 (0.29, Inf)	0.448
Lin, 2001	7/45 (16%)	88/319 (28%)	2.06 (0.87, 5.69)	0.151
Baker, 2013	~4/17 (24%)	~24/51 (47%)	2.85 (0.75,13.63)	0.151
Combined	11/70 (16%)	117/395 (30%)	2.38 (1.20, 4.70)	0.013

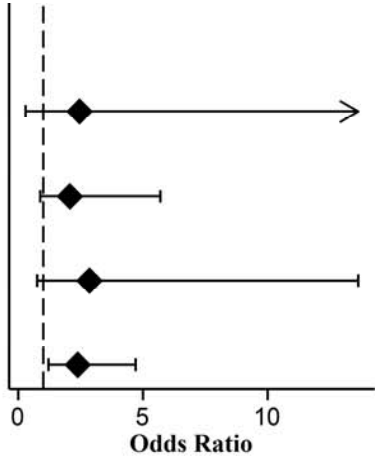


Figure 1.4: Proportion of mothers of infants with a capsular antibody concentrations ≥2 µg/mL for serotype Ia, a meta-analysis

Study	Cases	Controls	OR (95% CI)	pval
Baker, 1981	6/32 (19%)	33/45 (73%)	11.45 (3.54,43.02)	<0.001
Lin, 2004	15/26 (58%)	116/143 (81%)	3.16 (1.16, 8.26)	0.023
Baker, 2013	0/9 (0%)	~12/27 (45%)	9.00 (1.23, Inf)	0.023
Combined	21/67 (31%)	161/215 (75%)	6.56 (2.10,20.55)	0.001

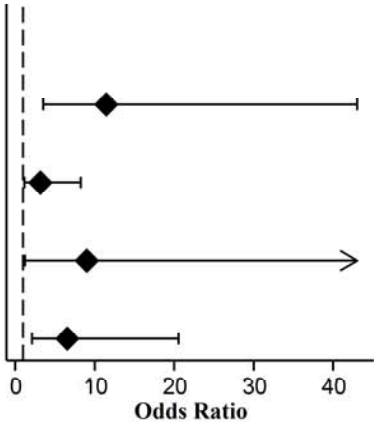


Figure 1.5: Proportion of mothers of infants with a capsular antibody concentrations ≥2 µg/mL for serotype III, a meta-analysis
Footnote: ~denotes approximation; pval is p-value

Furthermore, the quantitative determination of IgG capsular antibodies transferred from mother to infant is unlikely to be the only determinant of the correlate of protection; this measure should probably be supplemented with opsonophagocytic activity assays to measure antibody functionality (Hastings et al., 1985). The functionality of naturally occurring capsular antibodies in infants with invasive GBS disease cases and in pregnant women has been evaluated by some (Klesius et al., 1973, Hemming et al., 1976, Hastings et al., 1985, Kim et al., 1988); however, whether in vitro evaluation of opsonophagocytosis in neonatal serum correlates with in vivo protection is unclear because opsonophagocytic activity assays utilise exogenous components such as complement that may be physiologically deficient in the neonate (Edwards, 1986). Although opsonisation of GBS serotype III by naturally occurring antibodies was similar in colonized and non-colonized women (Hastings et al., 1985), natural acquired capsular antibody to serotype III in infants with invasive GBS disease did not demonstrate opsonophagocytosis activity (Hemming et al., 1976). An association between opsonophagocytosis and higher antibody concentrations in healthy neonates and donor sera has, however, been shown (Kim et al., 1988, Feldman et al., 1998), and opsonophagocytic activity increases post-GBS vaccination (Lancaster et al., 2011, Edwards et al., 2012).

This review also noted that a proportion of infants developed invasive GBS disease despite high antibody levels at birth in their mothers (Baker et al., 1977, Baker et al., 1981). This paradox may be due to maternal acquisition of GBS occurring just prior to delivery, resulting in a rapid increase of poorly functional antibodies being transferred to the foetus, which are inadequate to protect the neonate against invasive disease. The other possibility is that studies using RABA may have measured elevated IgM, rather than IgG, in the sera of mother's blood

with recently acquired GBS colonization. Furthermore, antibody concentrations required to protect against different serotypes may vary, as demonstrated in experimental animal-model and in-vitro studies (Klegerman et al., 1983, Hastings et al., 1985).

Further prospective cohort studies, in diverse settings, are needed to corroborate whether there is a possible sero-correlate of protection against invasive GBS disease. Such studies should also measure functional antibody, using opsonophagocytic activity assays, to improve the elucidation of the sero-correlate of protection against EOD and LOD. This could contribute to the licensure pathway of a GBS polysaccharide-protein conjugate vaccine without needing to undertake large scale efficacy trials in pregnant women.

1.10.2 Sero-correlates of protection to GBS surface-proteins

Most vaccines to GBS have elicited antibody responses to the antigens of the CPS-specific serotypes. However, even with a pentavalent CPS vaccine, serotype coverage may be limited in certain regions and non-typable strains of CPS range between 7–14% (Margarit et al., 2009, Madzivhandila et al., 2011). An alternate to capsular polysaccharide epitopes as vaccine targets against GBS, are the possibility of surface-protein antigens which are associated with virulence and genetically conserved across GBS strains. A number of GBS surface-proteins have been identified over the past few years and some have been shown to be immunogenic, inducing antibodies in animal studies and improving survival in challenge (Lindahl et al., 2005, Meinke et al., 2010) (Table 1.5).

Table 1.5: Vaccine candidate studies using Group B *Streptococcus* (GBS) surface-proteins

Protein name	**Biological function/ virulence potential**	**Vaccine-candidate studies**	
		Animal-model	**Human**
α-C protein	Unknown	Yes	Yes
β-C protein	Unknown	Yes	Yes
Rib[1]	Unknown	Yes	Yes
Alp2 (R28)	Adhesion to epithelial cells	Yes	No
Alp3	Unknown	No	No
C5 Peptidases	Inactivates human C5a; Adhesion to epithelial cells	Yes	No
Pilus Island	Directly adheres to host epithelium	Yes	No
FbsA[2]	Adherence to host epithelium by binding fibrinogen on cell membrane	Yes	No
FbsB[3]	Adherence to host epithelium by binding fibrinogen on cell membrane	Yes	No
BibA[4]	Assists in adherence to host epithelium	Yes	No
Lmb[5]	Adherence to host epithelium by binding laminin on cell membrane	No	No
Sip[6]	Unknown	Yes	Yes

[1]Rib- resistance to proteases immunity group B, [2]FbsA- Fibrinogen-binding protein A, [3]FbsB- Fibrinogen-binding protein B, [4]BibA- GBS Immunogenic Bacterial Adhesin, [5]Lmb- Laminin-binding protein, [6]Sip- surface immunogenic protein

The DEVANI project reported no association between PI antibodies and the risk of invasive GBS disease (Melin and Efstratiou, 2013). However, mice-model studies reported that vaccination of pregnant mice with PI antigens was associated with survival in their litters following inoculation with the GBS strains (Margarit et al., 2009). These studies used ELISA to measure antibodies to PI-2a and -2b (Maione et al., 2005, Margarit et al., 2009). It is possible that based on the coverage of the PI antigens amongst the various serotypes, PI vaccines may potentially protect against 94-99% of invasive GBS strains (Margarit et al., 2009, Martins et al., 2013). In addition, pili play a crucial role in adherence to mucosa, which is the primary step to vaginal-recto colonisation; and hence a PI based vaccine could potentially reduce GBS colonization in pregnant women and reduce mucosal acquisition of GBS in newborns (Margarit et al., 2009).

Similarly, animal model studies have identified FbsA and BibA as highly immunogenic and antibodies to these proteins protected mice from GBS inocula. (Santi et al., 2009, Meinke et al., 2010, Papasergi et al., 2013). Experimental models comprising of maternal immunization with subsequent neonatal pup challenge were conducted by vaccinating female mice with a fragment of the FbsA protein at three time points prior to mating. The pup litters were then injected with lethal doses of GBS inocula within 48 hours of birth. Vaccinating maternal mice conferred protection to 50% of their pups compared to no survival of pups born to unvaccinated mice (Papasergi et al., 2013). Similarly, maternal mice vaccinated with BibA surface-protein conferred protection to 68% their pups who survived a lethal GBS inoculum challenge. In addition, in-vitro opsonophagocytic assays demonstrated enhanced killing by polymorphonuclear cells using the vaccinated BibA sera in adult mice (Santi et al., 2009).

Although the PIs, FbsA and BibA surface-proteins seem to be attractive vaccine targets for preventing invasive GBS disease in humans, based on the animal-model studies above, this needs to be corroborated in humans. Studies on the association between other surface-protein antibodies and colonisation or disease in humans include that on antibodies to surface immunogenic protein (Sip), resistance to proteases immunity group B (Rib), αC-protein and βC proteins (Moyo et al., 2001, Lachenauer et al., 2002, Larsson et al., 2006, Manning et al., 2006, Pannaraj et al., 2007, Pannaraj et al., 2008) (Table 1.5). Overall, most studies of these human studies did not identify an association between surface-protein antibodies and the risk of EOD , with similar GMC's observed in mothers of neonates with invasive GBS disease compared to GBS colonised mothers of healthy neonates. In the first case-control study, measuring the IgG to the βC protein by ELISA, the GMC in the 5 cases (1.51 μg/mL, range: 0.49-7.07) was similar to the 13 colonised controls (1.88 μg/mL, range: 0.17-34.2)

(Lachenauer et al., 2002). Similarly, GMC's in 9 cases (0.97 μg/mL, 95% CI: 0.48-1.97) were similar to the 16 colonised controls (0.76 μg/mL, 95% CI: 0.49-1.19) in a separate study on βC protein (Pannaraj et al., 2007). Also for αC protein, GMC's were similar comparing 42 cases (371 ng/mL, 95% CI: 261-525) to 58 colonised controls (313 ng/mL, 95% CI: 231-424) (Pannaraj et al., 2008). The only study to have reported increased odds of disease associated with lower antibody thresholds, measured antibodies to Rib and αC in Rib expressed strains of invasive GBS disease compared to colonised controls (Larsson et al., 2006). Consequently, a correlate of protection has not been demonstrated in humans for any GBS surface-protein. Virulence potential and immunogenicity of surface-proteins may need further investigation and conclusions cannot be drawn from these studies which generally had small sample sizes.

1.11 The effect of HIV on Group B *Streptococcus* antibodies and the transplacental transfer

The efficacy of a GBS polysaccharide-protein conjugate vaccine may also be dependent on factors such as the effect of maternal HIV-infection on antibody levels and transplacental transfer to the foetus. Transplacental antibody transfer to the foetus is almost exclusively IgG antibody, with more efficient transfer of IgG1 than IgG2 (Chu and Englund, 2014). Maternal GBS capsular and surface-protein antibody has been shown to correlate with cord concentrations at birth, although maternal antibody concentrations are low in the majority of women (Baker et al., 1977, Boyer et al., 1984c, Lagergard et al., 1992, Lin et al., 2001, Larsson et al., 2006). The transfer of maternal IgG antibody mostly occurs in the third trimester of pregnancy, and infant to maternal ratios are estimated between 77-125% at term (≥37weeks), 50-75% between 34-36 weeks, 50% by 32-34 weeks and 30% at 28-30 weeks gestation.

The transfer of maternal antibody across the placenta is via an active transport mechanism utilizing Fc receptors (Leach et al., 1996, Kruczek et al., 2010). IgG must cross over from the syncytiotrophoblast and endothelium to pass from the maternal to foetal circulation (de Moraes-Pinto et al., 1996). It is postulated that HIV-infection, which is associated with a hyper-gammaglobulinaemia state, may result in a saturation of these receptors, hence impeding antibody transfer to the foetus (de Moraes-Pinto et al., 1996). Additional factors that also influence transplacental antibody transfer include placental integrity, IgG subclass, malaria, malnutrition, high parity, and gestational age (Cumberland et al., 2007, Kruczek et al., 2010, Chu and Englund, 2014). It is also thought that surface-protein antibody, which is predominantly IgG1 subtype, is transferred more efficiently across the placenta than antibody to polysaccharide which is predominantly IgG2 (Chu and Englund, 2014).

Low levels of maternal antibody to various epitopes have been reported in HIV-infected pregnant women (Jones et al., 2011, Gupta et al., 2014). Studies have also demonstrated lower transplacental transfer ratios of measles, varicella, tetanus, haemophilus, pertussis and pneumococcus antibodies in HIV-infected compared to HIV-uninfected maternal-newborn dyads (de Moraes-Pinto et al., 1996, Scott et al., 2005, Cumberland et al., 2007, Jones et al., 2011, Gupta et al., 2014). In 46 HIV-infected mother-newborn dyads, the transplacental transfer ratio of IgG to varicella, measles, pneumococcus and tetanus was 20-30% less than in the 53 HIV-uninfected mother-newborn dyads (de Moraes-Pinto et al., 1996). Geometric mean tetanus cord to maternal antibody ratios in 617 HIV-uninfected pairs was 0.92 (95% CI: 0.89–0.96) compared to 0.73 (95% CI: 0.65–0.82) in HIV-infected pairs (Cumberland et al., 2007). Similar results were reported for measles antibodies in the same setting (Scott et al., 2005). In South Africa, median cord to maternal antibody ratios demonstrated reduced transplacental

transfer of antibodies to *H*aemophil*us influenzae* type b (23%), pertussis (40%) and tetanus (27%) (Jones et al., 2011). This study reported no association between placental transfer of antibody and CD4+ T-lymphocyte or HIV-1 viral load counts. A subsequent analysis of this South African cohort (Le Doare et al., 2015), measured GBS antibody concentration and transplacental antibody transfer of 46 HIV-infected and 58 HIV-uninfected mother-infant pairs. HIV-infected mothers had lower baseline GMC's than HIV-uninfected for serotypes 1a (p=0.02), Ib (p=0.03), II (p=0.03), III (p=0.04) and V (p=0.04). There was also a reduction in the median cord to maternal ratio of capsular antibody between HIV-infected and HIV-uninfected mother-infant pairs for serotypes II (0.42 vs 1.00; p<0.01), V (0.51 vs 0.75; p=0.04) and III (0.54 vs 0.95; p=0.05), but not for serotypes Ia (0.66 vs 0.60; p=0.86) and Ib (0.48 vs 0.52; p=0.48) (Le Doare et al., 2015)

Antibody transfer from mother to foetus is crucial in the protection against various organisms during early neonatal life. The negative effect of HIV on maternal antibody concentrations and the transplacental transfer has been documented. This needs to be considered in GBS vaccine development, especially in setting with high maternal HIV-infection prevalence.

This study aims to: (i) Characterise the burden of invasive GBS disease and subsequent neurological sequelae thereof, including the effect of maternal HIV-infection on disease burden in infants; (ii) Determine the effect of maternal HIV-infection on antibody concentrations to GBS capsular and select surface-protein epitopes and transplacental transfer to newborns and (iii) Evaluate the association of maternal and infant serotype-specific capsular, pilus island, FbsA and BibA protein antibody concentrations and the risk of invasive GBS disease in young infants in a low-middle income setting.

2.0 Methods

2.1 Study Objectives

The objectives of my thesis were to undertake: (i) An epidemiological study describing the risk-factors, incidence and sequelae of invasive GBS disease in young infants; (ii) A cross-sectional study evaluating the effect of maternal HIV-infection on GBS capsular and surface-protein antibody levels and the transplacental transfer to the newborns; (iii) Determine whether a sero-correlate of protection against invasive GBS disease could be established for serotype-specific capsular antibody, as well as for selected GBS surface-protein epitopes. These objectives were addressed by a matched case-control study for the epidemiological and immunological studies (2.1.1 and 2.1.3); and a cross-sectional study for 2.1.2.

2.1.1 Epidemiology of Group B *Streptococcus*

1. To describe the incidence of early-onset and late-onset invasive GBS disease over a twelve month period (November 2012 to October 2013).
2. To evaluate the effect of maternal HIV-infection on the incidence of invasive GBS disease.
3. To investigate maternal and infant factors that are associated with invasive GBS disease.
4. To describe the serotype distribution of early-onset and late-onset invasive GBS disease.
5. To measure the mortality in infants with early-onset and late-onset invasive GBS disease.
6. To describe the short-term (3 and 6 month) neurodevelopment outcomes in infants who had invasive GBS disease compared to matched healthy controls.

2.1.2 The effect of maternal HIV-infection on Group B *Streptococcus* antibody levels and transplacental transfer

1. To determine the effect of maternal HIV-infection on serum serotype-specific capsular (Ia, Ib, III and V), pilus island (1, 2a and 2b), FbsA and BibA antibody concentrations.
2. To evaluate the effect of maternal HIV-infection on the transplacental transfer of serotype-specific capsular (Ia, Ib, III and V), pilus island (1, 2a and 2b), FbsA and BibA antibody concentrations to newborns.
3. To analyse the correlation between maternal CD4+ T-lymphocyte counts on maternal antibody concentration and transplacental transfer of serotype-specific capsular (Ia, Ib, III and V), pilus island (1, 2a and 2b), FbsA and BibA antibodies.
4. To analyse the correlation between maternal HIV-1 viral load counts on maternal antibody concentration and transplacental transfer of serotype-specific capsular (Ia, Ib, III and V), pilus island (1, 2a and 2b), FbsA and BibA antibodies.

2.1.3 Immunological correlates of protection against invasive Group B *Streptococcus*

1. To determine the association between naturally acquired maternal GBS capsular (Ia and III) IgG antibodies and invasive GBS disease in infants born at ≥34 weeks gestational age.
2. To evaluate the association between naturally acquired maternal GBS pilus island (1, 2a and 2b) antibodies and invasive GBS disease in infants born at ≥34 weeks gestational age.
3. To determine the association between naturally acquired maternal GBS FbsA and BibA antibodies and invasive GBS disease in infants born at ≥34 weeks gestational age.

2.2 Study Population

2.2.1 Description of the study population

South Africa has reported the highest incidence of invasive GBS disease in young infants globally over the last two decades (Dagnew et al., 2012, Edmond et al., 2012), mainly from studies conducted at Chris Hani Baragwanath Academic Hospital (CHBAH) (Madhi et al., 2003, Cutland et al., 2015). CHBAH is the only public hospital to the residents of Soweto, a peri-urban black African suburb in Johannesburg. Standard preventative and management practices of invasive GBS disease have remained unchanged since the incidence was first reported in 1997 in this setting. For the purposes of our study, we expanded on the sample population by including the three largest hospitals offering paediatric and obstetric care in the greater Johannesburg metropolitan area. The two additional secondary-tertiary level hospitals included are, Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) and Rahima Moosa Mother and Child Hospital (RMMCH). In pregnant women without access to private health insurance (80-90%), delivery of babies generally occurs either at a government funded public hospital or midwife obstetric units (MOU's) which are located within primary health care clinics in the community. These MOU's would refer women with complications during labour (for example: cephalopelvic disproportion or slow progress) to their respective public hospitals.

Of the six districts in the Gauteng province (see map- Appendix 5), CHBAH, CMJAH and RMMCH are located within the Johannesburg Metropolitan District but do receive referral of complicated cases of women in labour from hospitals in three other surrounding districts (West Rand District, Ekurhuleni District and Sedibeng District). The greater Johannesburg metropolitan is further divided into seven sub-districts, namely Regions A to G (see map-

Appendix 6). Region D citizens access health care at either CHBAH or five MOU's. A further two MOU's in Region G also refer complicated cases in labour to CHBAH. The CMJAH is situated in Region F with one MOU in this sub-district. The RMMCH is situated in Region B with no MOU in this sub-district. The single MOU in Region C refers to RMMCH and in region E refers either to CMJAH or RMMCH. CHBAH is predominantly utilized by black-Africans, whilst CMJAH and RMMCH cater for a more diverse racial mix of individuals in central and northern regions.

Within these sub-districts, infants with invasive GBS disease would be admitted to one of four regional hospitals that offer paediatric services, namely; CHBAH, CMJAH, RMMCH and Edenvale Hospital. Infants are not hospitalized at the MOU's or other primary level care facilities in these sub-districts. Table 2.1 outlines the GBS surveillance statistics from 2009 to 2011 for each hospital in the Gauteng region, with the majority of cases in the Johannesburg metropolitan being diagnosed at CHBAH, CMJAH, RMMCH hospitals, whilst there was only a single case reported at Edenvale hospital. This surveillance data excluded cases admitted at private facilities in this region, however, <10% of the Soweto population have medical insurance (Mayosi and Benatar, 2014). Therefore, it is highly probable that most neonates living in this region would be admitted to CHBAH.

Table 2.1: Overall number of Group B *Streptococcus* cases (all ages) reported in government funded hospitals within the six districts in Gauteng province between 2009 and 2011 (National Institute of Communicable Diseases, 2012).

District/region	Hospital	2009	2010	2011
JHB[1] Region D	Chris Hani Baragwanath Academic Hospital	109	108	99
JHB Region F	Charlotte Maxeke Johannesburg Academic Hospital	46	39	51
JHB Region B	Rahima Moosa Mother and Child Hospital	21	19	24
JHB Region B	South Rand Hospital (non-paediatric)	-	4	1
JHB Region B	Helen Joseph Hospital (non-paediatric)	4	11	3
JHB Region E	Edenvale Hospital	-	-	1
Ekurhuleni	Far East Rand Hospital	-	1	-
Ekurhuleni	Tambo Memorial Hospital	2	3	7
Ekurhuleni	Natalspruit Hospital	47	29	38
Ekurhuleni	Pholosong Hospital	-	2	-
Ekurhuleni	Tembisa Hospital	6	10	8
West rand	Leratong Hospital	18	19	16
West rand	Dr Yusuf Dadoo Hospital	6	1	2
West rand	Carletonville Hospital	1	-	-
Sedibeng	Sebokeng Hospital	10	9	7
Thswane	Tshwane District Hospital	1	3	1
Thswane	Steve Biko Academic Hospital	1	9	5
Thswane	Kalafong Hospital	5	13	10
Thswane	Dr George Mukhari Hospital	19	32	35

[1]JHB- Johannesburg metropolitan

2.2.2 Obstetric and paediatric care in the study population

Pregnant women are routinely evaluated at antenatal clinics close to their place of residence. At the first antenatal visit, the health of the mother and foetus is assessed by nurses and the gestational age of the foetus estimated. The mother is then followed up one to four weeks later (depending on the timing of her gestation), or referred to a hospital for continuation of antenatal care if assessed to have significant co-morbidities such as pregnancy induced hypertension, gestational diabetes, multiple pregnancies or previous complicated deliveries. Included at the first antenatal visit is screening for HIV and syphilis infection, and Rhesus blood group typing.

The standard-of-care for testing for HIV-infection status in pregnant women is a rapid HIV antibody screening test followed by a confirmatory rapid HIV antibody test (National Department of Health, 2010). If the results are indeterminate, an HIV ELISA is done to confirm her status. Following a positive HIV result, a CD4+ T-lymphocyte count test is done and the mother clinically staged according to the World Health Organization (WHO) staging. Prior to April 2013, women with a CD4+ T-lymphocyte count >350 cells/mm^3 and WHO stage 1 and 2 received antiretroviral prophylaxis with zidovudine (AZT) twice daily whilst those women with CD4+ T-lymphocyte count $\leq$350 cells/mm^3 or WHO stage 3 or 4 were initiated on triple antiretroviral therapy (ART), namely tenofovir (TDF), lamivudine (3TC) and nevirapine (NVP). From April 2013, the prevention of mother to child transmission (PMTCT) recommendation was amended so that all women regardless of their CD4+ T-lymphocyte count are started on triple therapy (National Department of Health, 2013). Furthermore, a fixed dose combination tablet, daily dose regimen was instituted. The change in the PMTCT management of HIV-infected pregnant women phased in over the next 3-6

months. During labour, the mothers on triple ART continue their treatment, whereas those on AZT prophylaxis take AZT 3 hourly and receive a single dose of NVP, followed by a single dose of combination TDF and emtricitabine (FTC) after delivery. Those pregnant women with a CD4+ T-lymphocyte count <350 cells/mm^3 are continued on ART, whereas if the CD4+ T-lymphocyte count is >350 cells/mm^3, the ART is continued until one week after cessation of breastfeeding. The infants are started on daily NVP for six weeks. Breastfed infants are to continue NVP as long as they are breastfeeding in mothers not on triple ART, but to stop NVP at six weeks if the mother is on triple ART or the infant is formula fed (National Department of Health, 2010) (National Department of Health, 2013). HIV-infected women in this setting receive their HIV care, which is fully funded by the State, at one of the antenatal clinics or at their respective hospitals.

Paediatric and obstetric standard-of-care practises are generally similar across the three hospitals, all of which are affiliated to the Faculty of Health Sciences of the University of Witwatersrand. Although these hospitals are designated to function as secondary or tertiary level hospitals, deficiencies in primary health centres results in these hospitals also rendering primary-level health care services to mothers and their children, including a high proportion of uncomplicated deliveries occur at these facilities. Complicated deliveries and sick newborns, including those with suspected sepsis and meningitis are also referred to one of the above three hospitals. At CHBAH, the largest of the hospitals, approximately one-third of deliveries occur by Caesarean section. Furthermore, approximately 15-18% of births weigh <2500 grams (i.e. low birth weight; Table 2.2).

Table 2.2: Estimated number of births at Chris Hani Baragwanath Academic Hospital (CHBAH) between 2005 and 2011 (Department of Obstetrics and Gynaecology, 2012)

	2005		2006		2007		2008		2009		2010		2011	
Birth weight	**Total**	**Live birth**	**Total**	**Live birth**	**Total**	**Live birth**	**Total**	**Live birth**	**Total**	**Live birth**	**Total**	**Live birth**	**Total**	**Live birth**
500-999	372	195	435	228	461	269	489	259	420	253	486	304	484	315
1000-1499	510	439	634	501	584	492	624	516	670	518	654	538	627	523
1500-1999	827	736	856	741	940	846	1063	939	934	831	1017	923	966	879
2000-2499	1758	1679	2039	1945	2236	2164	2353	2253	2188	2120	2465	2385	2336	2245
≥2500	16851	16718	18782	18645	19290	19147	19037	18882	18606	18472	18649	18515	18993	18841
Total	**20318**	**19767**	**22746**	**22060**	**23511**	**22918**	**23566**	**22849**	**22818**	**22194**	**23271**	**22665**	**23406**	**22803**
Caesarean Section	**5765 (28.4%)**		**6066 (26.7%)**		**6703 (28.5%)**		**7354 (31.2%)**		**7628 (33.4%)**		**8016 (34.4%)**		**8179 (34.9%)**	

In Johannesburg, the “risk-based” rather than “universal screening” strategy is used for reducing the risk of EOD. Intra-partum antibiotics are recommended for women in whom GBS is isolated from a sterile site during pregnancy or labour, or women with maternal fever, non-labour related abdominal tenderness or prolonged rupture of membranes. However, a recent study in the same setting showed that only 10-12% of vaginal deliveries, regardless of risk factors, delivering at CHBAH received IAP during labour (Cutland et al., 2012). This is in contrast to the universal screening approach that is adopted in many high-income countries, in which pregnant women are screened for GBS recto-vaginal colonization at 35-37 weeks gestation and prescribed IAP during labour and at least 4 hours prior to anticipated birth if colonised with GBS. The universal screening approach has been shown to be a more effective than the risk based strategy on preventing EOD and is recommended as standard-of-care in USA (Verani et al., 2010).

The care of newborns at the study hospitals include examination of all newborns with a birth weight of less than 2500 grams, newborns born before the mother reached hospital, newborns referred from the MOUs, or those with signs of respiratory distress or sepsis. First line antibiotics for management of neonatal sepsis at the hospitals are intravenous gentamicin and either penicillin or ampicillin. A full blood count and blood culture is routinely undertaken prior to initiating antibiotics. Furthermore, a lumbar puncture is undertaken to sample the cerebrospinal fluid (CSF) in all neonates with suspected sepsis, as well as if GBS is isolated from blood culture.

Neonates and older infants who had already been discharged home following birth that are suspected of subsequently having sepsis or meningitis are admitted to the general paediatric

wards at the hospitals. Markers of infection, including a white cell count and C-reactive protein (CRP) are usually measured in addition to blood and CSF cultures. These procedures are undertaken at the discretion of the attending physician; however, there is a low threshold for investigating for sepsis in this setting. First line antibiotics for young infants with suspected sepsis is gentamicin and either penicillin or ampicillin; whilst those with suspected meningitis are empirically treated with a third generation cephalosporin (usually cefotaxime).

2.3 Study Design and Method: Case-control study

2.3.1 Study Design

From November 2012 to February 2014, a matched case-control study was undertaken at the three University of the Witwatersrand-affiliated academic hospitals in Johannesburg, namely CHBAH, CMJAH and RMMCH. We planned on enrolling at least 80 cases of invasive GBS disease over twelve months, and aimed at recruiting five individually-matched controls for every case of invasive GBS disease (i.e. a 1:5 case-control ratio). We anticipated that from every five controls enrolled; at least one would be colonized with GBS (based on maternal GBS colonization data in this setting) (Kwatra et al., 2014).

2.3.2 Inclusion and Exclusion Criteria

Inclusion criteria for maternal participants

(i) Pregnant women delivering at CHBAH, CMJAH, RMMCH or referred to these facilities from their respective MOU's within 24 hours of delivery.

(ii) Able to understand and comply with planned study procedures.

(iii) Provides written informed consent.

Inclusion criteria for infants

(i) Identification of GBS from a normally sterile site in infants admitted to CHBAH, CMJAH, RMMCH

(ii) Infants < 90 days of age

Exclusion Criteria

(i) Refusal for study participation

(ii) Women on GBS vaccine trials

2.3.3 Study Method

2.3.3.1 Definitions of case and control subjects:

Case subjects were defined as an infant <90 days old with GBS confirmed by culture on blood, CSF or other normally sterile site, or by latex agglutination of CSF. These cases were identified by daily surveillance, with the assistance of microbiology and/or paediatric staff. EOD was defined as isolation of GBS within the first 6 days of life. LOD was defined as isolation of GBS from days 7-89 after birth.

Control subjects: To match for cases of EOD, healthy neonates were enrolled within the first 6 days of life and followed through to ensure they remained free of invasive GBS disease until 90 days of age. Secondly, to match for cases of LOD, we enrolled infants born within 14 days (but >7 days of life) to the day of the case subject and who did not develop invasive GBS disease. All controls were further matched to cases for gestational age at birth (within 2 weeks of the gestational age if the case was born prematurely (<37 weeks gestation) and to term if the case was born at ≥37 weeks gestational age, maternal HIV-infection status, maternal age (within 2.5 years of the case mother's age).

2.3.3.2 Assessment of gestational age:

In order to achieve accuracy, gestational age at birth was calculated using the following hierarchal tools.

1) The gestational age at birth was estimated from an ultrasound examination performed before 24 weeks gestational age.
2) If the mother did not have an ultrasound examination performed before 24 weeks gestational age, gestational age staging was undertaken using the Ballard score completed within 24 hours of birth by the attending doctor or study staff.
3) If the mother/infant did not have either of the above undertaken and was certain of her last normal menstrual period (LMP), this was used to stage the gestational age.
4) In the absence of any of the above methods for calculating the gestational age at birth, we used any available ultrasound examination (≥24 weeks) to calculate the gestational age.
5) If none of the above methods were available, then either the symphysis fundal height (SFH) at the time of labour was used, or the newborn was classified as being "term" if the birth weight was more than 2500 grams.

2.3.3.3 Method for recruitment of Case subjects:

Daily in-person audits with the National Health Laboratory Service (NHLS), Department of Microbiology and/or attending physicians were undertaken at CHBAH, CMJAH and RMMCH to identify cases of *Streptococcus agalactiae* from a sterile site. The mother was approached for consent and enrolment of her child and herself within 72 hours of the culture result becoming available. We attempted to enrol cases within a maximum period of 120 hours from the time the culture as delay in enrolment could have affected antibody measurements.

Procedures conducted at the time of enrolment (Table 2.3):

- Maternal and infant case report forms were completed by the study doctor or nurse.
- Maternal and delivery history including identifiable risk factors for GBS were obtained from the mothers and from the available clinical records. For those mothers who delivered at one of the site hospitals, the maternal files were extracted for data. Furthermore, examination of the mother, the use of IAP and maternal blood results including HIV status and CD4+ T-lymphocyte counts were recorded.
- Infant's history, examination, routine blood results and in-patient management were recorded from the infants hospital file.
- Bloods was taken from the mother (5mL) and infant (1-2mL) to measure antibodies to the four common CPS serotypes (Ia,Ib,III,V), and 5 proteins; 3 pilus proteins (PI-1, PI-2a and PI-2b), FbsA and BibA.
- The mother was swabbed (rectal and lower vaginal) for GBS culture and sero-typing.
- A clean-catch midstream urine specimen was taken for GBS culture.
- The mother was given a copy of the informed consent form and a card with a follow-up date to return at the next visit.
- Specimens were transported at room temperature to the laboratory at the Respiratory and Meningeal Pathogens Research Unit (RMPRU) for processing within 6 hours or otherwise refrigerated for 24-48 hours.

Table 2.3: Schedule of visits and procedures on participants in the study

Schedule of visits	Visit 1	Visit 2	Visit 3	Visit 4
Time Period	GBS[1] culture positive or Control (enrolment)	4 weeks post delivery (±1week) (21-35 days)	12 weeks post delivery (±1week) (77-91 days)	24 weeks post delivery (±1week) (161-175 days)
ICF[2] signed	X			
Inclusion/ exclusion/ Withdrawal criteria	X			
Medical history (Mom)	X			
Targeted physical exam (Mom)	X			
Medical history (infant)	X	X	X	X
Physical examination (infant)	X	X	X	X
Denver-II developmental assessment (infant)		X	X	X
Swabs (rectal &vaginal) for GBS culture (Mom)	X			
Urine culture (Mom)	X			
Maternal Blood for antibodies (Capsular, Pilus, FbsA and BibA)	X			
Cord/Infant Blood for antibodies (Capsular, Pilus, FbsA and BibA)	X			

[1]GBS-Group B *Streptococcus*; [2]ICF- Informed consent form

During the study, it was increasing difficult to enrol mothers whose newborn had demised from invasive GBS disease prior to the culture result becoming available. Once the culture result became available, the mothers were telephonically contacted to inform them of the disease as well as advise them on future preventative measures. The Ethics Committee also approved us retrospectively collecting clinical data from the medical records and run antibody analysis on residual sera at NHLS taken at the time of admission of the cases, in the event that consent was unavailable from the parent of the child.

Case recruitment started on the 7th November 2012 and ended on the 6th November 2013. Over the twelve month period, 126 cases of invasive GBS disease were identified from the three academic hospitals (Figure 2.1). We obtained consent from and enrolled 99 mother-infant pairs whilst data was retrospectively collected on a further 27 infants. Four subjects were excluded from the analysis, including two with recurrent episodes of invasive GBS disease, one case in which the culture result was revised to be *Streptococcus viridans* and one case which occurred in a 120 day old infant. Consequently, 122 invasive GBS disease cases were analysed, of which 119 isolates were serotyped. The cases in which serotypes were unavailable included two cases in which GBS was identified only on bacterial latex antigen and one case in which the isolate was not retrieved. For the antibody analysis, infant blood was unavailable on 10 subjects. Furthermore, we excluded two enrolled cases that had blood samples taken >120 hours from the date of culture and four infants in whom we were unable to enrol suitable matched controls. Therefore, serum was available from 103 infants (including 1 set of twins) and 89 mothers (Figure 2.1).

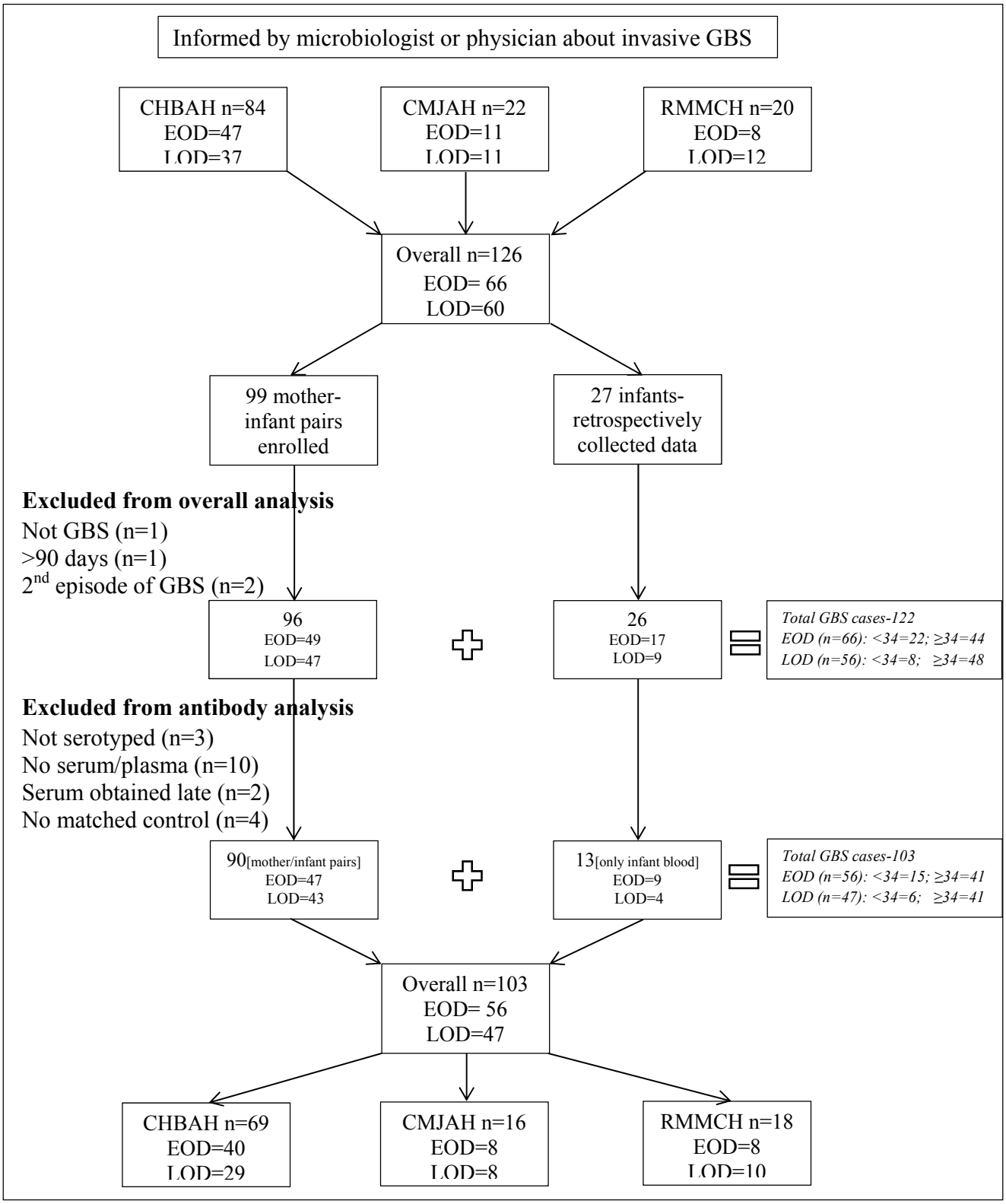


Figure 2.1: Schematic representation of cases presenting at the three sites

Footnote: CHBAH-Chris Hani Baragwanath Academic Hospital; CMJAH-Charlotte Maxeke Johannesburg Academic Hospital; RMMCH-Rahima Moosa Mother and Child Hospital; EOD-early onset disease; LOD-late onset disease.

2.3.3.4 Method for recruitment of control subjects matched for EOD

Controls were recruited over a fifteen month period from the 13th November 2013 to the 12th February 2014 during normal working hours at CHBAH only. Mothers in labour were screened to match the above criteria. Cord blood was collected during delivery and stored until the mother was clinically stable (±4 hours). Mothers of healthy neonates were identified, informed about the study, given an opportunity to read and sign the informed consent form and thereafter consented if willing to participate in the study. If the mother declined, the cord blood was discarded. In a minority (<5%) of deliveries, cord blood was not obtained and the mother was consented to obtain blood from the neonate within 72 hours of birth. The rest of the procedures conducted at the time of enrolment were the same as those mentioned in chapter 2.3.3.3 above. Audits of the microbiology laboratory continued three months after enrolment of controls ended to monitor whether any of the controls may have subsequently developed invasive GBS disease. Controls were also interviewed at the 3 month visit to confirm they were not hospitalized with sepsis.

2.3.3.5 Method for recruitment of control subjects matched for LOD

These infants were recruited from the community, except for the otherwise healthy premature infants who were also enrolled from the neonatal wards provided they had not been previously diagnosed with sepsis and the reason for hospitalization was only for feeding support and weight gain. Mothers of healthy infants were identified in the postnatal wards and given specific dates on which to return to the RMPRU clinic for enrolment. In addition, the maternity birth registers were used to identify mothers who were discharged with their baby after delivery and fulfilled the matching criteria. The contact numbers of these mothers were

sourced from the hospital patient registry. At the RMPRU clinic, mothers were informed about the study, given an opportunity to read the informed consent form and consented if agreeable to participate. The procedures carried out on the mother and infant were the same as for those listed for the cases in chapter 2.3.3.3 above.

2.3.3.6 Follow-up visits of cases and controls

Both cases and controls were followed up at 1, 3 and 6 months of chronological age at the RMPRU (Table 2.3). Those subjects who failed to attend the schedule visit within 14 days of the infants scheduled visit were excluded from the analysis of that age, whereas some were completely lost to follow-up because they did not attend any follow-up visits. For those children who did not attend follow-up, attempts were made to maintain the timing of the visit by contacting the next of kin, using alternative telephone numbers provided and by conducting home visits.

The follow-up visits were conducted by one of two trained research assistants, an enrolled nurse or a study doctor (myself or RMPRU medical officer). All cases and controls assessed as having developmental delay were additionally evaluated by a study doctor (myself or RMPRU medical officer). At these visits, a directed medical history and examination was conducted on the infant with particular neurodevelopmental focus. The Denver Developmental Screening Test II (Denver-II) (Appendix 7) was used to identify infants with suspected developmental delay. In addition, infants with hypertonia and/or hydrocephalus were categorised as having abnormal neurological findings. Infants diagnosed with neurological abnormalities were

referred for further care, including for occupational, physio and speech therapy. No blood samples were taken at these visits.

In 1992, the Denver-II was modified from the original Denver Developmental Screening Test which was developed in 1967 (Frankenburg et al., 1992). The Denver-II is a broadly accepted screening tool for developmental delay and has been approved by the American Academy of Pediatrics. The Denver-II, however, is not diagnostic or a predictor of later developmental delay. The Denver-II makes a valuable screening tool reaching sensitivities of 83% (Glascoe et al., 1992), which has been demonstrated to have high degree of intra- and inter-examiner correlation (Frankenburg et al., 1992). Furthermore, as the normal development of a child may be wide-ranging within ages, a percentile range based on growth and milestone curves in which each developmental test item may be accomplished is provided (Frankenburg et al., 1992, Shahshahani et al., 2010).

The Denver II includes 125 test items in 4 domains: gross-motor (32), fine-motor (29), language (39) and personal-social (25). An age line is drawn vertically corresponding to the age of the infant. For premature infants, an adjustment for the number of weeks to term is undertaken. At least three items to the left and three to the right of the age line are assessed in each of the four domains. Each test item is represented horizontally as a percentile age range (25-90%) for which it is estimated that the item can be achieved. The scoring system for the test items are graded as follows;

- “pass”- the infant performed the item or the caregiver reported this
- “fail”- the infant did not perform the item or the caregiver reported this

- "no opportunity"- the infant has not had the chance to perform the item as reported by the caregiver
- "refusal"- the infant refused to attempt the item.

A "fail" or "refusal" by the infant in an item to the left of the age line was classified as a "delay", whilst a "fail" or "refusal" by the infant in an item through the 75-90% age percentile was classified as a "caution" (Figure 2.2). The final result was then scored as "normal" (no delays or 1 caution) or "suspect" (≥2 cautions or ≥1 delay) in each of the four domains.

Figure 2.2: Interpretation of Denver-II scoring system (adapted from the Denver II screening test)

2.3.4 Laboratory Method

2.3.4.1 Blood and CSF collection and processing for GBS isolation.

Investigation for invasive GBS disease was undertaken at the discretion of attending physicians as part of the standard-of-care. Group B *Streptococcus* isolation by culture from otherwise sterile sites (blood or CSF) was undertaken by the NHLS. Blood was inoculated into a Bactec bottle at the infant's bedside by the attending physician at the time of admission and processed through a Bact/Alert microbial system (Organon Teknika, Durham, NC). A positive specimen was then plated on blood and chocolate agar incubated both aerobically and at 35ºC under 5-10% CO2, and observed for growth for a period of 72 hours. CSF specimens obtained

from a lumbar puncture of the infant were Gram stained and then directly plated onto blood or chocolate agar plates and inoculated into an enrichment broth (Brain Heart Infusion, Diagnostics Media Production) and observed for growth for 72 hours. These specimens were subjected to a GBS antigen agglutination test if culture did not yield growth and if the cell count was suggestive of meningitis. In addition, direct susceptibility was done as per laboratory standard operating procedure, according to Clinical and Laboratory Standards Institute (CLSI) guidelines. At the time of enrolment, the positive plate was retrieved from the NHLS microbiology laboratory and transported to the RMPRU laboratory for serotyping, pilus typing and storage of GBS isolate.

2.3.4.2 Maternal Vaginal, Rectal and Urine swab collection and isolation of GBS

A single recto-vaginal swab was used to identify GBS colonisation in the first 8 cases and 22 control mothers. This was amended to separate lower vaginal and rectal swabs subsequently to improve the sensitivity of GBS detection (Kwatra et al., 2013). The method of swabbing was otherwise consistent during the study period. Both vaginal and rectal specimens were collected using rayon tipped swabs (Medical Wire Equipment Co. Ltd. Cat: MW170). The rectal swab was inserted approximately 2 cm pass the anal verge and rotated against the rectal mucosa. The vaginal swab was inserted approximately 2 cm pass the introitus towards the lower vagina mucosal wall and rotated. Additionally, a swab of mid-stream urine sample collected in a sterile container was obtained for culture. All three swabs were inserted into the Amies transport medium without charcoal and transported to the RMPRU laboratory for processing. Swabs collected during normal working hours were processed within 24 hours, whereas swabs collected on weekends or public holidays were stored at 2-8°C and processed during laboratory hours.

Isolation of GBS from each swab was conducted according to the following procedures. Although conventional media detects GBS, we chose to use CHROMAgar StrepB as it is more sensitive in detecting GBS from rectal swabs and similar in detecting GBS from vaginal swabs (Kwatra et al., 2013). Furthermore, CHROMAgar StrepB has a higher specificity and detects non-haemolytic GBS as well (Morita et al., 2014). Swabs were plated out onto CHROMAgar StrepB (Media Mage Cat: M10155) in a semi-quantitative manner, by rubbing the swab onto the first quadrant of the agar plate, and then with a sterile loop streaking out across from the first quadrant to the second and then from the second to the third and from the third to the fourth quadrant. The CHROMAgar StrepB plates were incubated at 37°C for 18-24 hours in aerobic conditions and examined for growth of mauve GBS-like colony morphologies. If GBS-like colonies were not visible within 24 hours after incubation, the plates were incubated for a further 24 hours and re-examined for growth. If GBS like colonies were identified, they were subjected to further confirmatory tests, such as the catalase test, growth on bile esculin agar, inability to hydrolyze esculin, the Christie Atkinson Munch-Petersen (CAMP) test and the Welcogen bacterial latex agglutination test. Confirmed GBS isolates were stored in a medium containing skim milk, tryptone, glucose, and glycerin (STGG) at -70°C.

2.3.4.3 GBS serotyping and pilus-typing

The GBS isolates from the NHLS and the maternal swabs were serotyped and pilus-typed at RMPRU. Serotyping was performed by the latex agglutination method with specific antisera against types Ia, Ib and II to IX CPS antigens (Statens Serum Institute, SSI, Sweden) as described (Afshar et al., 2011). Briefly, GBS isolates stored in STGG storage medium were thawed and plated out on sheep blood agar supplemented with nalidixic acid and colistin. A

suspension was made of isolated GBS colonies that were picked off from sheep blood agar in 50µL of sterile saline in a sterile tube. Five microliters of the suspension were pipetted onto a glass slide with an equal volume of the latex bead suspensions, mixed for 5 to 10 seconds and observed for agglutination. Any agglutination or clumping seen after 30 seconds was not classified as a positive reaction. The process of serotyping commenced with the more common antigens first, in the hierarchal order of Ia, III, V, II, Ib, IV, VI, VII, VIII and IX, with no further testing undertaken once a serotype was identified.

Discordant serotype results from the rectal, vaginal or urine swab and those samples in which the invasive isolate serotype did not match the mothers colonising serotype were further typed by a single-plex polymerase chain reaction (PCR) method for serotypes Ia, Ib, II, III, IV and V using primer sequences described by Poyart et al. (Poyart et al., 2007). The *dlts* gene was used as a PCR positive control for GBS identification. Briefly, stored GBS isolates were sub-cultured on 5% blood agar supplemented with nalidixic acid and colistin and incubated for growth. Colonies were picked off and the Deoxyribonucleic Acid (DNA) was extracted using the NucliSENS® EasyMAG. The sample is then subjected to 40 PCR cycles to amplify the DNA. Each cycle goes through stages which activate the Taq DNA polymerase, denaturing of the DNA template, anneal the complementary primers to the target gene and extension of the DNA strand. The results were then interpreted by identifying the matching band size on agarose gel electrophoresis.

Pilus island typing of all invasive and colonizing GBS isolates were detected by real time PCR using TaqMan probes for PI-1, PI-2a and PI-2b, with primers that target the genomic regions

coding for the ancillary protein-1 of each PI as described previously (Madzivhandila et al., 2013). Briefly, GBS isolates were sub-cultured on sheep blood agar supplemented with nalidixic acid and colistin and incubated overnight at 37 °C in 5% CO2. A single GBS colony was suspended in 300 µL nuclease-free distilled water, heated at 95°C for 10 minutes, and centrifuged at 9000g for 1 min to pellet the cell debris. Four microliters of the supernatant was added to each PCR. The PCRs were run on an AB 7500 instrument (Applied Bio-systems; Singapore) in a 25 µL reaction volume with TaqMan universal PCR master (Applied Bio-systems, USA). The detection of PI-2b was performed as a single-plex reaction, and PI-1 and PI-2a were detected in duplex. GBS strains 2603 V/R (PI-1 and PI-2a) and COH1 (PI-2b) obtained from American type culture collection (ATCC) organisation were used as reference strains. A threshold C_T value is generated when the fluorescence passes through if amplification occurred. We did not investigate isolates for expression of FbsA and BibA.

2.3.4.4 Blood Sample collection, processing and storage

Maternal and infant blood sample were collected by venepuncture of a peripheral vein using sterile techniques. Five to ten millilitres of cord blood was collected at birth. The cord was clamped distally and the blood milked towards the distal end. Blood was aspirated into a syringe from the cord vessels. All blood samples were kept at room temperature to allow clotting; following which it was transported within 4-6 hours to the RMPRU lab for processing. The blood was stored in the 2-8ºC at RMPRU if not processed immediately for a maximum period of 24 hours. Blood was centrifuged for 5 min at a 3220 relative centrifugal force, equivalent to 4000 rpm (revolutions per minute). The serum was then aliquoted into 2-3 pre-labelled tubes and stored at –70ºC. Serum samples were processed in batches.

2.3.4.5 Antibody measurement in serum/plasma using a multiplex Luminex assay

Quantitative serum serotype-specific and surface-protein IgG antibody concentrations were measured with a multiplex Luminex platform. The multiplex Luminex assay is a fluorescence based micro-bead immunosorbent assay that utilizes differential dye-coded beads unique to each antigen against multiple antigens being tested in a single sample.

2.3.4.5.1 The Luminex multiplex assay versus ELISA

The multiplex Luminex is able to measure immune response simultaneously against multiple antigens, whereas the ELISA assay measures antibody responses individually for each antigen. The Luminex assay is consequently less labour intensive and requires less sample volume than the ELISA (Pang et al., 2005). The procedure of antibody determination by ELISA is undertaken by coating the well with the antigen and then adding the sample, incubating and allowing for antigen-antibody complexes to form. This is followed by a wash of the unbound antibody and the addition of enzyme-labelled secondary antibody and further incubation to allow binding. The final step is the addition the enzyme substrate which is metabolized by secondary antibody bound enzyme to form coloured product. The sample plate is inserted into the machine and the optical density of the colour products measured. In contrast, the Luminex assay utilises differential dye-coded beads specific to the antigen. These beads are inserted into the well with the sample and incubated for binding to take place. Thereafter, fluorescent dye-labelled secondary antibody is added and further incubated. After washing off unbound antibody, the plate is inserted into the machine to measure fluorescence intensity. The fluorescence intensity is measured as digital signals of red and near-infrared to the beads. Antibody concentration is then calculated based on the fluorescence intensity values specifically to that antigen.

2.3.4.5.2 Reference Serum

Capsular and pilus island protein antigens were kindly provided by Novartis Vaccines and Diagnostics (Italy), while BibA and FbsA protein antigens were provided by Valneva Austria GmbH. Capsular polysaccharides were coupled to the microsphere beads (Bio-Rad, CA, USA) with the crosslinking agent 4-(4,6 dimethoxy[1,3,5]triazin-2-yl)-4- methyl-morpholinium (DMTMM), while protein antigens were coupled to beads with a two-step carbodiimide reaction (Schlottmann et al., 2006, Ditse et al., 2013). Polygam (purified pooled commercial gammaglobulin; National Bioproducts, South Africa) was used as an in-house reference serum. Antibody concentrations against GBS capsular polysaccharides were assigned to a reference by calibrating them with standard GBS reference serums provided by an academic collaborator, Dr Carol J Baker from the USA. These concentrations are shown in Table 2.4. For the proteins, however, no international reference serum is available and thus laboratories assign in house arbitrary concentrations to the reference standard. An arbitrary concentration of 10 000 units/mL for each protein antigen was assigned to the reference. For PI, antibody was measured to the backbone or ancillary protein antigenic targets on the surface of the pilus island, i.e. GBS-80 for PI-1, GBS-67 for PI-2a and SAN1518 for PI-2b (Figure 1.2). For BibA, we measured antibodies to the BibA-COH1 antigen.

Table 2.4: Antibody concentrations (against capsular polysaccharides) of the reference serum supplied by Dr Carol J Baker

Serotype	IgG (µg/mL)
Ia	11.72
Ib	4.33
III	8.78
V	6.41

2.3.4.5.3 Validation of multiplex assay

The Luminex multiplex assay used to determine the antibody titres was validated with standard quality assurance validation steps including establishing the linearity of the assay, a multiplex versus single-plex comparison, determining the upper and lower limits of detection and the specificity of the in-house reference serum.

The first step in the validation process was to develop the linearity and range of the assay. The standard curves were developed using Polygam reference at different dilutions for each of the tested antibodies (Figure 2.3.1-2.3.9). The dilutions in the figures below are represented by "S" and were as follows: S1- 1:100, S2- 1:400, S3- 1:1600, S4- 1:6400, S5- 1:25600, S6- 1:102400 and S7- 1:409600.

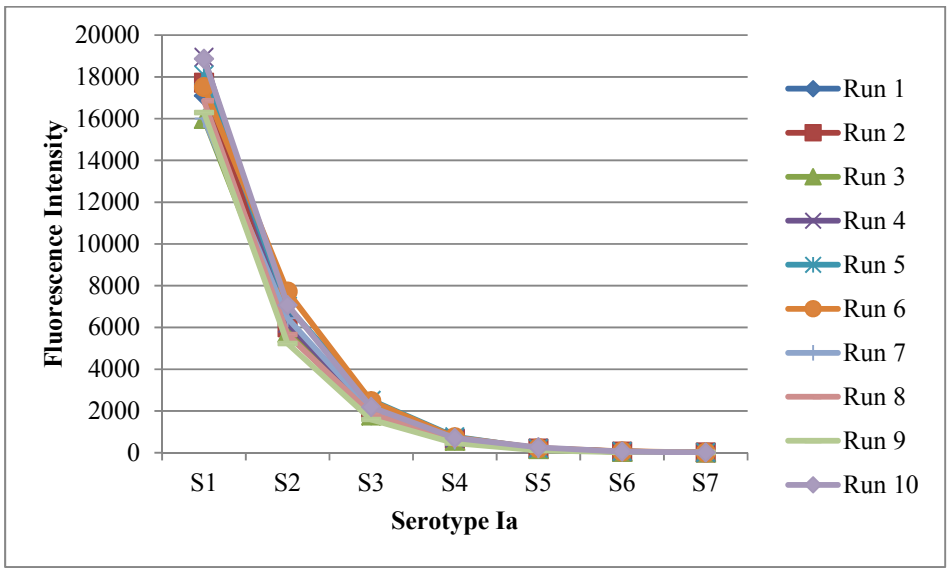


Figure 2.3.1: Standard linearity curves for serotype Ia

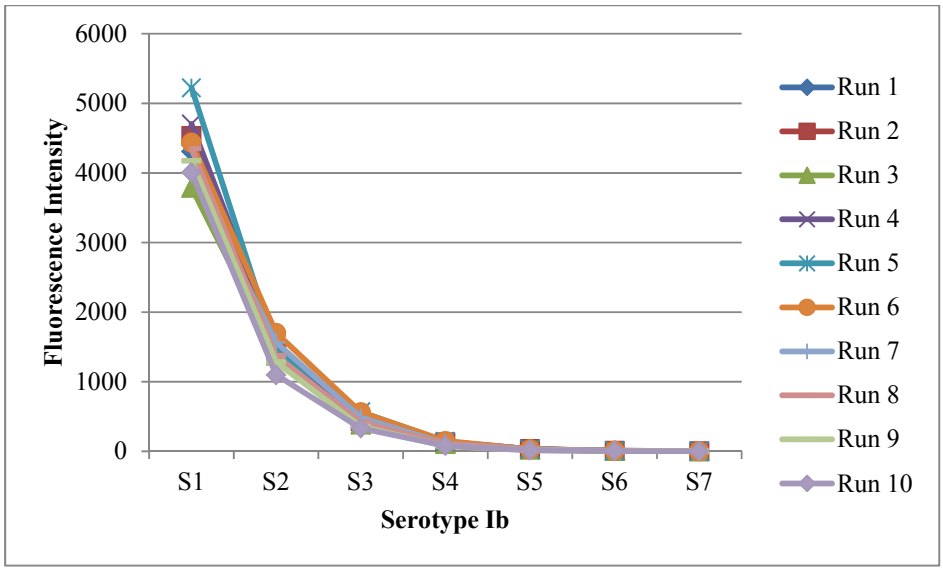


Figure 2.3.2: Standard linearity curves for serotype Ib

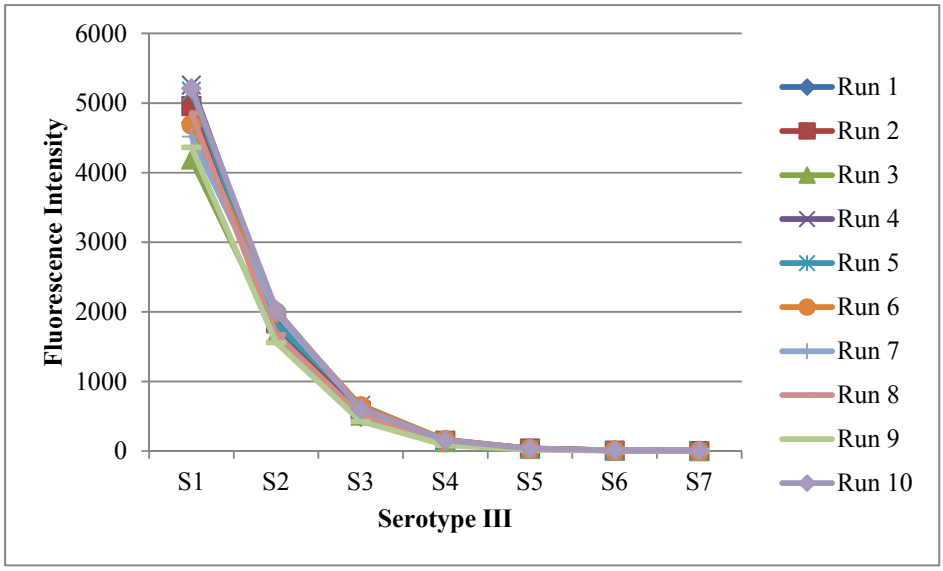


Figure 2.3.3: Standard linearity curves for serotype III

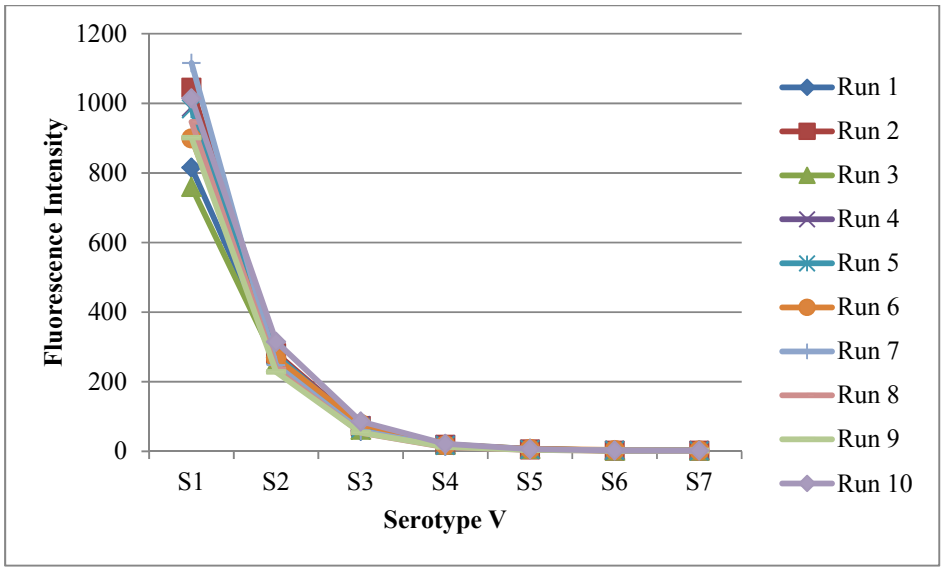


Figure 2.3.4: Standard linearity curves for serotype V

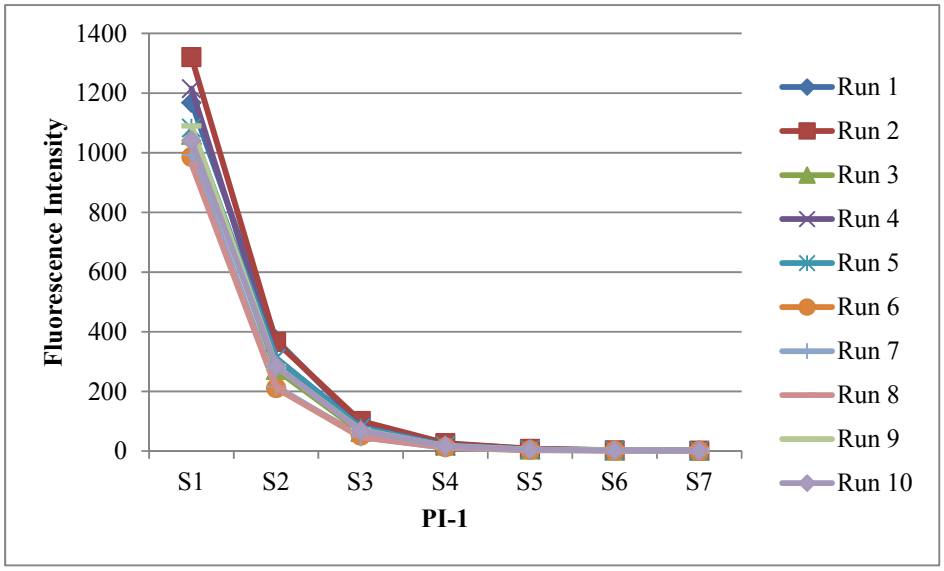


Figure 2.3.5: Standard linearity curves for pilus island-1 (PI-1)

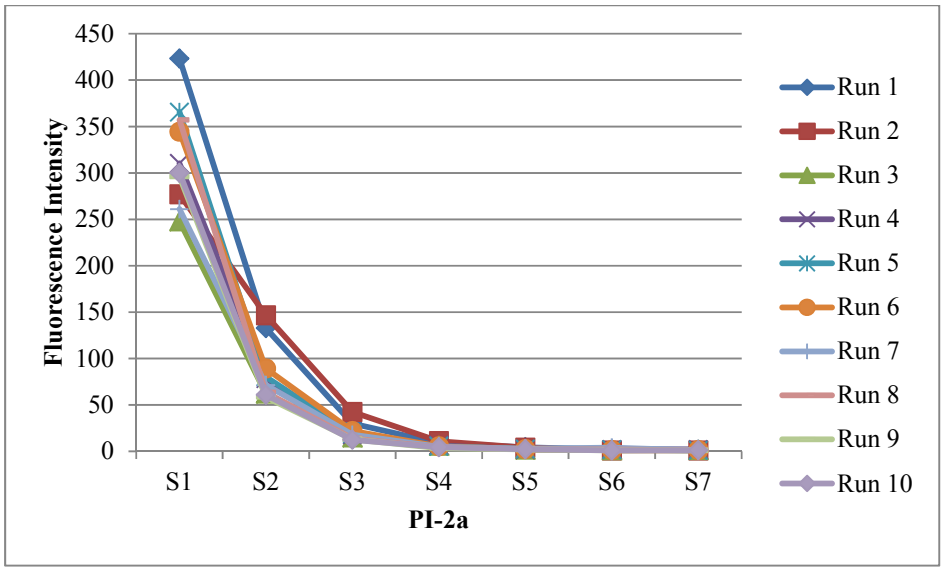


Figure 2.3.6: Standard linearity curves for pilus island-2a (PI-2a)

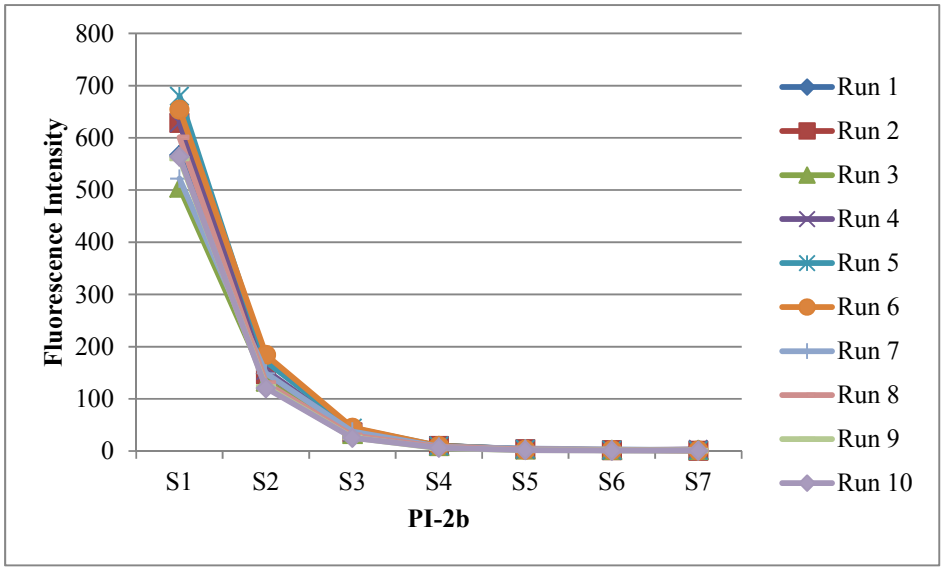


Figure 2.3.7: Standard linearity curves for pilus island-2b (PI-2b)

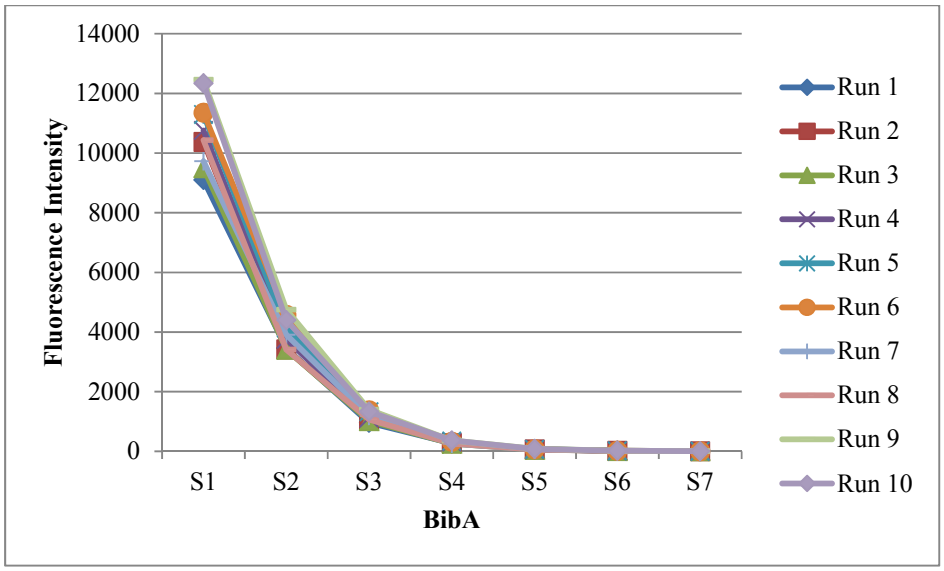


Figure 2.3.8: Standard linearity curves for GBS Immunogenic Bacterial Adhesin (BibA)

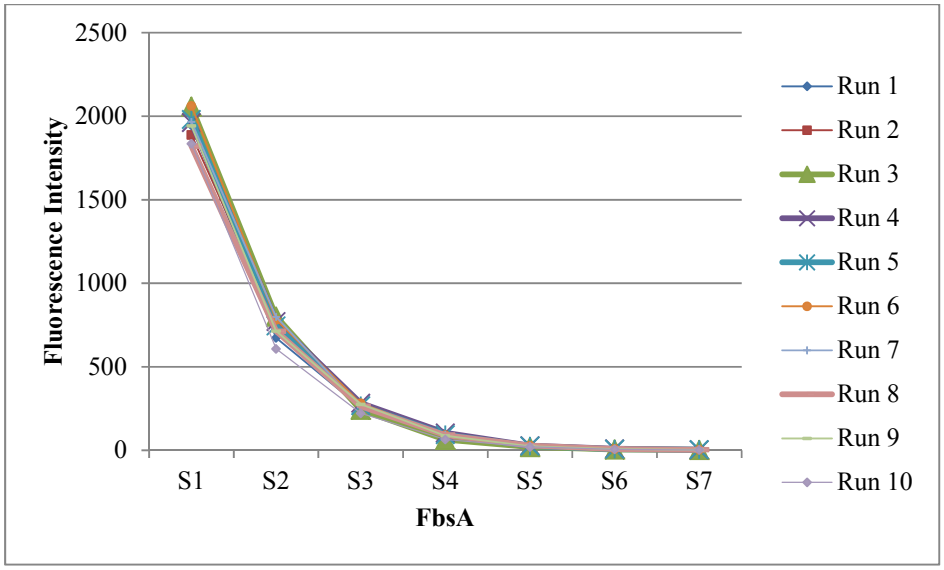


Figure 2.3.9: Standard linearity curves for fibrinogen-binding protein A (FbsA)

The Luminex assay was also validated by comparing the median fluorescence intensity (MFI) values for reference serum (Polygam 1:100 dilution) obtained with the multiplex assay as compared to those obtained by single-plex assays. We observed a maximum variation of 20% in the results (Table 2.5). These were not undertaken for FbsA and BibA.

Table 2.5: Median fluorescence intensity (MFI) values obtained for reference serum at 1:100 dilutions with the multiplex assay as compared to those obtained by single-plex assays for IgG antibodies

Antigen	**Median %**	**Min %**	**Max %**
Ia	100	86	111
Ib	85	79	97
III	101	84	110
V	103	82	112
PI-1	105	80	122
PI-2a	105	82	119
PI-2b	106	81	120

Lower limits of detection (LLD) for the multiplex assay were calculated from the fluorescence of the mean blank value plus 3 standard deviations whilst lower limits of quantification (LLQ) were calculated from the fluorescence of the mean blank value plus 10 standard deviations. The LLD and LLQ values were converted to antibody concentration from an "averaged" reference curve consisting of the mean fluorescence values at each concentration and the concentrations for the cut off were determined relative to the reference. For statistical purposes, any value that falls below the LLD was assigned a value of half of the LLD (Table 2.6).

Table 2.6: Lower limits of detection for capsular and surface-protein antibody concentrations

	ug/mL				AU/mL				
Serum IgG	Ia	Ib	III	V	PI-1	PI-2a	PI-2b	BibA	FbsA
Mean (FI[1])	2.20	1.73	1.97	1.62	1.54	1.5	1.4	2.21	2.47
Standard deviation SD (FI)	0.57	0.38	0.38	0.48	0.41	0.34	0.32	0.59	0.76
Mean + 3SD (FI)	3.91	2.86	3.11	3.07	2.78	2.52	2.35	3.99	4.75
Mean + 10 SD (FI)	7.91	5.52	5.78	6.46	5.68	4.90	4.58	8.14	10.06
LLD[2] (Mean + 3SD)	0.0008	0.002	0.004	0.016	41	110	46	6	19
LLQ[3] (Mean + 10 SD)	0.0017	0.0189	0.0343	0.194	83.5	250.7	100	9.6	61
½ of LOD	0.0004	0.001	0.002	0.008	20.5	55	23	3	9.5

[1]FI-Fluorescence intensity; [2]LLD- lower limits of detection; [3]LLQ- lower limits of quantification.

To assess analytical specificity and to verify antigenic integrity of each GBS antigen–microsphere set, each GBS antigen (100 µg/mL) was incubated at 37˚C for 2 hours as an inhibitor to different wells containing the multiplexed GBS–microsphere mix and reference serum added at 1:100 dilutions. Following incubation, the serology assay was performed. Specificity results for each GBS antigen–microsphere using homologous and heterologous inhibition were determined by calculating the percent inhibition in MFI signal in the presence of the GBS inhibitor relative to the FI signal in the absence of the inhibitor using the following equation.

$$\% \text{ inhibition} = 100 \times \frac{(\text{FI of reference serum}) - (\text{FI of reference serum} + \text{inhibitor antigen})}{\text{FI of reference serum}}$$

We achieved >87% specificity with the CPS antigens. Cross-reactivity was observed between serotype Ia and Ib antigens and between serotype III and V antigens. No significant cross-reactivity was shown between surface-proteins. The specificity of FbsA was low (Table 2.7).

Table 2.7: Specificity and cross reactivity of capsular and surface-protein antibody using the multiplex assay

Antigen	Ia	Ib	III	V	PI-1	PI-2a	PI-2b	BibA	FbsA
Ia	100%	31%	9%	8%	3%	2%	3%	-10%	-1%
Ib	2%	98%	1%	3%	-3%	-2%	-2%	-13%	-6%
III	2%	1%	96%	17%	1%	-1%	0%	-11%	-4%
V	3%	11%	3%	88%	7%	6%	5%	-5%	5%
PI-1	-6%	-16%	-6%	-24%	96%	2%	-1%	-9%	1%
PI-2a	-12%	-18%	-12%	-25%	2%	95%	-2%	-23%	-16%
PI-2b	-2%	-4%	1%	-9%	2%	4%	91%	-6%	9%
BibA	0%	2%	-2%	5%	11%	11%	12%	93%	13%
FbsA	-1%	-10%	-7%	-35%	-15%	-23%	-15%	-17%	32%

High and low controls were screened by using samples with high and low antibody titres from a previous study at the RMPRU (Kwatra et al., 2015) . Antibody concentrations were assigned to the high and low controls using the in-house reference (Table 2.8).

Table 2.8: High and low control capsular and surface-protein antibody concentrations

Control	µg/mL				AU/mL				
	Ia	Ib	III	V	PI-1	PI-2a	PI-2b	BibA	FbsA
High	7.72	1.17	8.58	3.86	4994	4227	3422	8135	5383
Low	0.05	0.12	0.22	0.80	768	1568	725	3338	2213

With regard to the reproducibility of the assay, the coefficient of variation was 9.83, 10.94, 12.32 and 9.53 for capsular serotypes Ia, Ib, III and V, and 8.29, 12.48, 8.49, 8.04 and 16.95 for surface-proteins PI-1, PI-2a, PI-2b, BibA and FbsA, respectively. Once the validation process was completed, coating and counting of the beads were done at the beginning and repeated as required.

2.3.4.5.4 The procedure for measuring antibody concentrations using the Luminex assay

The following steps were undertaken to run serum samples:

1. The dilution plate was labelled according to the samples to be tested
2. Standards prepared from reference serum were serially diluted in four-fold dilutions beginning at 1:100.
3. High and low controls were diluted at 1:100 dilution.
4. The sample was diluted to 1:100, but this was modified to 1:200 after the 3rd run as many samples were over range.
5. All samples, standards and controls were prepared in true duplicates
6. The filter plate was saturated with 100µL of assay buffer (Phosphate buffer solution 7.2, 10% Foetal Bovine serum and 0.05% Sodium Azide) and the plate was vacuumed
7. The antigen coated beads were prepared in assay buffer.
8. Each well would roughly contain 3500 beads of each of the nine tested antigens.
9. The plate was then washed twice with wash buffer (Phosphate buffer solution 7.2, 0.5% Tween, 0.02% Sodium Azide).Washing is simply pipetting the wash buffer solution in the wells followed by vacuum of the plate.
10. 50µL of the sample, standards and controls were added into its corresponding well.

11. The plate was incubated with the sample at room temperature for 60minutes on a shaker.
12. The secondary antibody was prepared in wash buffer (1:100)
13. After incubation, the plate vacuumed and washed three times with wash buffer
14. 50µL of the secondary antibody was added to each well.
15. The plate was further incubated with the sample at room temperature for 30 min on a shaker.
16. After incubation, the plate vacuumed and washed three times with wash buffer
17. Then we added 130µL of wash buffer to each well.
18. Thereafter, 100-110 µL of wash buffer containing beads were transferred to the reading plate.
19. The Luminex machine was calibrated and setup with the plate format
20. The bead numbers were allocated with respect to coating antigen.
21. Antigen specific antibody concentration for the standard and controls were entered into the machine.
22. Samples information with respect to dilution and sample identity were entered into the machine.
23. The plate was inserted into the machine for the run.
24. Acquisition software (Bio-Plex Manager) was used to acquire and analyse data using a 5 Parameter Logistic curve fit.
25. Only 38 samples could be run at a time and took approximately an hour to run.

2.3.4.5.5 Quality Assurance of tested samples

The quality assurance of each tested plate of samples included identifying the over range samples which were then re-tested at higher dilutions (1:300-1:1000). In addition, concentration for high and low controls for each plate were within acceptance criteria (i.e. ± 30% of their original concentration), as well as a <20% coefficient variability was accepted between the true duplicates. Cross-checking of results was overseen by a senior medical scientist [Gaurav Kwatra] at the RMPRU.

2.3.5 Data Analysis

Data was collected on study-specific data collection forms and entered into specially designed Microsoft Access and Excel databases. Data were analysed using STATA version 13.1 (College Station, Texas, USA), R version 2.15 (Vienna, Austria), JAGS (Plummer, 2003) and GraphPad Prism version 6.05 for Windows (GraphPad Software, California USA).

2.3.5.1 Group B Streptococcus epidemiology

Cases of GBS were stratified as EOD and LOD. The incidence (per 1,000 live births) of invasive GBS disease in the twelve month period was calculated as the number of EOD or LOD GBS cases of black-African descent, specifically residing in Region D (n=28755) and Region G (n=2749), among whom hospitalization occurs predominantly at CHBAH. Maternal HIV-infection was reported in 8827 (Region D: 8072 and Region G: 755) live births. We did not undertake incidence calculation for the other regions, due to the overlap of utilization of other health care facilities not under surveillance in those regions (Table 2.9).

Table 2.9: Live birth estimates for the Johannesburg metropolitan in regions D and G (District Research Committee, 2014)

		2012		2013												
Region	**Hospital/MOU**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**April**	**May**	**June**	**Jul**	**Aug**	**Sept**	**Oct**	**Total**	**Total (region D & G**	**HIV-infected**
	Hospital														31504	8827 (28.0%)
D	CHBAH[1]	1707	1705	1982	1662	1902	1777	1804	1700	1951	2072	1837	1715	21814		
	MOU's[2]															
D	Lilian Ngoyi	201	197	204	181	160	180	182	189	185	152	185	186	2202		
D	Mofolo	77	86	73	92	88	79	66	58	78	86	86	67	936		
D	Chiawelo	90	95	83	67	112	83	110	110	100	101	110	100	1161		
D	Zola	75	114	112	99	114	99	100	90	116	119	103	107	1248		
D	Itereleng	110	122	123	113	113	110	104	144	118	110	112	115	1394		
G	Stretford	87	91	104	109	111	111	116	109	131	101	102	97	1269		
G	Lenasia South	110	114	115	124	124	123	106	119	127	139	149	130	1480		

[1]CHBAH- Chris Hani Baragwanath Academic Hospital; [2]MOU's- midwife obstetric units

Demographic and clinical characteristics were compared between cases of EOD and LOD. Odds ratios for proportions were reported using the Chi-square or Fisher's exact test. Medians were reported for non-parametric variables and compared using the Mann-Whitney test. Serotype distributions were reported as proportions of the total number of cases serotyped and stratified by EOD and LOD

Maternal and infant risk factors were compared between cases and matched controls. The primary objective of the case-control study was to compare neurodevelopmental outcomes and evaluate for serum capsular antibody thresholds associated with protection against invasive GBS disease, rather than to compare risk factors. Thus, using conditional logistics regression, multivariate odds ratios were calculated for identifying risk factors, which included adjusting for criteria that we matched controls to, as well as including risk factors identified in the univariate analysis with p-value <0.15.

Survival analysis was conducted on infants with GBS at the 90 day of life time-point and stratified by gestational age, EOD and LOD. Kaplan-Meier survival estimates were constructed. Case fatality ratios were calculated as the proportion of deaths during hospitalization by the total number of GBS cases.

Univariate and adjusted odds ratios (aOR) were also reported on predictors of mortality using chi-square and logistic regression test, respectively. In the multivariate analysis, an adjustment was made for those variables in which the univariate analysis reported p-value <0.15. Neurodevelopment parameters were measured for invasive GBS cases and matched controls at 3 and 6 months of age. The proportion of cases and controls with a "suspect" score on the

Denver-II were reported. Infants with increased tone or and with evidence of hydrocephalus on cranial sonar or computed tomography (CT) brain scan were regarded as having abnormal neurological findings. Using conditional logistic regression, multivariate odds ratios were used to compare abnormal Denver-II and neurological findings between cases and controls. An adjustment was made for factors that may impact on neurodevelopment; including, gender, gestational age, birth weight <2500 grams, perinatal asphyxia, mechanical ventilation, infant HIV-exposure status and previous non-GBS hospitalizations.

2.3.5.2 Association between capsular antibody levels and invasive Group B Streptococcus disease

In keeping with previous studies (Lin et al., 2001, Lin et al., 2004), infants <34 weeks gestation were excluded from the analysis as they are likely to have low transpalcental antibody transfer (Boyer et al., 1984c, Christensen et al., 1984). Furthermore, the analysis was restricted to serotypes Ia and III that were the dominant serotypes causing disease (Madzivhandila et al., 2011). For the primary analysis, we compared controls in which the mother was colonized with the same serotype (i.e. homotypic controls) that caused the disease in cases. A secondary analysis was conducted on controls in which the mothers were either non-colonized or colonized with GBS serotypes heterotypic to the case serotype (i.e. non-homotypic controls). The primary analysis focused on maternal rather than infant antibody concentrations which could have been affected by possible antibody absorption related to the invasive GBS disease in cases.

Using a method described by Kleinbaum and Klein, matched sets of cases and controls were pooled and the number of strata was reduced by combining interchangeable sets (Kleinbaum

DG and Klein M, 2002). Each stratum contained a case and a homotypic colonized control that was matched for all of the following five variables: (i) serotype, (ii) EOD or LOD, (iii) maternal HIV-status, (iv) maternal age as <25 years, 25-<35 years and ≥35 years and (v) gestational age as 34-<37 weeks and ≥37 weeks (Table 2.10). Conditional logistic regression was used to compare the proportion of stratum matched cases to homotypic colonized controls, and stratum matched cases to non-homotypic controls at different antibody thresholds using <0.1 μg/mL as a referent. An adjustment was made for those variables in which the p-value was <0.20 when comparing demographic and risk factors. Odds ratios and 95% confidence interval were reported. Two-tailed p-values <0.05 were considered statistically significant.

Table 2.10: Stratum matched interchangeable sets of cases and controls

			Number of matched strata			
Maternal HIV status	Maternal age (years)	Gestational age (weeks)	Ia-EOD[1]	Ia-LOD[2]	III-EOD	III-LOD
Negative	<25	34-<37	1	7		
	25-<35		2			
	≥35					
	<25	≥37	3	8	11	15
	25-<35		4		12	16
	≥35					
Positive	<25	34-<37				17
	25-<35				13	
	≥35					
	<25	≥37	5	9		18
	25-<35		6	10	14	19
	≥35					

[1]EOD- Early-onset disease, [2]LOD- Late-onset disease.

Demographic characteristics and commonly reported risk factors for invasive GBS disease were compared between stratum matched cases and homotypic colonized controls, and stratum matched cases and non-homotypic controls using Chi-square, Fisher's exact or Mann-Whitney test. Median antibody concentrations were reported and stratified by EOD, LOD and maternal

HIV-status. Median infant to maternal ratios was compared between stratum matched EOD cases and controls using the Mann-Whitney test.

To explore the association between antibody concentration and invasive GBS disease, we used a Bayesian model (Carey et al., 2001) to calculate the probability that a woman with serotype-specific IgG concentration greater than or equal to *c*, gives birth to a neonate who would develop EOD or LOD due to the homotypic serotype, P(D|Ab>=*c*). We assumed antibody concentrations from cases and controls follow a Weibull distribution and placed non-informative priors on the Weibull parameters. To adjust for the case-control design of the study, the model allocates a common stratum-specific marginal risk of disease to cases and controls from the same stratum. A Beta (25, 2500) was used for the prior distribution of the marginal probability of disease P(D), corresponding to a most probable marginal risk of disease equal to 0.4% with the central 95% mass falling within 0.25% and 0.60%. The marginal risk of serotype-specific disease was calculated as the proportionate risk of disease in colonized women for that serotype (i.e. based on the incidence of serotype-specific disease in this population and the maternal GBS colonization prevalence). Further details regarding the model have been described (Carey et al., 2001). Under the Bayesian framework, we obtain possible values for the quantity of interest, referred to as the posterior distribution, given the data and the prior information. The posterior mode and a range where the centre mass of the distribution lies are usually reported. In our setting, we are interested in P(D|Ab>=*c*). For each value *c*, we plot the posterior mode and the range from the 25^{th} to 75^{th} percentile of the posterior distribution.

2.3.5.3 Association between surface-protein antibody concentrations and invasive Group B Streptococcus disease

The analysis plan for surface-protein antibody was similar to that undertaken for capsular antibody, except for the differences outlined below. For the primary analysis for FbsA and BibA antibody, we compared cases to controls whose mothers were colonized with GBS. For the primary analysis for PI-proteins, we compared cases in which the specific PI was identified from the invasive isolate to controls whose mothers were colonized with GBS strains with the homotypic PI (irrespective of serotype or the presence of two pilus island units in a sample). The secondary analysis compared cases to non-colonized controls. Each stratum contained a case and a colonized control that was matched for all of the following: (i) pilus-type (for pilus protein analysis), (ii) EOD or LOD, (iii) maternal HIV-status, (iv) maternal age as <25 years, 25-<35 years and ≥35 years and (v) gestational age as 34-<37 weeks and ≥37 weeks. Conditional logistic regression was used to compare the proportion of stratum matched cases to colonized controls, and stratum matched cases to non-colonized controls at different antibody thresholds. The referent was determined by visual analysis of the separation point between the cases and controls from the reverse cumulative plots. We adjusted for possible confounding variables in which the p-value was <0.20 in the univariate analysis.

Bayesian modelling was undertaken to determine the absolute risk of disease per 1,000 live births. We assumed that the antibody concentrations follow a Weibull distribution. The most probable marginal risk of disease was equal to 1% with the central 95% mass falling within 0.64% and 1.41%. The marginal risk was calculated as the proportionate risk of disease and maternal GBS colonization reported in this population. We plotted the posterior mode and the range from the 25th to 75th percentile of the posterior distribution. Further details regarding the model have been described in chapter 2.3.5.2 above.

2.3.6 Quality Control

For all cases, data of clinical information from the hospital records were extracted by myself. The majority of control subjects were enrolled by study assistants or enrolled nurses. Data was captured in real time onto specifically designed Microsoft Access databases. Source documentation underwent quality checks on average every second month. The database underwent a complete quality check with source document referencing between October 2013 and January 2014 by a RMPRU employed data clerk.

2.3.7 Ethics

The study was approved by the University of Witwatersrand Human Research Ethics Committee (HREC) on the 28th September 2013 (HREC number: M120963; Appendix 8) and registered on the South African National Clinical Trial Register (DOH-27-0113-4309). Informed consent was obtained from women at the time of study-enrolment.

Amendments to the protocol were made in January and April 2013 and included the following:

1. "The mother of this infant will be approached for consent and enrolment of her child and herself within 72hours of the culture result."

This was amended to a week instead of 72 hours as this gave us more time to enrol the patient.

2. "The mothers will be consented for her HIV status together with her CD4+ T-lymphocyte and HIV-1 viral load counts undertaken in the past three months"

The time frame of 'past three months' was removed as many mothers had their CD4+ T-lymphocyte test done early in pregnancy.

3. “Five controls, matched for gestational age of the case (±2 week), maternal HIV status, maternal age (±2 years) and race will be identified and recruited within 21 days of the case.”

The criteria for matching controls was amended for the maternal age from ‘±2 years’ to ±3 years as we had difficulty in identifying controls with our strict matching criteria. We amended the time-frame for recruitment of the control from ‘within 21 days of the case’ to as close to the case as possible.

4. “The positive plate will be retrieved from the NHLS microbiology laboratory for serotyping and storage”

The standard practise of the NHLS laboratory was to discard the agar plate after 5 days without serotyping the organism due to the high cost of serotyping. This was an essential component when evaluating the antibody levels in the infant as the antibody levels are directly related to the serotype causing disease.

5. “For GBS isolation, swabs will be inoculated onto CNA agar (5 % horse blood with 10μg/mL colistin and 15μg/mL nalidixic acid) and into 2mL of Todd-Hewitt broth supplemented with 8 μg/mL gentamycin and 15 μg/mL nalidixic acid, followed by inoculation onto 5 % horse blood agar”

Based on research done in the unit, it was found that CHROMAgar is more sensitive at detecting GBS than the above-mentioned CNA agar and thus we decided to use the CHROMAgar for this study.

2.4 Study Design and Method: Cross-sectional study

2.4.1 Study Design

A cross-sectional study was undertaken on pregnant mothers at the CHBAH from 29th January 2013 to 17th July 2014 to determine the effect of maternal HIV-infection on GBS antibody concentration and the transplacental transfer to the newborn. The HIV-1 sero-prevalence amongst pregnant women in this setting was 28.4% during the study period

2.4.2 Sample Size Calculation

The sample size was premised on evaluating the differences in the ratio of antibody transfer between HIV-infected and HIV-uninfected mothers to their foetus with an 80% power

$$\text{Ratio of antibody transfer} = \frac{Antibody\ level\ in\ cord\ blood}{Antibody\ level\ in\ maternal\ blood}$$

It was hypothesized that the range of ratio of transplacental antibody transfer in HIV-uninfected mother-newborn dyads is between 80-120% based on reported data (Boyer et al., 1984c, Lagergard et al., 1992, Lin et al., 2001). From this, the sample population for both HIV-infected and HIV-uninfected was calculated for a 20%, 30%, 40% and 50% difference in antibody transfer (Table 2.11). These calculations are based on assuming that log (transfer ratio) is normally distributed with a standard deviation of approximately 0.5. An estimate sample of 79 HIV-infected and 79 HIV-uninfected pregnant women was required to detect at least 20% difference in transplacental transfer between HIV –infected compared to HIV-uninfected women (Table 2.11).

Table 2.11: Sample size calculations based on differences in antibody transfer ratio

Transfer Ratio for HIV-uninfected		***1.2***	***1***	***0.8***
difference=0.2	HIV-infected	119	79	48
	HIV-uninfected	119	79	48
difference =0.3	HIV-infected	48	31	18
	HIV-uninfected	48	31	18
difference =0.4	HIV-infected	24	16	9
	HIV-uninfected	24	16	9
difference =0.5	HIV-infected	14	9	5
	HIV-uninfected	14	9	5

2.4.3 Inclusion and Exclusion Criteria

Inclusion criteria

(i) Infant weight ≥2500grams

(ii) Known maternal HIV-status

Exclusion Criteria

(i) Unwilling to consent to the study

(ii) Unable to obtain maternal or cord/ infant blood

(iii) Mothers currently on GBS vaccine trials

(iv) Previously vaccinated mothers against GBS

2.4.4 Study Method

Study staff members were stationed in the maternity wards during normal working hours from Monday to Friday at CHBAH. Gestational age was estimated using the same hierarchy of methods used in chapter 2.3.3.2. Cord blood was collected on potential participants at the time of birth. The mother was approached once clinically stable after delivery and consented to enrol in the study if she met the inclusion criteria. The cord blood was discarded if the mother declined participation.

Procedures conducted at the time of enrolment:

- Maternal and infant case report forms were completed by the study nurse, research assistant or study doctor.
- Maternal and delivery history including identifiable risk factors for GBS were obtained from the mothers and from the available clinical records. Furthermore, examination of the mother, the use of IAP and maternal blood results including HIV status and CD4+ T-lymphocyte counts were recorded.
- Infant's history, examination, routine blood results and in-patient management were recorded from the infants hospital file.
- Blood was collected within 24 hours of delivery from the mother (5mL) in addition to the cord blood at birth (5mL) for antibodies to the 4 common serotypes (Ia, Ib, III and V), and 5 proteins; 3 pilus proteins (PI-1, PI-2a and PI-2b), FbsA and BibA. Newborns were not tested for HIV-1 infection immediately after delivery.
- Blood (2mL) was taken from HIV-infected mother for HIV-1 viral load testing.
- The mother was swabbed (rectal and lower vaginal) for GBS culture within 24 hours of delivery.
- The mother was given a copy of the informed consent form.
- Specimens were transported at room temperature to the RMPRU laboratory for processing within 6 hours or otherwise refrigerated at 2-8°C for 24-48 hours.

2.4.5 Laboratory Method

The laboratory methods (i.e. maternal vaginal and rectal swab collection and isolation of GBS, serotyping and pilus-typing, and antibody measurement using a multiplex Luminex assay) for this study were the same as for the case-control study (chapter 2.3.4.2 to 2.3.4.5.).

In addition, for HIV-1 viral load testing, blood was collected in an EDTA containing tube, following which it was centrifuged for 5 min at 3220 relative centrifugal force, equivalent to 4000rpm (revolutions per minute) to separate the plasma and buffy coat (leucocytes and platelets) from the erythrocytes and then stored at -70ºC.

HIV-1 viral loads were measured using the real-time PCR COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, version 2.0. This was an in vitro nucleic acid amplification test for the quantitation of Human Immunodeficiency Virus Type 1 (HIV-1) RNA in human plasma using the Roche COBAS®. The test quantitates HIV-1 RNA over the range of 20-10,000,000 copies/mL. Values below the detectable range of 20copies/mL were assigned the value of 20.

2.4.6 Data Analysis

Data was collected on study-specific data collection forms and entered into specially designed Microsoft Access and Excel databases. Data was analysed using STATA version 13.1 (College Station, Texas, USA) and GraphPad Prism version 6.05 for Windows (GraphPad Software, California USA). Two-tailed p-values <0.05 were considered statistically significant.

Maternal and cord blood IgG antibody concentrations were measured, and cord blood to maternal ratio calculated to compare the efficiency of transplacental antibody transfer between HIV-exposed and HIV-unexposed newborns. Demographic characteristics were compared between HIV-uninfected and HIV-infected mother-newborn dyads using Chi-square or Fisher's exact test for proportions whilst the Mann-Whitney test was used to compare the

medians. Antibody concentrations remained non-parametric after log transformation, thus median concentrations are reported.

Median maternal antibody concentrations were compared between HIV-uninfected and HIV-infected women at delivery and cord blood antibody concentrations between HIV-unexposed and HIV-exposed newborns using the Mann-Whitney test. Using quantile regression, median maternal antibody concentrations, cord blood antibody concentrations and cord to maternal ratios between HIV-uninfected and HIV-infected women were compared, and adjusted for overall colonization, colonizing serotype for homotypic capsular antibodies, maternal age and parity. The proportions of HIV-infected and HIV-uninfected women with serotype-specific capsular antibody concentrations (above different thresholds proposed to be protective against invasive GBS disease in their infants) was compared (Chapter 1.10.1). In HIV-infected women, CD4+ T-lymphocyte counts and HIV-1 viral loads were correlated with maternal antibody concentrations and cord to maternal ratios using Spearman's test. Furthermore, the maternal antibody concentrations and cord to maternal ratios at varying CD4+ T-lymphocyte counts and HIV-1 viral load thresholds were compared using the Mann-Whitney test.

2.4.7 Quality Control

Quality control was carried out as outlined in chapter 2.3.6.

2.4.8 Ethics

The study was approved by the University of Witwatersrand Human Research Ethics Committee on the 7th November 2012 (HREC number: M120905, Appendix 9) and registered

as an observational study on the South African National Clinical Trial Register (DOH-27-0113-4310). Written informed consent was obtained from the women at time of study-enrolment.

The HREC approved the study on condition that the manner in which we recruited the participants was amended. Instead of consenting mothers in labour, the HREC suggested that we obtain the cord blood which is normally discarded as standard practise and then consent the mother once she has settled clinically post-delivery. Should the mother decline participation, the cord-blood would have been discarded in the appropriate manner.

In addition, an amendment to the protocol was made in February 2013. The South African PMTCT group had moved away from doing HIV-1 viral load testing in pregnant women routinely. In the protocol, we indicated that the HIV-1 viral load results will be obtained as part of the standard of care. HIV-1 viral load testing is thought to be an important determinant of placental antibody transfer in pregnant women. As this was no longer standard practice, we requested approval to obtain 1-2 mL of the mothers' blood to do the HIV-1 viral load test.

3.0 Burden of invasive Group B *Streptococcus (GBS)* disease and subsequent neurodevelopmental outcome in South African infants

An estimated 680 000 neonatal deaths from severe bacterial infections occurred globally in 2012 (Seale et al., 2014). Group B *Streptococcus* is a leading cause of sepsis and meningitis in neonates, despite the decline in incidence of EOD following widespread use of IAP for pregnant women recto-vaginally colonized in the USA (Thigpen et al., 2011, Weston et al., 2011, Schrag and Verani, 2013). In contrast, the burden of invasive GBS disease in low-middle income such as South Africa, where screening for GBS during pregnancy coupled with IAP is not standard of care, has remained unchanged over the past two decades (Haffejee et al., 1991, Madhi et al., 2003, Cutland et al., 2015). Additionally, maternal HIV-infection has been associated with increased risk of invasive GBS disease in their infants (Epalza et al., 2010, Cutland et al., 2015). In this chapter, the epidemiology of invasive GBS disease in South African infants was described (the published paper is attached as Appendix 2)

3.1 Results

3.1.1 Participant selection and demographic characteristics

We identified 122 invasive GBS disease cases in infants <90 days of age over a 12 month period, including 82 (67.2%) at CHBAH, 22 (18.0%) at CMJAH and 18 (14.8%) at RMMCH. In addition, two infants (1.6%) had a recurrence of invasive GBS disease associated with the same serotype (i.e. Ia and Ib) at 5 and 8 days following completion of 10 and 7 days of intravenous antibiotics, respectively. Sixty-six (54.1%) infants had EOD, 95.5% (n=63) of which were identified within 24 hours of life, and all of them had bacteraemia. A higher proportion of cases occurred in males (55.7%) and 116 of 122 (95.1%) were of black-African descent, including 95.1% (n=78) of cases at CHBAH. The prevalence of premature birth

(<37 weeks gestational age) was more common among EOD (45.4%) than LOD cases (25.0%; p=0.019; Table 3.1); including when stratified to <34 weeks of gestational age (33.3% vs. 14.3% respectively; p=0.015). The odds of presenting with meningitis were 45.91 (95% CI: 10.04-410.36; p<0.001) times greater in LOD (58.9%) compared to EOD cases (3.0%), the latter presenting predominantly as sepsis (Table 3.1). Maternal HIV-infection was 3.50 (95% CI: 1.53-8.09) fold greater among LOD (55.4%) compared to EOD cases (25.8%; p<0.001). Only, 1 case and 1 control were diagnosed as HIV-infected at 6 weeks of age.

3.1.2 Incidence and serotype distribution of invasive GBS disease

Of 31 504 live births, there were 75 cases of invasive GBS disease in black-African infants residing in regions D and G; 73 (89.0%) infants presented to CHBAH and 2 (11.1%) to RMMCH. The overall incidence (per 1,000 live births) of invasive GBS disease was 2.38 (95% CI: 1.87-2.98); the incidences of EOD (n=43) and LOD (n=32) were 1.37 (95% CI: 0.99-1.84) and 1.02 (95% CI: 0.70-1.43) respectively. The estimated incidence of disease was significantly higher in HIV-exposed than in HIV-unexposed infants [3.40 (95% CI: 2.29-4.85) versus 1.94 (95% CI: 1.41-2.60) respectively; p=0.016]. The incidence of EOD was similar in HIV-exposed (1.13; 95% CI: 0.54-2.08) and HIV-unexposed (1.46; 95% CI: 1.00-2.04; p=0.487) infants but the incidence risk ratio of LOD was 4.67 (95% CI: 2.24-9.74) greater in HIV-exposed (2.27; 95% CI: 1.39-3.50) compared to HIV-unexposed infants (0.49; 95% CI: 0.24-0.87; p<0.001). Overall, serotypes Ia, Ib and III constituted 75.8% and 92.5% of EOD and LOD cases, respectively (Figures 3.1 and 3.2). Serotype Ia (48.5%) was the commonest cause of EOD and serotype III (64.2%) for LOD. Serotype V was the third commonest serotype, including 18.2% of EOD and 7.6% of LOD cases.

Table 3.1: Demographic characteristics of infants with invasive Group B *Streptococcal* (GBS) disease

	All cases, n=122	EOD[1], n=66	LOD[2], n=56	OR(95% CI)[3]	p-value[4]
Gestational Age					
≥37 weeks	78 (63.9)	36 (54.6)	42 (75.0)	0.40 (0.17-0.93)	0.019
<37 - ≥34 weeks	14 (11.5)	8 (12.1)	6 (10.7)	1.15 (0.32-4.31)	0.808
<34 weeks	30 (24.6)	22 (33.3)	8 (14.3)	3.00 (1.13-8.56)	0.015
Birth Weight					
≥2500 grams	77 (63.1)	38 (57.6)	39 (69.6)	0.59 (0.26-1.33)	0.169
1500-2499 grams	27 (22.1)	14 (21.2)	13 (23.2)	0.89 (0.35-2.30)	0.791
1000-1499 grams	10 (8.2)	7 (10.6)	3 (5.4)	2.10 (0.45-13.12)	0.292
≤999 grams	8 (6.6)	7 (10.6)	1 (1.8)	6.53 (0.79-299.28)	0.068
Gender					
Male	68 (55.7)	35 (53.0)	33 (58.9)	0.79 (0.36-1.72)	0.513
Race					
Black	116 (95.1)	62 (93.9)	54 (96.4)	0.57 (0.05-4.20)	0.526
Mixed race	6 (4.9)	4 (6.1)	2 (3.6)		
Maternal HIV status					
HIV-infected	48 (39.4)	17 (25.8)	31 (55.4)	0.27 (0.12-0.64)	<0.001
HIV-uninfected	73 (59.8)	48 (72.7)	25 (44.6)	2.67 (1.15-6.24)	0.012
HIV-unknown	1 (0.8)	1 (1.5)			
Mode of delivery					
Caesarean-section	29 (23.8)	20 (30.3)	9 (16.1)	2.27 (0.87-6.25)	0.066
Vertex delivery	91 (74.6)	45 (68.2)	46 (82.1)	0.47 (0.18-1.18)	0.078
Unknown	2 (1.6)	1 (1.5)	1 (1.8)		
GBS isolation					
Blood only	87 (71.3)	64 (97.0)	23 (41.1)	45.91 (10.04-410.36)	<0.001
CSF[5] only	13 (10.7)		13 (23.2)		<0.001
Blood and CSF	22 (18.0)	2 (3.0)	20 (35.7)	0.06 (0.01-0.26)	<0.001
Infant age at presentation					
Median(range)	0 (0-74)	0 (0-5)	15 (7-74)		
<24hours	63 (51.6)	63 (95.5)			
1-6 days	3 (2.5)	3 (4.5)			
7-28 days	41 (33.6)		41 (73.2)		
>28 days	15 (12.3)		15 (26.8)		

[1]EOD- Early-onset disease, [2]LOD- Late-onset disease, [3]OR(95% CI)- calculated odds ratio with 95% confidence comparing EOD to LOD, [4]p-value- using Chi-squared, Fisher exact or Wilcoxon rank-sum (Mann-Whitney) test, [5]CSF- Cerebrospinal fluid.

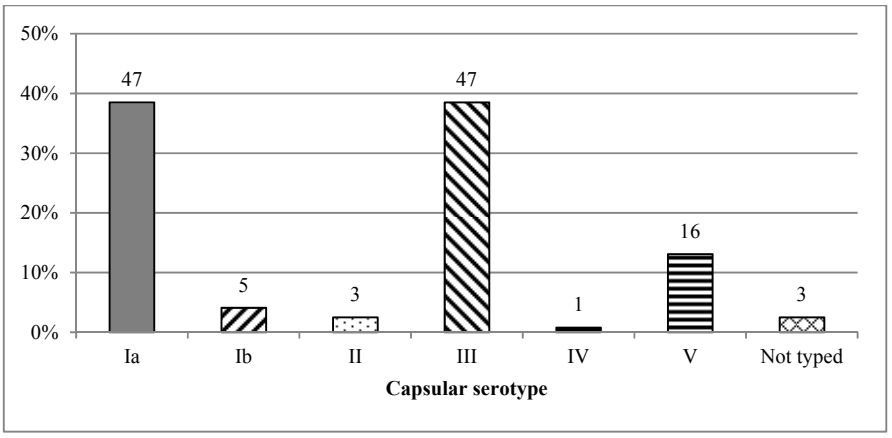


Figure 3.1: Serotype distributions of infants with Group B *Streptococcus* (GBS) disease

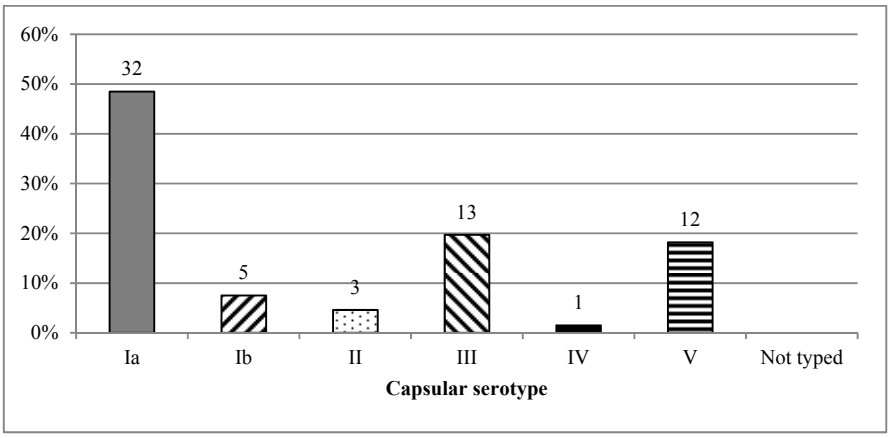


Figure 3.2: Serotype distributions of infants with early-onset Group B *Streptococcus* (GBS) disease

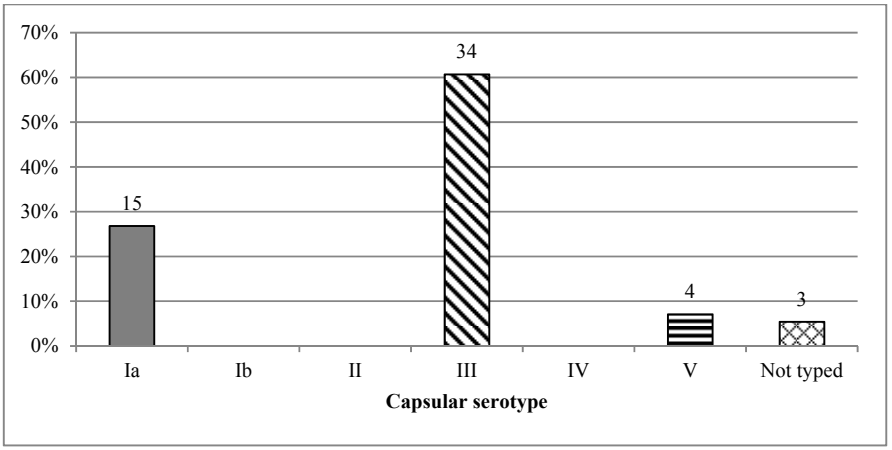


Figure 3.3: Serotype distributions of infants with late-onset Group B *Streptococcus* (GBS) disease

3.1.3 Risk factors for invasive GBS disease

For cases born at ≥34 weeks gestational age, at least 5 controls (mean; 7) were matched for EOD and 3 (mean; 5) for LOD, however, we only managed to enrol between 1 to 4 controls (mean; 2) for cases born at <34 weeks gestational age. Offensive draining liquor (aOR: 27.37; 95% CI: 1.94-386.50) was a risk factor for EOD, whereas maternal GBS bacteriuria was a risk factor for EOD (aOR: 8.41; 95% CI: 1.44-49.15) and LOD (aOR: 3.49; 95% CI: 1.17-10.40; Table 3.2). Maternal fever (≥38°C) was observed in only one case. Although the occurrence prolonged (>18 hours prior to delivery) rupture of membranes (PROM) was more common in EOD cases than controls, no increased risk was found in the multivariate analysis (p=0.213; Table 3.2). Although thirteen (12.8%) mothers of invasive GBS disease cases were not swabbed at enrolment, the prevalence of maternal GBS colonization was higher in mothers of EOD cases (74.5%) than controls (25.1%). Maternal risk factors were not different in HIV-infected and HIV-uninfected mothers (Table 3.3).

Intra-partum antibiotic prophylaxis (IAP) was not administered to most mothers who had at least one risk factor (per Center for Disease Control risk based criteria for IAP; i.e. gestation <37 weeks, PROM and maternal intra-partum fever) predisposing to invasive GBS disease (Verani et al., 2010). Among EOD cases, 5 (16.1%) of 31 mothers with at least one risk factor received IAP ≥4 hours prior to delivery, two (6.5%) received IAP within 4 hours of delivery and 24 (77.4%) did not receive IAP during labour (Table 3.2). Among controls, 36 (34.6%) of 104 mothers with at least one risk factor received IAP ≥4 hours prior to delivery, four (3.9%) received IAP within 4 hours of delivery and 64 (61.5%) did not receive IAP during labour. For infants born to mothers with risk factors, who received IAP at least 4 hours before delivery, the odds of developing EOD was 0.36 (95% CI: 0.10-1.08; Table 3.2).

Table 3.2: Risk factors for invasive Group B *Streptococcal* (GBS) disease in early-onset and late-onset disease cases and matched controls

	Cases	Controls	Univariate-OR (95% CI)[1]	p-value	Multivariate-OR (95% CI)[2]	p-value
Early-onset disease	n=56	n=323				
Maternal GBS colonization	35/47 (74.5)	81/323 (25.1)	8.71 (4.15-19.23)	<0.001	3.38 (0.77-14.83)	0.107
Prolonged ROM (>18hours)[3]	14/49 (28.6)	32/313 (10.2)	3.51 (1.57-7.54)	<0.001	2.08 (0.61-7.08)	0.239
Maternal fever (≥38.0 °C)[4]	1/50 (2.0)	0/319 (0)		0.136		
Offensive liquor	10/52 (19.2)	1/317 (0.3)	75.24 (10.05-3274.04)	<0.001	27.37 (1.94-386.50)	0.014
Maternal GBS Bacteriuria	27/47 (57.5)	22/220 (10.0)	12.15 (5.51-26.79)	<0.001	8.41 (1.44-49.15)	0.018
Any IAP[5]	7/31 (22.6)	40/104 (38.5)	0.47 (0.16-1.26)	0.103		
IAP ≥4 hours prior to delivery	5/31 (16.1)	36/104 (34.6)	0.36 (0.10-1.08)	0.074		
No IAP	24/31 (77.4)	64/104 (61.5)	2.14 (0.80-6.41)	0.103		
Late-onset disease	n=46	n=212				
Maternal GBS colonization	28/42 (66.7)	64/212 (30.2)	4.63 (2.17-10.11)	<0.001	2.44 (0.88-6.79)	0.088
Prolonged ROM(>18hours)[3]	2/35 (5.7)	18/204 (8.8)	0.63 (0.07-2.83)	0.746		
Offensive liquor	2/38 (5.3)	3/203 (1.5)	3.70 (0.30-33.27)	0.178		
Maternal GBS Bacteriuria	18/42 (42.9)	25/212 (11.8)	5.61 (2.48-12.46)	<0.001	3.49 (1.17-10.40)	0.025
Any IAP	1/12 (8.3)	16/56 (28.6)	0.23 (0.01-1.84)	0.269		
IAP ≥4 hours prior to delivery	1/12 (8.3)	10/56 (17.9)	0.42 (0.01-3.59)	0.674		
No IAP	11/12 (91.7)	40/56 (71.4)	4.40 (0.54-201.01)	0.269		

[1]Univariate-OR(95% CI)- calculated odds ratio with 95% confidence using Fisher exact test comparing cases and controls, [2] Multivariate-OR(95% CI)- calculated odds ratio with 95% confidence of disease using conditional logistic regression (For early-onset disease: adjusted for HIV-status, maternal age at delivery, gestational age, maternal GBS colonization, prolonged ROM, offensive liquor, maternal temperature>38, GBS bacteriuria and any intra-partum antibiotics. For late-onset disease: adjusted for HIV-status, maternal age at delivery, gestational age, maternal GBS colonization and GBS bacteriuria), [3] Prolonged ROM (>18 hours) - prolonged rupture of membranes, [4]Maternal fever during labour, [5]IAP-Intrapartum antibiotic prophylaxis to pregnant women that met risk-based criteria (gestation <37 weeks, PROM and maternal intra-partum fever).

Table 3.3: Risk factors for invasive Group B *Streptococcus* (GBS) disease in HIV-infected and HIV-uninfected mothers of GBS cases

Risk factors	HIV-infected, n=41	HIV-uninfected, n=61	OR (95% CI)[1]	p-value
Prematurity (<37 weeks)	12 (29.3)	21 (34.4)	0.79 (0.30-2.00)	0.585
Prolonged ROM (>18hours)[2]	5/31 (16.1)	11/53 (20.8)	0.73 (0.18-2.63)	0.775
Maternal fever (≥38.0 °C)	0/25 (0)	1/52 (1.9)		0.999
Offensive liquor	3/32 (9.4)	9/58 (15.5)	0.56 (0.09-2.52)	0.527
GBS Bacteriuria	23/38 (60.5)	22/51 (43.1)	2.02 (0.79-5.20)	0.105

[1]OR(95% CI)- calculated Odds ratio with 95% confidence using Fischer exact test comparing cases and controls,
[2] Prolonged ROM(>18 hours)- prolonged rupture of membranes.

3.1.4 Clinical presentation of invasive GBS disease

Infants with EOD presented frequently with respiratory distress (83.3%) and 59.3% had a CRP >10 mg/l at time of investigation (Table 3.4). In contrast, other clinical and laboratory signs of sepsis were less frequent (<15%), including leukopenia in only 12.5% of EOD cases. Pyrexia (39.3% vs 3.0%; p<0.001) was more prevalent in LOD cases than EOD. As compared to EOD, infants with LOD also had an increased odds of presenting with poor feeding (OR: 20.71; 95% CI: 4.54-187.69), irritability (OR: 16.65; 95% CI: 5.03-69.74) and lethargy (OR: 3.37; 95% CI 1.17-10.51). Also, LOD cases were more likely to have CRP>40 mg/l (58.7% vs 30.5%; p=0.004) and leukopenia (37.5% vs 12.5%; p=0.001) than EOD cases (Table 3.4).

Table 3.4: Clinical and laboratory features of infants with invasive Group B *Streptococcal* (GBS) disease

	All cases, n=122	EOD[1], n=66	LOD[2], n=56	OR(95% CI)[3]	p-value[4]
Signs and symptoms					
Respiratory distress	75 (61.5)	55 (83.3)	20 (35.7)	0.11 (0.04-0.28)	<0.001
Poor feeding	24 (19.7)	2 (3.0)	22 (39.3)	20.71 (4.54-187.69)	<0.001
Irritability	33 (27.1)	4 (6.1)	29 (51.8)	16.65 (5.03-69.74)	<0.001
Lethargy	23 (18.9)	7 (10.6)	16 (28.6)	3.37 (1.17-10.51)	0.012
Apnoea	13 (10.7)	6 (9.1)	7 (12.5)	1.43 (0.38-5.50)	0.543
Seizures	13 (10.7)	6 (9.1)	7 (12.5)	1.43 (0.38-5.50)	0.543
Increased tone	21/119 (17.7)	9/63 (14.3)	12/56 (21.4)	1.63 (0.57-4.82)	0.308
Decreased tone	16/119 (13.5)	9 /63 (14.3)	7/56 (12.5)	0.86 (0.25-2.82)	0.776
Temperature					
Median(range)	36.8 (33.2-40)	36.6 (33.2-38)	37.5 (35.8-40)		<0.001
≥38°C	24 (19.7)	2 (3.0)	22 (39.3)	20.71 (4.54-187.69)	<0.001
≤35.5°C	5 (4.1)	5 (7.6)	0 (0)		0.062
Intensive/High care					
Mechanical Ventilation	19 (15.6)	10 (15.2)	9 (16.1)	0.93 (0.31-2.84)	0.889
CPAP[5]	6 (4.9)	6 (9.1)	0 (0)		0.031
Inotropic support	8 (6.6)	5 (7.6)	3 (5.4)	0.69 (0.10-3.75)	0.725
Markers of infection					
WCC[6]	n=120	n=64	n=56		
Median(range)x10^9/L	11.8 (1.2-36.2)	13.7 (2.4-36.2)	8.0 (1.2-35.7)		0.003
WCC>20x10^9/L	27 (22.5)	17 (26.6)	10 (17.9)	0.60 (0.22-1.57)	0.255
WCC<5x10^9/L	29 (24.2)	8 (12.5)	21 (37.5)	4.20 (1.56-12.08)	0.001
CRP[7]	n=105	n=59	n=46		
Median(range)mg/L	31.0 (0-351.0)	18.0 (0-277.0)	43.5 (1.0-351.0)		0.002
CRP>10mg/L	69 (65.7)	35 (59.3)	34 (73.9)	1.94 (0.78-4.96)	0.118
CRP>40mg/L	45 (42.9)	18 (30.5)	27 (58.7)	3.24 (1.34-7.87)	0.004

[1]EOD- Early-onset disease, [2]LOD- Late-onset disease, [3]OR(95% CI)- calculated odds ratio with 95% confidence comparing LOD to EOD i.e. LOD was used as the reference comparison group, [4]p-value- using Chi-squared, Fisher exact or Wilcoxon rank-sum (Mann-Whitney) test, [5]CPAP- Continuous positive airway pressure, [6]WCC- White cell count, [7]CRP- C-reactive protein.

3.1.5 Mortality and neurological outcomes of invasive GBS disease

Overall, 23 (18.9%) cases were admitted for intensive care, of whom 19 (10 EOD and 9 LOD) required mechanical ventilation and 8 (5 EOD and 3 LOD) required inotropic support. The mortality ratio among cases ventilated was 60.0% (n=6) of EOD and 55.6% (n=5) for LOD; and 87.5% (n=7) cases who required inotrope support died. The overall case fatality ratio among cases was 18.0% (22/122), including 22.7% for EOD (15/66) and 12.5% for LOD (7/56). Most fatalities (14/22; 63.6%) occurred within 48 hours of presentation to hospital or birth, including 11/14 (78.6%) of deaths among the EOD cases. Significant infant predictors of mortality were gestational age <34 weeks (aOR: 9.45; 95% CI: 2.11-42.29), apnoea at presentation (aOR: 16.54; 95% CI: 1.55-176.33), seizures (aOR: 6.71; 95% CI: 1.07-42.24) and need for inotropic support (aOR: 281.93; 95% CI: 7.32-10864.64; Table 3.5). HIV-exposed infants were not at increased risk of death (aOR: 0.14; 95% CI: 0.02-0.79).

Table 3.5: Predictors of mortality from invasive Group B *Streptococcus* (GBS) disease

	Demised n=22	Survived n=100	Univariate-OR (95% CI)[1]	p-value	Multivariate-OR (95% CI)[2]	p-value
Timing of disease						
Early-onset disease	15 (68.2)	51 (51.0)	2.06 (0.71-6.47)	0.143	1.31 (0.29-5.95)	0.726
Late-onset disease	7 (31.8)	49 (49.0)	0.49 (0.16-1.41)	0.143		
Mode of presentation						
Meningitis	5 (22.7)	30 (30.0)	0.69 (0.18-2.18)	0.608		
Gestational age						
<34 weeks	11 (50.0)	19 (19.0)	4.26 (1.42-12.58)	0.002	9.45 (2.11-42.29)	0.003
HIV-exposure						
HIV-exposed	4 (18.2)	44 (44.0)	0.28 (0.07-0.95)	0.030	0.14 (0.02-0.79)	0.027
HIV-unexposed	17 (77.3)	56 (56.0)	2.67 (0.85-9.92)	0.092		
HIV-unknown	1 (4.5)					
Gender						
Male	11 (50.0)	57 (57.0)	0.75 (0.27-2.12)	0.549		
Clinical features						
Apnoea	7 (31.8)	6 (6.0)	7.31 (1.79-29.7)	<0.001	16.54 (1.55-176.33)	0.020
Seizures	5 (22.7)	8 (8.0)	3.38 (0.76-13.34)	0.058	6.71 (1.07-42.24)	0.043
High/intensive care						
Mechanical Ventilation support	11 (50.0)	8 (8.0)	11.5 (3.31-40.06)	<0.001	0.34 (0.03-3.77)	0.376
Inotropic support	7 (31.8)	1 (1.0)	46.2 (5.09-2101.36)	<0.001	281.93 (7.32-10864.64)	0.002
Lab markers						
WCC[3](<5x10^9/l)	6 (27.3)	23 (23.0)	1.26 (0.36-3.88)	0.670		
CRP[4](>40mg/l)	6 (27.3)	39 (39.0)	0.59 (0.17-1.76)	0.302		

[1]OR(95% CI)- calculated odds ratio with 95% confidence comparing infants that demised versus survivors of invasive GBS disease using Chi-squared or Fisher exact test , [2] Multivariate-OR(95% CI)- calculated odds ratio with 95% confidence using logistic regression (adjusted for timing of disease, HIV-exposure, prematurity (<34 weeks), ventilation, inotropic support, apnoea, seizures), [3]WCC- White cell count, [4]CRP- C-reactive protein.

Survival data was unavailable for 17/122 (13.9%) infants at 90 days of age. Reasons for data being unavailable included: ten cases who were unable to continue study participation and seven cases that were lost to follow-up; these were censored. Figures 3.3-3.6 demonstrate the Kaplan-Meier survival curves at the 90 days of age time-point for infants who had invasive GBS disease. We observed an increased rate of mortality in premature infants.

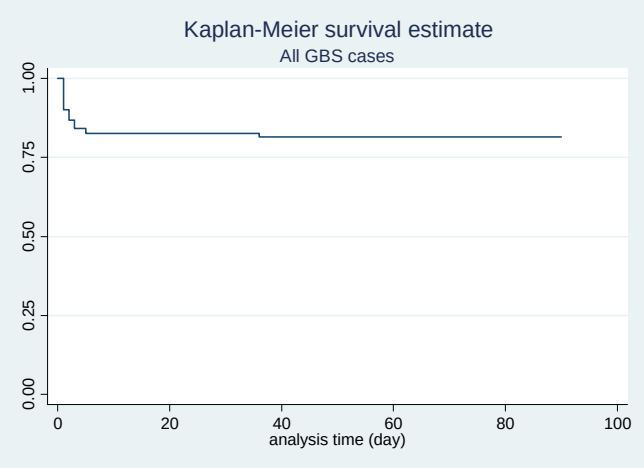


Figure 3.4: Kaplan-Meier survival curve at 90 days of age for infants who had invasive Group B *Streptococcus* (GBS) disease

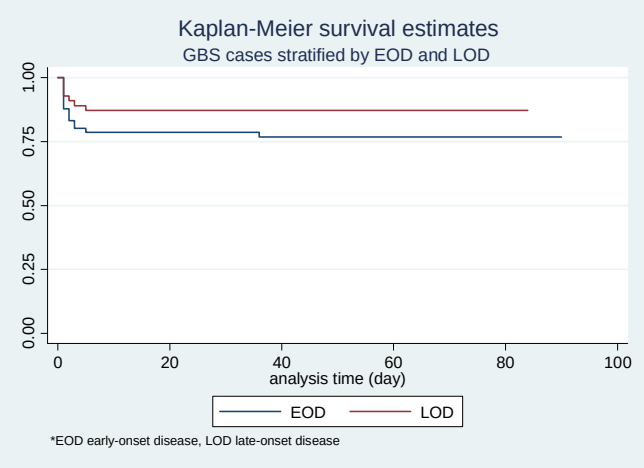


Figure 3.5: Kaplan-Meier survival curve at 90 days of age for infants who had invasive Group B *Streptococcus* (GBS) disease stratified by early-onset (EOD) and late-onset (LOD) disease

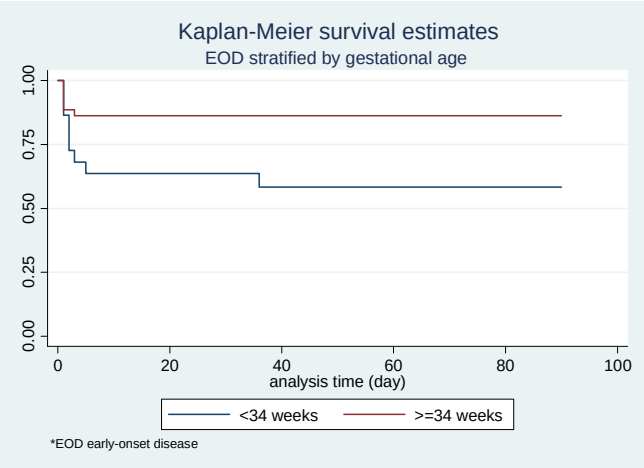


Figure 3.6: Kaplan-Meier survival curve at 90 days of age for infants who had early-onset invasive Group B *Streptococcus* (GBS) disease stratified by gestational age

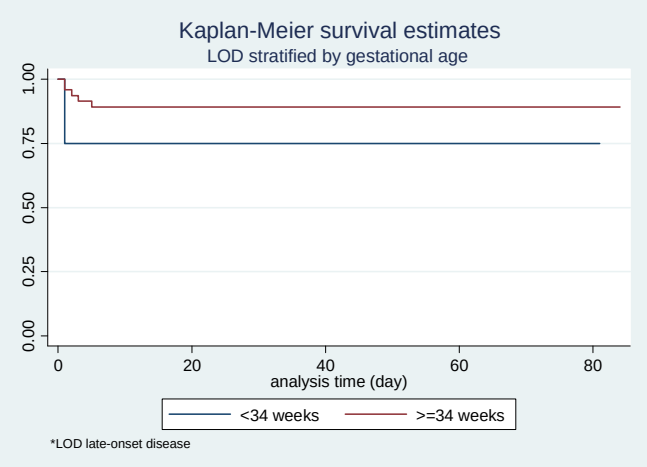


Figure 3.7: Kaplan-Meier survival curve at 90 days of age for infants who had late-onset invasive Group B *Streptococcus* (GBS) disease stratified by gestational age

Of the 100 surviving cases discharged from hospital, both the three and six monthly follow-ups were completed for 63 cases and 214 controls; whilst a further 10 cases and 66 controls only attended one of the two visits. Reasons for follow-up data being unavailable in the remaining cases included 6 whose parents declined study participation, 4 cases born to women considered unable to provide informed consent and 17 cases were lost to follow-up. Demographic and clinical characteristics were similar between cases and controls (Table 3.6). At 3 months of age, there were concerns about normal neurological development in 9 of 68 (13.2%) infants with invasive GBS disease and 1 of 262 (0.4%) control infants (Table 3.7). GBS affected infants were 21.48 (95% CI: 2.58 179.15; p=0.005) times more likely have neurological sequelae than controls. Three cases one with hypertonia and one with personal-social delay on Denver-II subsequently showed signs of recovery from neurological impairment at 6 months, whilst one case did not attend the visit.

At 6 months of age, four additional cases had an abnormal Denver-II screening test. Amongst the cases; two had fine-motor delay only, one had gross-motor delay only, one had gross and fine-motor delay and one had gross, fine-motor and personal-social delay. Four cases had hypertonia and/or hyper-reflexia on neurological examination with a normal Denver-II assessment. The only control with an abnormal Denver-II screening test had gross motor delay. GBS-affected infants were 13.18 (95% CI: 1.44 120.95; p=0.023) times more likely have neurological sequelae than controls. Neurological abnormalities were detected in a greater proportion of GBS affected infants with meningitis (23.5%) than sepsis (9.8%). Hydrocephalus was confirmed in two infants with meningitis (Table 3.7).

Table 3.6: Baseline demographic characteristics of Group B *Streptococcus* (GBS) cases and matched controls at 3 and 6 month visits

	All			EOD[1]			LOD[2]		
3 month visit	**Cases, n=68**	**Controls, n=262**	**p-value**	**Cases, n=37**	**Controls, n=109**	**p-value**	**Cases, n=31**	**Controls ,n=153**	**p-value**
Gestation									
≥37 weeks	48 (70.6)	193 (73.7)	0.611	24 (64.9)	72 (66.1)	0.895	24 (77.4)	121 (79.1)	0.836
<37 weeks	20 (29.4)	69 (26.3)		13 (35.1)	37 (33.9)		7 (22.6)	32 (20.9)	
Median(Range)	39.5 (28.0-43.0)	38.8 (28.0-44.0)	0.525	38.4 (28.0-42.0)	38.2 (30.0-44.0)	0.959	40.0 (29.3-43.0)	39.1 (28.0-44.0)	0.134
Birth weight									
≥2500 grams	48 (70.6)	204 (77.9)	0.208	26 (70.3)	73 (67.0)	0.711	22 (71.0)	131 (85.6)	0.047
<2500 grams	20 (29.4)	58 (22.1)		11 (29.7)	36 (33.0)		9 (29.0)	22 (14.4)	
Median(Range)	2903 (870-4155)	3008 (1195-4315)	0.066	2895 (870-4155)	2870 (1195-3955)	0.973	2915 (1415-3610)	3105 (1465-4315)	0.010
Gender									
Male	41 (60.3)	134 (51.2)	0.178	20 (54.1)	62 (56.9)	0.765	21 (67.7)	72 (47.1)	0.036
HIV exposure									
HIV-exposed	29 (42.6)	122 (46.6)	0.563	10 (27.0)	30 (27.5)	0.953	19 (61.3)	92 (60.1)	0.904
HIV-unexposed	39 (57.4)	140 (53.4)		27 (73.0)	79 (72.5)		12 (38.7)	61 (39.9)	
Clinical presentations									
GBS meningitis	19 (27.9)			2 (5.4)			17 (54.8)		
Perinatal asphyxia	9 (13.2)			9 (24.3)					
Ventilated	3 (4.4)			2 (5.4)			1 (3.2)		
6 month visit	**Cases, n=68**	**Controls, n=232**	**p-value**	**Cases, n=36**	**Controls, n=96**	**p-value**	**Cases, n=32**	**Controls ,n=136**	**p-value**
Gestation									
≥37 weeks	50 (73.5)	185 (79.7)	0.274	24 (66.7)	71 (74.0)	0.406	26 (81.3)	114 (83.8)	0.725
<37 weeks	18 (26.5)	47 (20.3)		12 (33.3)	25 (26.0)		6 (18.7)	22 (16.2)	
Median(Range)	40.0 (28.0-43.0)	39.0 (26.2-44.0)	0.588	38.2 (28.0-42.0)	38.4 (30.0-44.0)	0.446	40.0 (28.0-43.0)	39.2 (26.2-44.0)	0.042
Birth weight									
≥2500 grams	51 (75.0)	189 (81.5)	0.241	26 (72.2)	71 (74.0)	0.841	25 (78.1)	118 (86.8)	0.217
<2500 grams	17 (25.0)	43 (18.5)		10 (27.8)	25 (26.0)		7 (21.9)	18 (13.2)	
Median(Range)	2920 (870-4155)	3039 (1170-3955)	0.072	2903 (870-4155)	2920 (1405-3955)	0.690	2938 (1200-3610)	3093 (1170-395)	0.039
Gender									
Male	43 (63.2)	124 (53.5)	0.153	21 (58.3)	55 (57.3)	0.914	22 (68.8)	69 (50.7)	0.066
HIV exposure									
HIV-exposed	27 (39.7)	100 (43.1)	0.618	9 (25.0)	24 (25.0)	0.999	18 (56.3)	76 (55.9)	0.970
HIV-unexposed	41 (60.3)	132 (56.9)		27 (75.0)	72 (75.0)		14 (43.7)	60 (44.1)	
Clinical presentations									
GBS meningitis	17 (25.0)			2 (5.6)			15 (46.9)		
Perinatal asphyxia	9 (13.2)			9 (25.0)			-		
Ventilated	4 (5.9)			2 (5.6)			2 (6.3)		

[1]EOD- Early-onset disease, [2]LOD- Late-onset disease.

Table 3.7: Neurological sequelae of infants with invasive Group B *Streptococcus* (GBS) disease at 3 and 6 month visits

	Cases			Controls	Univariate-OR (95% CI) [1]	p-value	Multivariate-OR (95% CI) [2]	p-value
	Sepsis	Meningitis	Overall					
3 months	**n=49**	**n=19**	**n=68**	**n=262**				
Overall[3]	3 (6.1)	6 (31.6)	9 (13.2)	1 (0.4)	39.81 (5.27-1751.09)	<0.001	21.48 (2.58-179.15)	0.005
Abnormal Denver-II assessment[4]	2 (4.1)	1 (5.3)	3 (4.4)	1 (0.4)				
Hypertonia/hyper-reflexia[5]	1 (2.0)	5 (26.3)	6 (8.9)	0				
6 months	**n=51**	**n=17**	**n=68**	**n=232**				
Overall	5 (9.8)	4 (23.5)	9 (13.2)	1 (0.4)	35.24 (4.66-1550.57)	<0.001	13.18 (1.44-120.95)	0.023
Abnormal Denver-II assessment	4 (7.8)	1 (5.9)	5 (7.4)	1 (0.4)				
Hypertonia/hyper-reflexia	1 (2.0)	3 (17.6)	4 (5.9)	0				

[1] Univariate-OR(95% CI)- calculated Odds ratio with 95% confidence using Fisher exact test comparing overall cases and controls, [2] Multivariate-OR(95% CI)- calculated Odds ratio with 95% confidence using conditional logistic regression (adjusted for gender, gestational age, birth weight ≥2500, perinatal asphyxia, ventilation at presentation, HIV-status and previous non-GBS admissions), [3]Number (%) of cases and controls with neurological sequelae based on abnormal Denver-II assessments and hypertonia/hyper-reflexia, [4]Abnormal Denver-II assessments in four tested domains (Gross Motor, Fine Motor, Language and Personal/Social), [5]Hypertonia and/or hyper-reflexia on neurological examination of infant with a normal Denver-II assessment.

3.2 Discussion

The overall incidence (per 1,000 live births) of invasive GBS disease in this study was 2.38 (95% CI: 1.87-2.98), which is double that reported for Africa (1.21; 95% CI: 0.50-1.91), and even greater compared to other regions as determined in a recent meta-analysis (Edmond et al., 2012). Though maternal HIV-infection status was not associated with any difference in incidence of EOD, we observed a five-fold greater risk of LOD in HIV-exposed compared to HIV-unexposed infants. The observed case fatality ratio (18.0%) was similar to that previously reported in the mid-1990s (Madhi et al., 2003), however, lower than reported for Kenya (46%) and Malawi (33%) but almost double that in high income settings (7-11%) (Dagnew et al., 2012, Edmond et al., 2012). Neurological sequelae were noted in 13.2% of infants with invasive GBS disease surviving to 6 months-of-age.

Compared to the period 1997-1999 from the same setting, there was a marginal decline in EOD incidence (1.37; 95% CI: 0.99-1.84 vs. 2.06; 95% CI: 1.54-2.76 in 1997-99) (Madhi et al., 2003), nevertheless, the incidence was similar to that reported in USA in the early 1990's prior to the implementation of IAP (Schrag et al., 2000). Despite the majority of births in our setting (99%) occurring in health-care facilities, the implementation of the risk-based IAP strategy, recommended at the hospitals has not been effectively implemented, with only 23.2% of women for whom it was indicated actually receiving IAP timeously. The reasons for this need to be investigated further and could include lack of recognition of risk-factors or failure to administer IAP by birth-attendants, very late arrival at delivery-facilities in relation to birth, or under-staffing at the delivery-facility (average of 60 deliveries occur at CHBAH daily) which results in oversight in effective implementation of even a risk-based IAP strategy. This challenge of risk-based IAP implementation, further accentuates the logistical challenges that

South Africa would face in implementing a more expensive and resource intensive strategy of screening women for GBS recto-vaginal colonization at 35-37 weeks of gestational age, coupled to IAP of colonized women at least 4 hours prior to delivery (Verani et al., 2010). In addition to the common risk factors for invasive GBS disease such as PROM and maternal fever, we also identified a higher proportion of GBS bacteriuria in mothers of infants with EOD compared to controls. The prevalence of maternal GBS bacteriuria has been reported to be 2-7% in pregnancy and is considered to be a surrogate marker of heavy recto-vaginal colonization, as well as a risk factor for EOD (Verani et al., 2010). This was corroborated by our study and supports the CDC recommendation to provide IAP to women who are identified to have GBS growth in urine during pregnancy (Verani et al., 2010). Furthermore, GBS bacteriuria was identified in a greater proportion to mothers of LOD cases (43%) than their controls (12%). Of the 18 cases of LOD whose mothers had GBS bacteriuria, 16 (88.9%) of isolates was the same serotype to that causing disease in the infant. These finding strongly support that IAP should be provided to mothers with GBS bacteriuria as it may be a risk factor for LOD as well (Verani et al., 2010).

In keeping with the higher morbidity caused by infectious diseases in HIV-exposed infants in low-middle income countries (Koyanagi et al., 2011, Landes et al., 2012), the high maternal HIV prevalence (29.5%) may account in part for the high burden of invasive GBS disease in South Africa. Although the incidence of LOD among HIV-unexposed infants in our setting (0.49; 95% CI: 0.24-0.87) is similar to that seen in the Americas (0.31; 95% CI: 0.16-0.89) and globally (0.24; 95% CI: 0.17-0.30) (Edmond et al., 2012), we found that HIV-exposed infants were at a greater risk of developing LOD compared to HIV-unexposed infants. This finding had also been observed in a smaller number of cases from Belgium, and more recently

in another study reporting on invasive GBS disease from 2004-2008 from Soweto, South Africa (Epalza et al., 2010, Cutland et al., 2015). The reasons for the increased susceptibility to invasive GBS disease could be due to perturbations of the infant immune system caused by exposure to HIV virion *in-utero* or maternal ART (Afran et al., 2014); or lower levels of transferred maternal capsular antibody (Jones et al., 2011). Notably, no significant difference in incidence was observed when comparing CD4+ T-lymphocyte counts amongst mothers of cases of LOD and controls (data not shown).

Significant predictors for invasive GBS disease related death in our study included premature birth and presentation with apnoea or seizures, which are important warning signs of severe illness in neonates (World Health Organization, 2005). Contrary to previous reports, in our study, HIV-exposure did not predict mortality in infants with invasive GBS disease (Landes et al., 2012). These findings were unexpected and need to be verified with larger sample sizes before definitive conclusion can be drawn. Although the neonatal immune system differs to older children, there isn't any clear evidence to support an association between mortality and cytokines or other deficiencies (Filteau, 2009). The majority of deaths (63.6%) occurred within 48 hours of hospitalization, highlighting the fulminant course of invasive GBS disease in young infants even in secondary-tertiary level care hospitals. This may explain the failure of recognition of invasive GBS disease as a major cause of morbidity and mortality in low-resource settings, where there are logistical constraints to timeously accessing health-care facilities and many of the cases might have died prior to an opportunity for investigating for invasive disease. Furthermore, the potentially fulminant course of invasive GBS disease, coupled with the observation that 95.5% of EOD occurred within 24 hours of birth in our setting, highlights a further possible reason for under-ascertainment of the burden of EOD in

low-income settings where large number of births occur outside of health care settings or where there is limited laboratory infrastructure to investigate for invasive GBS disease at the time of birth. Also, the high proportion of EOD presenting within 24 hours of birth, predominantly with signs of respiratory distress at the time of birth, suggest that GBS infection possibly occurred whilst *in-utero*. This supports the notion that GBS infection may occur *in-utero* from ascending infection into the amniotic cavity even in the presence of macroscopically intact amniotic sac (Whidbey et al., 2013), which could be aspirated by the foetus resulting in congenital pneumonia and sepsis manifesting as respiratory distress at birth.

In addition to the high mortality ratio, survivors of invasive GBS disease were more likely to have neurological sequelae at 6 months of age (13.2%) than controls (0.4%). The proportion of GBS-meningitis cases who survived with neurological sequelae (23.5%) was similar to that reported at discharge in infants with GBS meningitis in the USA between 1998 and 2006 (22%, 11/50) (Levent et al., 2010). The relatively low overall risk of neurological sequelae in our setting may also be related to the high mortality in these infants. Furthermore, in the absence of screening for auditory and visual deficits, as well as the early assessments, we are likely to have underestimated the number of infants with neurological sequelae from invasive GBS disease. There have been previous reports of long-term neurological sequelae in 26-50% of GBS meningitis survivors at 3-18 years of age (Edwards et al., 1985, Wald et al., 1986, Bedford et al., 2001, Libster et al., 2012), and we are continuing with follow-up of our cohort to report on long-term neurological outcomes.

Compared to earlier studies in South Africa, we have observed a shift in the distribution of serotypes causing EOD, with serotype Ia being most common now (48.5%) compared to serotype III (49%-58%) in previous studies (Madhi et al., 2003, Madzivhandila et al., 2011). Also, there is an increase in proportion of EOD caused by serotype V (18.2% of EOD and 7.6% of LOD), compared to the previous years in the same setting (5.8% of EOD and 1.9% of LOD) (Madzivhandila et al., 2011), which is similar to the proportion of invasive GBS disease cases reported from high-income countries (14-18% of EOD and 14% of LOD) (Zaleznik et al., 2000, Phares et al., 2008). Although there are differences in the invasive potential of different GBS serotypes, with serotype III being most invasive (Madzivhandila et al., 2011), temporal changes in serotype distribution associated with recto-vaginal colonization are mirrored by changes in their relative contribution to EOD as observed with serotype Ia over a twenty-year surveillance period in United Kingdom (Lamagni et al., 2013). Nevertheless, the majority of serotypes causing EOD (76%) and LOD (93%) in our study were due to serotypes Ia, Ib and III, which are included in a trivalent GBS polysaccharide-protein conjugate vaccine targeted at immunization of pregnant women currently in clinical trials (Madhi et al., 2013)

Limitations of our study include case enrolments over a single year; nevertheless, we identified a large number of invasive GBS disease cases and report a persistently high incidence. Due to study constraints, we did not blind examiners performing neurodevelopmental screening tests but plan to do so at future visits. Although other developmental screening test are available (i.e. Bailey), we were limited to using the Denver-II screening test which has been shown to be reliable in young infants (Frankenburg et al., 1992). Furthermore, we currently only report on neurological sequelae up to 6 months of age, and did not have any follow-up outcomes on 27% of cases discharged from hospital. The short-term

follow-up for neurological sequelae could fail to identify mild development delay or learning problems that manifest later in life, or conversely may over-estimate the long-term sequelae as the neurological system matures in children (Eyre, 2003).

Our study emphasizes the need to consider alternate strategies for the prevention of invasive GBS disease in settings such as ours, where screening for recto-vaginal GBS colonization and even risk-based approaches coupled to IAP is not being effectively implemented or not logistically feasible. This includes the potential of preventing invasive GBS disease in newborns and young infants through targeted vaccination of pregnant women. Maternal vaccination aimed at the protection of young infants against infectious disease has been demonstrated to be effective against neonatal tetanus, influenza illness and pertussis until 6 months of age (Steinhoff, 2013, Amirthalingam et al., 2014, Madhi et al., 2014). Considering the increased risk of LOD in HIV-exposed infants, the immunogenicity of a GBS polysaccharide-protein conjugate vaccine would also need to be evaluated in this population.

4.0 HIV-1 is associated with lower Group B *Streptococcus* (GBS) capsular and surface-protein IgG antibody levels and reduced transplacental antibody transfer in pregnant women

A meta-analysis of studies undertaken from 2000 to 2011, reported the highest incidence of invasive GBS disease to be in low-middle income countries from Eastern and Southern Africa (Edmond et al., 2012). Maternal and infant GBS serotype-specific capsular antibody has been associated with protection against homotypic serotype invasive GBS disease in infants (Baker and Kasper, 1976). Furthermore, GBS surface-proteins which facilitate adherence to host epithelium such as PI-1, PI-2a, PI-2b, FbsA and BibA have been shown to be immunogenic, and induce antibodies in animal-model studies that improved survival following systemic GBS inoculation challenges (Lindahl et al., 2005, Margarit et al., 2009, Meinke et al., 2010).

Although maternal HIV-infection is not associated with higher prevalence of recto-vaginal GBS colonization during pregnancy or at birth (El Beitune et al., 2006, Mavenyengwa et al., 2010, Gray et al., 2011, Shah et al., 2011, Cutland et al., 2012), a greater risk of invasive GBS disease have been reported in HIV-exposed compared to HIV-unexposed infants (Epalza et al., 2010, Cutland et al., 2015). The basis for the increased susceptibility to invasive GBS disease in HIV-exposed infants remains to be ascertained and could include maternal HIV-infection being associated with lower concentrations of protective GBS antibodies or impaired transplacental antibody transfer (Afran et al., 2014). In this chapter, the effect of maternal HIV-infection on GBS capsular and surface-protein antibody concentrations, as well as the effect on transplacental antibody transfer is described (the published paper is attached as Appendix 3)

4.1 Results

4.1.1 Participant selection and demographic characteristics

Of the 320 women screened, 70 refused consent and 76 failed to meet the inclusion criteria. We therefore enrolled 174 mother-newborn dyads, ten of whom were subsequently excluded (including nine dyads where the newborn gestational age was ≤36 weeks, and one dyad in whom maternal blood was taken >12 hours following delivery). Thus, 164 mother-newborn dyads were analysed, including 81 HIV-uninfected and 83 HIV-infected women all of whom had singleton births. Except for HIV-infected women being older (median 30.7 vs 26.0 years; p=0.006), they were otherwise similar in demographic characteristics compared to HIV-uninfected women (Table 4.1). Among the 83 HIV-infected women at the time of delivery, 36 (43.4%) were on triple ART, 46 (55.4%) on AZT only and one (1.2%) had not received any ART. The median duration on triple ART from initiation to delivery was 13.4 weeks (range: 1.4 - >44) and 17.1 weeks (range: 2.4 - 42.7) for women on AZT only. Overall, 49 (29.9%) of 164 women were colonized with GBS; colonization was similar in HIV-uninfected (27.2%) and HIV-infected (32.5%; p=0.453) women (Table 4.1). The commonest colonizing serotype was Ia (59.1% of all serotypes) in HIV-uninfected women and III (40.7% of serotypes) in HIV-infected women (Table 4.1).

All women had detectable antibody levels to all four GBS serotypes, however, cord blood antibody levels were not detected in two samples for serotype Ia and in five samples each for serotypes Ib, III and V. Regarding surface-protein antibodies, only one woman had undetectable antibody levels to PI-2a. For cord blood samples, antibody levels were undetectable on two for BibA, four for FbsA, five for PI-1 and PI-2b, and six for PI-2a. The final analysis included all samples as results were similar when the undetectable samples were excluded from the analysis (data not shown).

Table 4.1: Demographic and recto-vaginal colonization characteristics of HIV-uninfected and HIV-infected mother-newborn dyads

	All mother-newborn dyads (n=164)	HIV-uninfected mother-newborn dyads (n=81)	HIV-infected mother-newborn dyads (n=83)	p-value[1]
Mother				
Age: Median (IQR)	28.0 (23.1-33.7)	26.0 (22.1-31.2)	30.7 (23.7-35.6)	0.006
Parity: Median (IQR)	1 (0-2)	1 (0-2)	1 (0-2)	0.079
Black-African race	157 (98.1)	78 (96.3)	83 (100.0)	0.118
GBS colonization				
Colonized mothers[2]	49 (29.9)	22 (27.2)	27 (32.5)	0.453
Ia	21 [42.9][3]	13 [59.1]	8 [29.6]	0.131[4]
Ib	2 [4.1]	0 [0]	2 [7.4]	
II	4 [8.2]	2 [9.1]	2 [7.4]	
III	14 [28.6]	3 [13.6]	11 [40.7]	
V	9 [18.4]	5 [22.7]	4 [14.8]	
Newborn				
Male gender	90 (54.9)	45 (55.6)	45 (54.2)	0.863
Gestational age: Median (IQR)	40.0 (38.3-40.3)	40.0 (38.2-40.4)	40.0 (38.4-40.2)	0.997
Birth weight: Median (IQR)	3063 (2878-3363)	3130 (2895-3385)	3034 (2852-3275)	0.194

[1]p-value after comparing HIV-uninfected and HIV-infected mother-newborn dyads using Chi-square, Fisher's exact or Mann-Whitney test, [2]number of GBS rectal/vaginal colonized mothers stratified by colonizing serotype (an HIV-uninfected mother was dual colonized with Ia and V), [3]serotype proportion of colonized mothers, [4]Multiple two-way comparisons using Fisher's exact test.

4.1.2 Maternal HIV-infection status and capsular antibodies

Median capsular antibody concentrations (μg/mL) were lower in HIV-infected than HIV-uninfected women for serotypes Ib (0.06 vs. 0.09; p=0.033) and V (0.40 vs.0.59; p=0.040); similar trends were observed for serotype Ia (0.13 vs. 0.36; p=0.077), but this difference was not significant (Figure 4.1 A-D, Table 4.2). Median cord blood capsular antibody concentrations (for all serotypes) were significantly lower in HIV-exposed than in HIV-unexposed newborns; the respective antibody concentrations (μg/mL) for serotypes Ia, Ib, III and V were 0.07 vs. 0.26 (p=0.005), 0.07 vs. 0.15 (p=0.013), 0.15 vs. 0.25 (p=0.005) and 0.34 vs.0.57 (p=0.004) (Figure 4.1 A-D, Table 4.2).

Table 4.2: Median antibody concentrations in HIV-infected and HIV-uninfected mother and cord/newborn sera

	Mother			Cord/ Newborn		
	HIV-uninfected Median(IQR)[1] n=81	HIV-infected Median(IQR) n=83	p-value[2]	HIV-unexposed Median(IQR) n=81	HIV-exposed Median(IQR) n=83	p-value[2]
Capsular serotypes (μg/mL)						
Ia	0.36 (0.05-4.20)	0.13 (0.05-0.81)	0.077	0.26 (0.04-3.43)	0.07 (0.02-0.47)	0.005
Ib	0.09 (0.06-0.22)	0.06 (0.04-0.15)	0.033	0.15 (0.07-0.26)	0.07 (0.03-0.22)	0.013
III	0.25 (0.11-0.79)	0.21 (0.10-0.42)	0.261	0.25 (0.11-0.62)	0.15 (0.06-0.32)	0.005
V	0.59 (0.27-1.07)	0.40 (0.25-0.71)	0.040	0.57 (0.28-1.33)	0.34 (0.16-0.76)	0.004
Surface-proteins (AU/mL)						
PI-1	1020 (327-4913)	549 (264-1865)	0.016	1177 (264-2799)	502 (195-1917)	0.039
PI-2a	1972 (845-4765)	1130 (485-3866)	0.015	1966 (609-6167)	1560 (424-5747)	0.541
PI-2b	1072 (610-2543)	611 (306-1704)	0.015	865 (457-2674)	478 (259-1427)	0.024
BibA	4790 (2527-8412)	3829 (1769-8174)	0.236	4350 (1610-8596)	2943 (1606-6870)	0.266
FbsA	2169 (1418-4914)	1444 (711-2886)	<0.001	2758 (1305-5611)	1717 (794-3797)	0.010

[1]Interquartile range, [2]Mann-Whitney test.

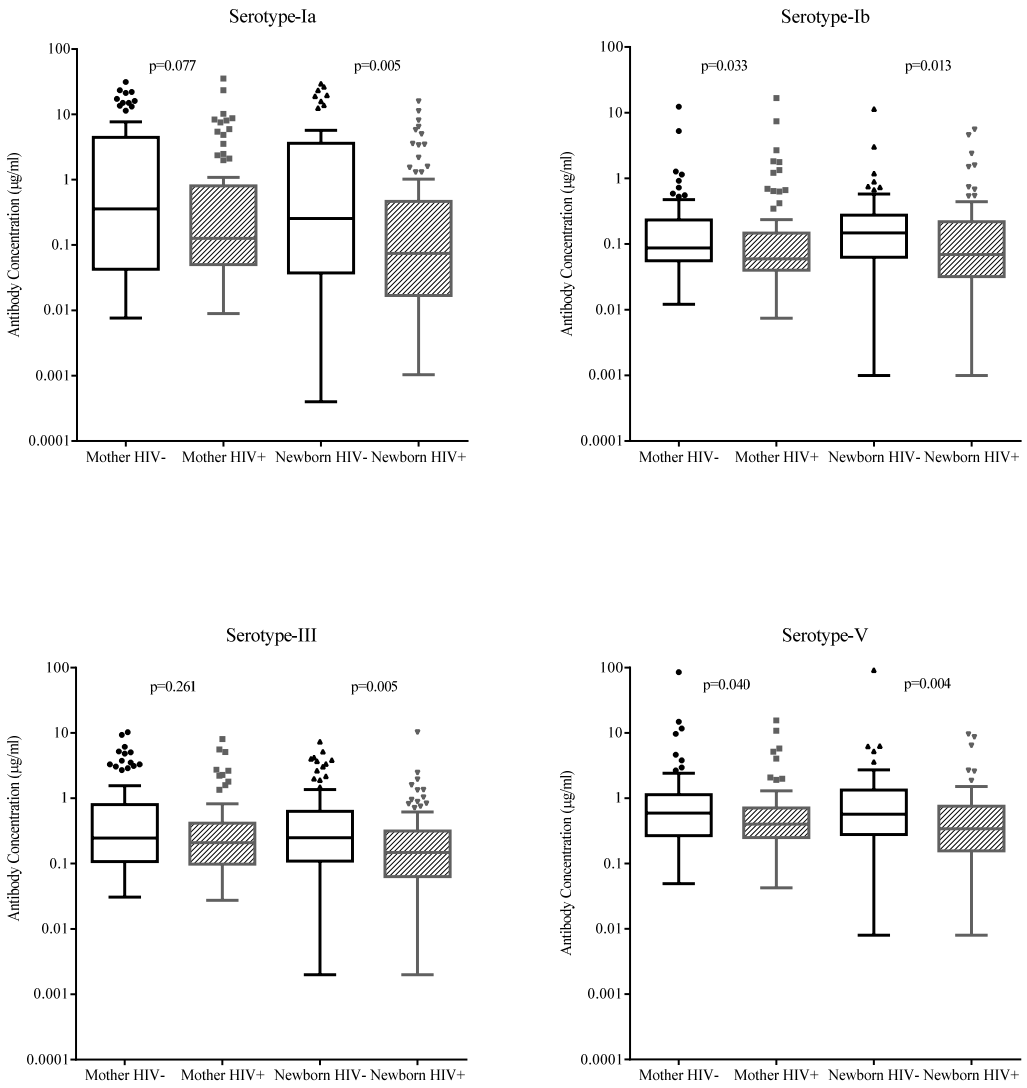


Figure 4.1: Tukey box and whisker plots comparing capsular antibody concentrations of serotype-Ia, serotype-Ib, serotype-III and serotype-V between HIV-uninfected and -infected mothers, and HIV-unexposed and -exposed newborns

Footnote: Mother HIV- denotes HIV-uninfected and HIV+ denotes HIV-infected, Newborn HIV- denotes HIV-unexposed and Newborn HIV+ denotes HIV-exposed. The y-axis has been log10 scaled. For the box and whisker plots; the box represents the distance of the 25th and 75th percentile with the median represented by the solid line within the box. The upper whisker represents 1.5 times the interquartile distance from the 75th centile, while the lower whisker represents 1.5 times the interquartile distance from the 25th centile. The dot symbols represent outliers above the upper whisker.

After adjusting for confounding factors, we compared maternal antibody concentrations between HIV-infected and HIV-uninfected women at multiple percentiles using quantile regression analysis. Significant differences in antibody concentrations for serotypes Ia, III and V between HIV-infected and HIV-uninfected women were found at higher percentiles (above 65^{th}), suggesting that HIV-infected women also tended to have lower antibody concentrations that HIV-uninfected at higher percentiles (Table 4.3). Corroborating this, we demonstrated that a lower proportion of HIV-infected women had capsular antibody concentrations above thresholds of ≥1 μg/mL and ≥2 μg/mL for serotypes Ia and III (Table 4.4). Using multivariate analysis, with an antibody concentration of <0.5 μg/mL as a referent, the adjusted odds of having capsular antibody concentration ≥2 μg/mL in HIV-infected compared to HIV-uninfected women were 0.33 (95% CI: 0.15-0.75; p=0.008) and 0.34 (95% CI: 0.12-1.00; p=0.049) for serotypes Ia and III, respectively (Table 4.4).

Notably, two infants born to HIV-infected women developed late-onset GBS meningitis from serotypes Ia and III at 19 and 22 days of age, and among whom their mother's antibody concentrations were 0.08 and 0.12 for the homotypic serotypes and the transplacental ratio was 0.14 and 0.69, respectively.

Table 4.3: P-values comparing maternal antibody concentrations between HIV-infected and HIV-uninfected pregnant women using quantile regression analysis at different percentiles

Percentiles	Ia	Ib	III	V	PI-1	PI-2a	PI-2b	BibA	FbsA
0.5	0.634	0.279	0.795	0.263	0.241	0.21	0.139	0.314	0.084
0.6	0.236	0.128	0.963	0.159	0.098	0.223	0.126	0.242	0.049
0.7	0.008	0.124	0.055	0.029	0.023	0.136	0.014	0.493	0.026
0.8	0.002	0.666	0.005	0.035	0.01	0.047	0.09	0.649	0.117
0.9	<0.001	0.241	<0.001	0.002	<0.001	0.002	0.188	0.032	0.152

Footnote: Adjusted for overall colonization, colonizing serotype for capsular antibodies, maternal age and parity

Table 4.4: Proportion of HIV-infected and HIV-uninfected women with capsular antibody concentrations (μg/mL) above different thresholds

Antibody concentration (μg/mL)	HIV-infected n=83	HIV-uninfected n=81	aOR (95% CI)[1]	p-value
Ia				
<0.5	59 (71.1)	46 (56.8)	Referent	
≥0.5	24 (28.9)	35 (43.2)	0.44 (0.22-0.89)	0.021
≥1	17 (20.5)	30 (37.0)	0.37 (0.16-0.72)	0.005
≥2	14 (16.9)	26 (32.1)	0.33 (0.15-0.75)	0.008
Ib				
<0.5	72 (86.7)	72 (88.9)	Referent	
≥0.5	11 (13.3)	9 (11.1)	1.34 (0.51-3.52)	0.550
≥1	7 (8.4)	4 (4.9)	2.11 (0.57-7.78)	0.261
≥2	3 (3.6)	2 (2.5)	1.95 (0.30-12.59)	0.482
III				
<0.5	64 (77.1)	55 (67.9)	Referent	
≥0.5	19 (22.9)	26 (32.1)	0.48 (0.23-1.02)	0.058
≥1	10 (12.1)	17 (21.0)	0.37 (0.14-0.95)	0.038
≥2	7 (8.4)	14 (17.3)	0.34 (0.12-1.00)	0.049
V				
<0.5	49 (59.0)	37 (45.7)	Referent	
≥0.5	34 (41.0)	44 (54.3)	0.58 (0.30-1.11)	0.099
≥1	14 (16.9)	23 (28.4)	0.46 (0.21-1.03)	0.059
≥2	6 (7.2)	10 (12.3)	0.50 (0.16-1.54)	0.228

[1]Adjusted-OR (95% CI)- calculated odds ratio with 95% confidence of disease using logistic regression (adjusted for parity, maternal age and serotype-specific colonization)

Overall, median cord to maternal ratios for capsular antibody ranged between 75% to 119% in HIV-uninfected mother-newborn dyads and 47% to 93% among HIV-infected mother-newborn dyads (Table 4.5). In the multivariate model, after adjusting for overall colonization, serotype-specific colonization, maternal age and parity, the cord to maternal ratio was 37.4% ($p<0.001$) and 32.5% ($p=0.027$) lower for serotypes Ia and III in HIV-infected compared to HIV-uninfected mother-newborn dyads (Table 4.5).

Table 4.5: Transplacental antibody transfer (cord to maternal blood ratio) between HIV-uninfected and HIV-infected mother-newborn dyads

	HIV-uninfected mother-newborn dyads Median CMR[1] (IQR)[2] n=81	HIV-infected mother-newborn dyads Median CMR (IQR) n=83	Reduction, %[3]	p-value[4]
Capsular serotypes				
Ia	0.749 (0.562-1.021)	0.469 (0.322-0.754)	37.4	<0.001
Ib	1.187 (0.730-1.959)	0.930 (0.593-1.574)	21.7	0.483
III	0.902 (0.605-1.229)	0.609 (0.407-0.976)	32.5	0.027
V	0.954 (0.677-1.310)	0.825 (0.543-1.158)	13.5	0.084
Surface-proteins				
PI-1	1.056 (0.835-1.453)	0.948 (0.669-1.431)	10.2	0.379
PI-2a	0.904 (0.545-1.317)	1.262 (0.613-3.000)	NR[5]	0.213
PI-2b	1.006 (0.598-1.588)	0.904 (0.562-1.521)	10.1	0.500
BibA	0.860 (0.687-1.139)	0.759 (0.539-1.126)	11.7	0.207
FbsA	0.964 (0.601-1.695)	1.159 (0.454-2.347)	NR	0.385

[1]CMR-cord to maternal ratio, [2]Interquartile range, [3]Reduction in cord to maternal ratio comparing HIV-infected and HIV-uninfected mother-newborn dyads; calculated as the ratio of the cord to maternal ratio from HIV-infected/HIV-uninfected women, subtracted from 1, [4]Using quantile regression (adjusted for overall colonization, colonizing serotype for capsular antibodies, maternal age and parity), [5]No reduction.

4.1.3 Maternal HIV-infection status and surface-protein antibodies

As compared to HIV-uninfected women, HIV-infected women had lower median antibody concentrations (AU/mL) against surface-protein PI-1 (549 vs. 1020; p=0.016), PI-2a (1130 vs. 1972; p=0.015), PI-2b (611 vs. 1072; p=0.015) and FbsA (1444 vs.2169; p<0.001), but not significantly so for BibA (3829 vs. 4790; p=0.236) (Figure 4.2 A-E, Table 4.2). Cord blood median surface-protein antibody concentrations were lower in HIV-exposed compared to HIV-unexposed newborns for PI-1 (502 vs. 1177; p=0.039), PI-2b (478 vs. 865; p=0.024) and FbsA (1717 vs.2758; p=0.010) (Figure 4.2 A-E, Table 4.2). The median cord to maternal ratios (range 76%-126%) were similar for all antibodies directed against surface-proteins between HIV-uninfected and HIV-infected mother-newborn dyads (Table 4.5).

4.1.4 Effect of CD4+ T-lymphocyte and HIV-1 viral load counts on GBS antibody in HIV-infected women

In HIV-infected women, 71 of 83 (85.5%) had a CD4+ T-lymphocyte count measured within 6 months before delivery with a median CD4+ T-lymphocyte count of 423 cells/mm^3 (IQR: 264-594). The median HIV-1 viral load in 79/83 (95.2%) participants was 96 copies/mL (IQR: 20-3841) and undetectable in 28 of the 79 (35.4%) samples. There was no correlation between CD4+ T-lymphocyte counts and maternal antibody concentrations or between CD4+ T-lymphocyte counts and cord to maternal ratios for any of the nine measured antibodies. Furthermore, median maternal antibody concentrations and cord to maternal ratios were similar when stratified by different thresholds of CD4+ T-lymphocyte counts (Table 4.6 and 4.7). Similarly, there was no correlation between maternal HIV-1 viral load and maternal antibody concentration or cord to maternal ratios for any of the nine measured antibodies (Table 4.6 and 4.7).

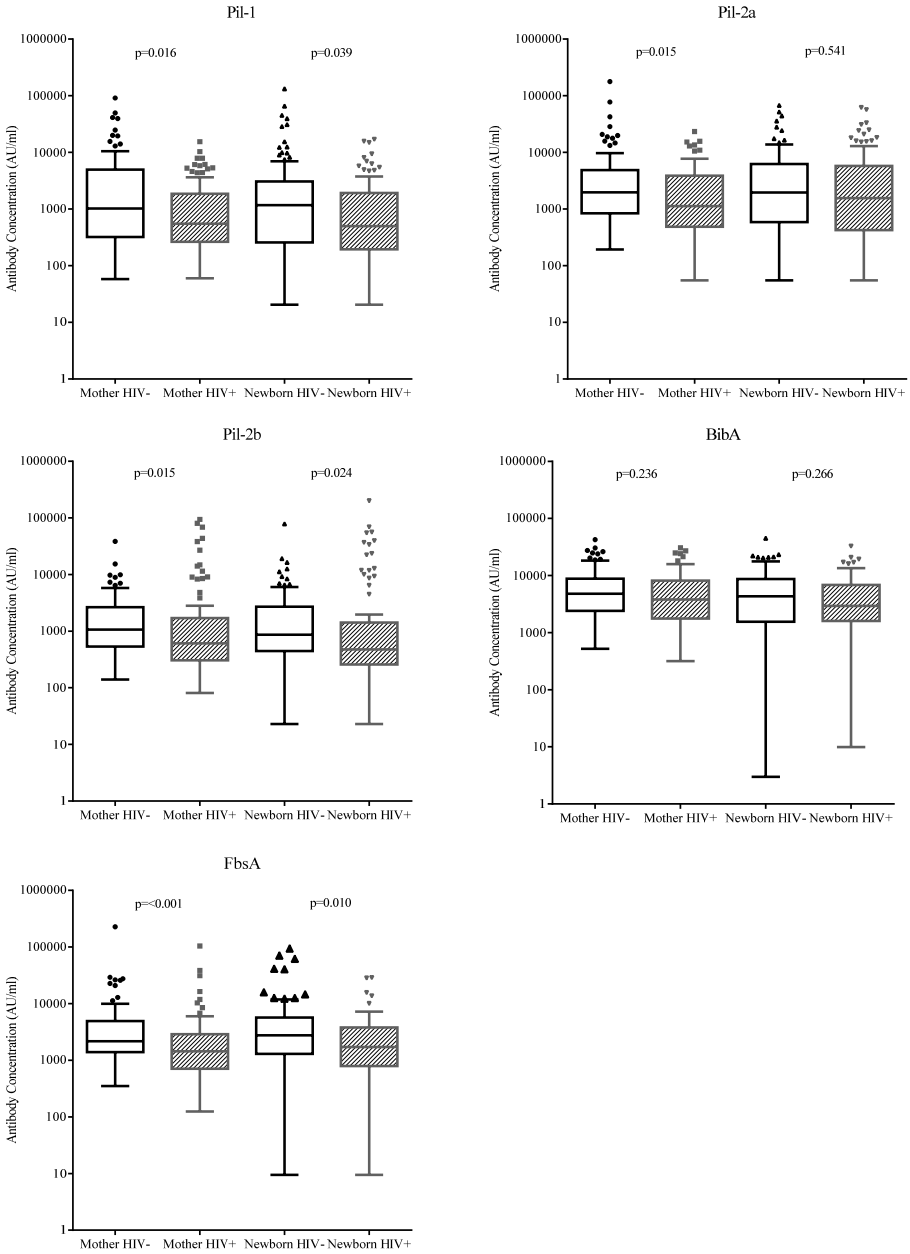


Figure 4.2: Tukey box and whisker plots comparing surface-protein antibody concentrations of PI-1, PI-2a, PI-2b, BibA and FbsA between HIV-uninfected and -infected mothers, and HIV-unexposed and -exposed newborns

Footnote: Mother HIV- denotes HIV-uninfected and HIV+ denotes HIV-infected, Newborn HIV- denotes HIV-unexposed and Newborn HIV+ denotes HIV-exposed. Arbitrary units is abbreviated AU. The y-axis has been log10 scaled. For the box and whisker plots; the box represents the distance of the 25th and 75th percentile with the median represented by the solid line within the box. The upper whisker represents 1.5 times the interquartile distance from the 75th centile, while the lower whisker represents 1.5 times the interquartile distance from the 25th centile. The dot symbols represent outliers above the upper whisker.

Table 4.6: Median (interquartile range) capsular (µg/mL) and protein (AU/mL) antibody concentrations stratified by CD4+ T-lymphocyte counts and HIV-1 viral load in HIV-infected women

	Ia	Ib	III	V	PI-1	PI-2a	PI-2b	BibA	FbsA
CD4+ (cells/mm3)									
<200 (n=7)	0.11 (0.06-0.81)	0.10 (0.05-0.42)	0.21 (0.19-0.35)	0.57 (0.44-0.71)	224 (196-4616)	2235 (362-5577)	604 (326-892)	7342 (1760-18105)	1444 (525-5117)
200-350 (n=19)	0.19 (0.06-0.69)	0.11 (0.04-0.19)	0.27 (0.15-0.36)	0.44 (0.27-0.72)	480 (265-1983)	772 (493-2291)	539 (242-2025)	3210 (1723-8174)	1563 (778-2733)
350-500 (n=21)	0.09 (0.03-0.87)	0.07 (0.03-0.14)	0.23 (0.10-0.55)	0.39 (0.10-1.01)	581 (361-1227)	2123 (700-4203)	732 (411-1895)	2894 (1732-6301)	1074 (584-2426)
>500 (n=24)	0.13 (0.04-0.49)	0.06 (0.04-0.09)	0.13 (0.07-0.63)	0.37 (0.23-0.68)	451 (249-1636)	733 (415-3014)	502 (298-876)	3723 (1731-7213)	1227 (761-1710)
p-value[1]	0.637	0.089	0.369	0.178	0.671	0.369	0.777	0.219	0.571
Viral load (copies/mL)									
<40 (n=31)	0.14 (0.07-0.75)	0.06 (0.04-0.17)	0.27 (0.12-0.69)	0.43 (0.25-0.71)	446 (217-2037)	1074 (457-2557)	411 (277-4822)	4309 (1760-9098)	1454 (542-2886)
40-1000 (n=21)	0.09 (0.05-0.45)	0.06 (0.04-0.12)	0.19 (0.10-0.33)	0.48 (0.28-0.71)	549 (273-1417)	866 (542-2123)	735 (406-1761)	3829 (1723-7280)	1228 (754-2426)
1000-10 000 (n=14)	0.06 (0.04-1.01)	0.03 (0.02-0.09)	0.15 (0.09-0.45)	0.20 (0.07-0.68)	452 (196-979)	1490 (273-3744)	446 (255-604)	3081 (1354-3979)	1110 (393-3702)
>10 000 (n=13)	0.18 (0.07-0.87)	0.12 (0.05-0.23)	0.28 (0.15-0.35)	0.48 (0.31-0.61)	686 (484-1227)	3866 (772-5577)	728 (538-1322)	5645 (3046-13400)	1559 (897-4053)
p-value[2]	0.529	0.580	0.709	0.738	0.263	0.108	0.348	0.177	0.598

[1]comparing median antibody concentration in mothers with CD4+ T-lymphocyte counts <200 and CD4+ T-lymphocyte counts >500 using Mann-Whitney test; [2]comparing median antibody concentration in mothers with HIV-1 viral load <40 and HIV-1 viral load >10 000 using Mann-Whitney test.

Table 4.7: Cord to maternal median (interquartile range) ratios stratified by CD4+ T-lymphocyte counts and HIV-1 viral load in HIV-infected mother-newborn dyads

	Ia	Ib	III	V	PI-1	PI-2a	PI-2b	BibA	FbsA
CD4+ (cells/mm3)									
<200 (n=7)	0.45 (0.38-0.54)	1.54 (0.59-1.96)	1.00 (0.53-1.28)	0.83 (0.65-1.41)	0.80 (0.58-1.25)	1.98 (0.56-4.76)	1.17 (0.79-2.20)	1.13 (1.03-1.31)	1.79 (0.78-4.39)
200-350 (n=19)	0.48 (0.18-0.94)	1.17 (0.84-1.97)	0.61 (0.40-0.98)	0.79 (0.54-1.20)	0.75 (0.33-1.43)	0.93 (0.39-2.71)	0.78 (0.24-1.71)	0.67 (0.55-1.31)	1.16 (0.28-2.48)
350-500 (n=21)	0.45 (0.37-0.65)	0.79 (0.51-1.04)	0.52 (0.38-0.78)	0.81 (0.56-1.03)	1.09 (0.70-1.63)	1.67 (1.13-3.47)	1.08 (0.75-1.31)	0.78 (0.53-1.17)	1.75 (0.74-3.13)
>500 (n=24)	0.50 (0.32-0.70)	1.01 (0.66-1.66)	0.73 (0.45-0.99)	0.89 (0.53-1.21)	1.04 (0.72-1.66)	1.26 (0.65-2.53)	0.92 (0.64-1.50)	0.75 (0.65-1.02)	1.14 (0.62-2.10)
p-value[1]	0.887	0.850	0.321	0.539	0.395	0.603	0.603	0.047	0.422
Viral load (copies/mL)									
<40 (n=31)	0.45 (0.28-0.87)	1.00 (0.59-2.09)	0.81 (0.51-1.13)	1.03 (0.56-1.33)	1.09 (0.70-1.40)	1.68 (0.71-3.51)	1.12 (0.69-1.58)	0.93 (0.57-1.35)	1.75 (0.51-3.73)
40-1000 (n=21)	0.57 (0.38-0.71)	1.02 (0.62-1.26)	0.56 (0.41-0.81)	0.65 (0.54-0.83)	0.75 (0.58-1.43)	0.65 (0.46-1.36)	0.78 (0.46-1.05)	0.70 (0.57-0.82)	0.83 (0.43-1.23)
1000-10 000 (n=14)	0.51 (0.35-0.82)	0.98 (0.51-1.51)	0.53 (0.32-0.98)	0.87 (0.58-2.04)	1.07 (0.78-2.16)	2.03 (1.34-3.62)	1.03 (0.90-2.17)	0.83 (0.54-1.33)	1.38 (0.81-2.19)
>10 000 (n=13)	0.52 (0.38-0.74)	0.82 (0.75-1.24)	0.61 (0.40-0.78)	0.77 (0.59-1.03)	0.86 (0.67-1.35)	1.32 (0.59-2.19)	0.78 (0.50-1.12)	0.55 (0.48-0.88)	1.03 (0.52-2.13)
p-value[2]	0.969	0.464	0.185	0.389	0.728	0.403	0.232	0.139	0.479

[1]comparing median cord to maternal ratios in mothers with CD4+ T-lymphocyte counts <200 and CD4+ T-lymphocyte counts >500 using Mann-Whitney test;
[2]comparing median cord to maternal ratios in mothers with viral load <40 and viral load >10 000 using Mann-Whitney test.

4.2 Discussion

The findings from our study suggest that the possible mechanisms for the increased susceptibility to invasive GBS disease in HIV-exposed infants may relate to lower maternal capsular and surface-protein antibody concentrations, and inefficient transplacental transfer of capsular antibody to the foetus of HIV-infected women. HIV-infected women had lower GBS capsular antibody concentrations than their HIV-uninfected counterparts, and notably a lower proportion of HIV-infected women had capsular antibodies above the putative "protective" thresholds that has been reported to protect against invasive GBS disease in their infants (Chapter 1.10.1). The lower GBS antibody concentrations in HIV-infected women could represent waning of natural acquired antibody or reduced humoral immune responsiveness to recto-vaginal colonization which likely induces the antibody responses (Kwatra et al., 2015). Additionally, reduced maternal exposure to GBS may also result in lesser antibody production to various serotype-specific epitopes (Dangor et al., 2015). This is supported by some studies which reported a lower prevalence of GBS colonization in HIV-infected women, including previously in our setting (Gray et al., 2011, Cutland et al., 2012); although this was not observed in the current study cohort.

The transplacental transfer of antibodies to serotypes Ia and III, which account for the majority (72%) of invasive GBS disease globally (Edmond et al., 2012), was 37.4% and 32.5% lower in HIV-exposed compared to HIV-unexposed newborns. Additionally, maternal capsular antibody concentrations were lower in HIV-infected women compared to HIV-uninfected women for serotypes Ib and V, with a trend towards being lower for serotype Ia, but not for serotype III. Serotype III, which has the highest invasive potential, is the least immunogenic of

all serotypes (Davies et al., 2001, Madzivhandila et al., 2011) and this may explain why concentrations were similar in HIV-infected and HIV-uninfected women. Furthermore, the trend toward higher colonization prevalence of serotype III in HIV-infected compared to HIV-uninfected women in our study may have contributed to similar serotype III antibody concentrations between the women (Table 4.1).

We also measured antibody concentrations to select GBS surface-proteins which induce antibody responses and could be possible vaccine epitopes. There is, however, a paucity of data on these GBS surface-protein antibody concentrations and no international reference standards exist. Thus, we can only report on the comparisons using in-house reference serum employed consistently across all samples. HIV-infected women had lower median concentrations for all GBS surface-proteins, although antibody differences to BibA were not significant. In addition, we observed that contrary to the capsular antibody transfer, the transfer of surface-protein antibodies from mother to foetus was more efficient, and similar between HIV-infected and HIV-uninfected mother-newborn dyads. This may occur because surface-protein antibodies, which are mainly subclass IgG1, are more efficiently transferred than capsular antibodies, which are predominantly of subclass IgG2 (Palmeira et al., 2012).

Our results are consistent with reports showing reduced transplacental transfer of maternal antibodies directed against epitopes of varicella (31% reduction), measles (35% reduction), pneumococcus (24-30% reduction), *Haemophilus influenzae* type b (23% reduction), pertussis (40% reduction) and tetanus (27-52% reduction) in HIV-infected compared to HIV-uninfected mother-newborn dyads (de Moraes-Pinto et al., 1996, Scott et al., 2005, Cumberland et al.,

2007, Jones et al., 2011, Gupta et al., 2014). However, no difference in transplacental antibody transfer between HIV-infected and HIV-uninfected women for pathogens such as herpes, some pneumococcal serotypes and influenza have also been reported (de Moraes-Pinto et al., 1996, Gupta et al., 2014, Madhi et al., 2014). Transplacental IgG antibody transfer is thought to occur via an active transport mechanism utilizing neonatal Fc receptors found on the placenta (Leach et al., 1996, Kruczek et al., 2010, Palmeira et al., 2012). The decrease in transplacental antibody transfer in HIV-infected women is thought to be as a consequence of maternal hyper-gammaglobulinaemia which saturates the neonatal Fc receptors (de Moraes-Pinto et al., 1999). Other reasons for the variation in transplacental antibody transfer may relate to differences in IgG subclass and mechanism of transfer of antibody (i.e. active or passive transport) (Palmeira et al., 2012).

Although our study did not identify a significant association between CD4+ T-lymphocyte counts and HIV-1 viral loads on maternal antibody and cord to maternal ratios among HIV-infected women, the study was not powered (with a sample size of 79) to detect a significant relationship when the true correlation is between -0.35 and 0.35. Similarly, no association has been observed between maternal CD4+ T-lymphocyte counts and transplacental transfer of pneumococcal, *Haemophilus influenzae* type b, pertussis and tetanus antibodies in HIV-infected women (Jones et al., 2011, Gupta et al., 2014), whereas a positive correlation with CD4+ T-lymphocyte counts and maternal antibody concentrations was reported to antibodies to pertussis, pneumococcus and tetanus (Jones et al., 2011). More recently, a large European cohort study reported an increased risk of bacterial infections in HIV-exposed infants, particularly if born to women with low CD4+ T-lymphocyte counts (Taron-Brocard et al.,

2014). Most pregnant women in our setting had undetectable HIV-1 viral load and had immune reconstituted at the time of antibody sampling. A study conducted in Nairobi in HIV-infected women reported a 44% decrease of measles antibody transfer with every $\log_{10}$ increase in HIV-1 viral load, indicating that infants born to women with advanced maternal HIV-infection may be at increased risk of disease due to reduced acquisition of maternal antibody concentrations (Farquhar et al., 2005).

Limitations of our study include that we did not match for age and colonization status in HIV-infected and HIV-uninfected women, however, we adjusted for these factors in the multivariate analysis and findings remained consistent. Furthermore, we did not quantify the effect of cross reactivity of serotype Ib with Ia, as previously documented by Brigsten et al. (Brigtsen et al., 2002), may have had on the absolute antibody concentration for serotype Ib. The assay was, however, applied consistently to both HIV-infected and HIV-uninfected dyads and hence is unlikely to alter the differences observed between HIV-infected and HIV-uninfected women in our study. Also, our study only measured IgG antibodies, whilst IgA antibodies may also be transplacentally transferred; and have been associated with protection against invasive GBS disease in animal model studies (Shen et al., 2000, Meinke et al., 2010). Additionally, CD4+ T-lymphocyte counts were measured as part of standard-of-care at any time within 6 months (mean: 2.8 months) of delivery and the study was not specifically powered to address whether immunological status or different HIV-1 viral load were associated with differences in maternal antibody or transplacental antibody transfer.

The lower GBS antibody concentrations and reduced transplacental antibody transfer in HIV-infected women, which places their infants at risk for invasive GBS disease, may be mitigated by maternal GBS vaccination. An investigational trivalent GBS polysaccharide-protein conjugate vaccine was however reported to be less immunogenic in HIV-infected than HIV-uninfected pregnant women (Heyderman et al., 2014). Therefore, in HIV-burden settings, maternal vaccination may require modified formulations or dosing schedules in HIV-infected women.

5.0 Association between capsular antibody concentrations and invasive Group B *Streptococcus* (GBS) disease in South African infants

The burden of invasive GBS disease in young infants is highest in low-middle income countries where an alternative to IAP for GBS-colonized pregnant women is required (Dagnew et al., 2012, Edmond et al., 2012). Vaccinating women during pregnancy with tetanus, influenza and pertussis vaccines, which increases the transplacental transfer of protective antibodies to the newborns, prevents illness during early infancy (Steinhoff, 2013, Amirthalingam et al., 2014, Madhi et al., 2014) Similarly, maternal GBS vaccination could prevent invasive GBS disease in young infants. The identification of serological correlates of protection against invasive GBS disease could expedite the licensure of GBS vaccines, without needing to undertake large efficacy trials (Madhi et al., 2013). Although maternal serotype-specific capsular antibody levels are associated with protection against invasive GBS disease in high-income settings (Chapter 1.10.1) , this association has not been assessed in low-middle income countries. In this chapter, the association between naturally acquired serotype Ia and III GBS capsular antibody levels and invasive GBS disease in infants born at ≥34 weeks gestational age in a low-middle income setting was investigated (the paper is in press, *Vaccine* 2015).

5.1 Results

5.1.1 Participant selection and demographic characteristics

Over a twelve month period, 122 (66 EOD and 56 LOD) infants were diagnosed with invasive GBS disease. The clinical characteristics of these infants are reported in chapter 3.1 Sixty-three (51.6%) cases were excluded from the analysis: 30 (24.6%) infants were <34 weeks gestation, blood samples were unavailable for 15 cases and only obtained >72 hours in 2

infants after confirmation of disease, 13 infants had disease caused by serotypes other than Ia or III, 2 infants were diagnosed by a positive CSF latex agglutination test, and the GBS isolate was not retrieved for one infant (Figure 5.1). Of 544 controls, 57 infants were excluded (50 were infants <34 weeks gestation and suitable matched controls were unavailable for a further 7). Maternal GBS colonization was confirmed in 135 (27.7%) of the remaining 487 controls, of whom 53 (39.3%) were colonized with serotype Ia and 39 (28.9%) with serotype III.

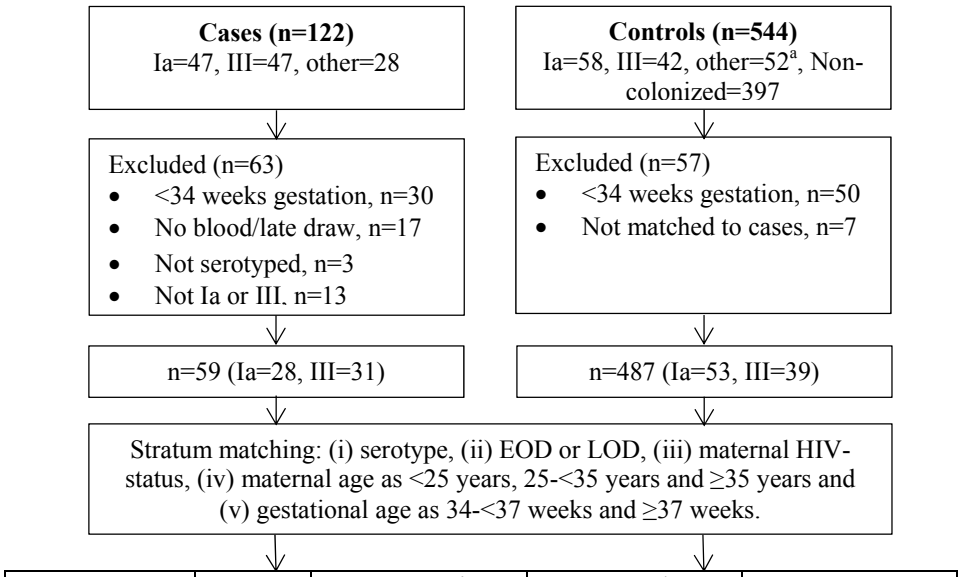
Final antibody comparisons, after strata-matching cases and controls, was conducted on 27 (EOD-15, LOD-12) mother-infant pairs for serotype Ia and 29 (EOD-7, LOD-22) for serotype III. These were matched to 43 homotypic and 360 non-homotypic (65 colonized with other serotypes and 295 non-colonized) controls for serotype Ia, and on 31 homotypic and 351 non-homotypic (75 colonized with other serotypes and 276 non-colonized) controls for serotype III (Figure 5.1).

5.1.2 Comparison of cases to homotypic controls

Cases and homotypic controls had similar maternal and infant demographic characteristics, as well as risk factors for invasive GBS disease such as parity, race/ethnicity, the risk of prolonged rupture of membranes and the use of intrapartum antibiotic prophylaxis (Table 5.1).

The median maternal and infant serotype Ia antibody concentrations (in μg/mL) were 0.05 (IQR: 0.02-0.24) and 0.01 (IQR: 0.01-0.07) in cases and 0.29 (IQR: 0.06-1.60) and 0.19 (IQR: 0.05-1.54) in homotypic controls, respectively (Figures 5.2, Tables 5.2 and 5.3). The median maternal and infant serotype III antibody concentrations (in μg/mL) were

0.14 (IQR: 0.08-0.33) and 0.04 (IQR: 0.02-0.08) in cases, and 0.29 (IQR: 0.13-0.58) and 0.15 (IQR: 0.06-0.44) in homotypic controls, respectively (Figures 5.2, Tables 5.2 and 5.3). Stratified by EOD and LOD, serotype Ia and III maternal and infant antibody concentrations were lower in cases compared to homotypic controls (Tables 5.2 and 5.3). Maternal homotypic controls matched to LOD cases had similar antibody concentrations as homotypic EOD controls enrolled at birth (p=0.958)



Serotype	Cases	Homotypic colonized controls	Controls colonized with other serotypes	Non-colonized controls
Ia	27	43	65	295
III	29	31	75	276

Figure 5.1: Diagrammatic representation of participant enrolment and exclusion

Footnote: [*]Five mothers of controls were colonized by more than 1 serotype. .

Table 5.1: Demographic characteristics and invasive Group B *Streptococcus* (GBS) disease risk factors in matched cases and homotypic controls ≥34 weeks of age

	Serotype Ia			**Serotype III**		
	Cases n=27 [EOD[1]=15, LOD[2]=12]	Controls n=43 [EOD=30, LOD=13]	p-value[3]	Cases n=29 [EOD=7, LOD=22]	Controls n=31 [EOD=16, LOD=15]	p-value
Maternal						
HIV-infected	9 (33.3)	13 (30.2)	0.786	17 (58.6)	16 (51.6)	0.586
HIV-uninfected	18 (66.7)	30 (69.7)		12 (41.4)	15 (48.4)	
Median age in years (IQR[4])	23.9 (19.7-30.0)	24.2 (21.4-29.0)	0.286	26.8 (24.4-31.3)	25.1 (21.4-31.7)	0.510
Median parity (IQR)	0 (0-1)	1 (0-2)	0.155	1 (1-2)	1 (0-2)	0.227
Black-African Race	25 (92.6)	42 (97.7)	0.555	29 (100)	30 (96.8)	0.999
Fever	0/21 (0)	0/41 (0)	0.999	0/17 (0)	0/29 (0)	0.999
PROM[5] (>18 hours)	4/24 (16.7)	3/41 (7.3)	0.409	1/21 (4.8)	3/29 (10.3)	0.630
IAP[6]	3/27 (11.1)	3/42 (7.1)	0.672	0/29 (0)	3/30 (10.0)	0.237
Infant						
Median gestation in weeks (IQR)	39.0 (37.0-40.0)	39.3 (38.0-40.3)	0.562	40.0 (40.0-40.2)	39.2 (38.0-40.4)	0.050
Median birth weight in grams (IQR)	2925 (2720-3275)	3035 (2830-3450)	0.372	3110 (2800-3320)	3095 (2740-3520)	0.636
Male gender	16 (59.3)	22 (51.2)	0.508	15 (51.7)	17 (54.8)	0.809
Day of life at enrolment						
EOD-Median (IQR)	4 (3-5)	1 (1-1)	<0.001	3 (3-5)	1 (1-1)	<0.001
LOD-Median (IQR)	20 (15-29)	20 (16-22)	0.870	17 (11-23)	24 (14-27)	0.193

[1]EOD- Early-onset disease, [2]LOD- Late-onset disease, [3]p-value- using Chi-squared, Fisher exact or Mann-Whitney test, [4]IQR-Interquartile range, [5]PROM- prolonged rupture of membranes, [6]IAP-Intrapartum antibiotic prophylaxis.

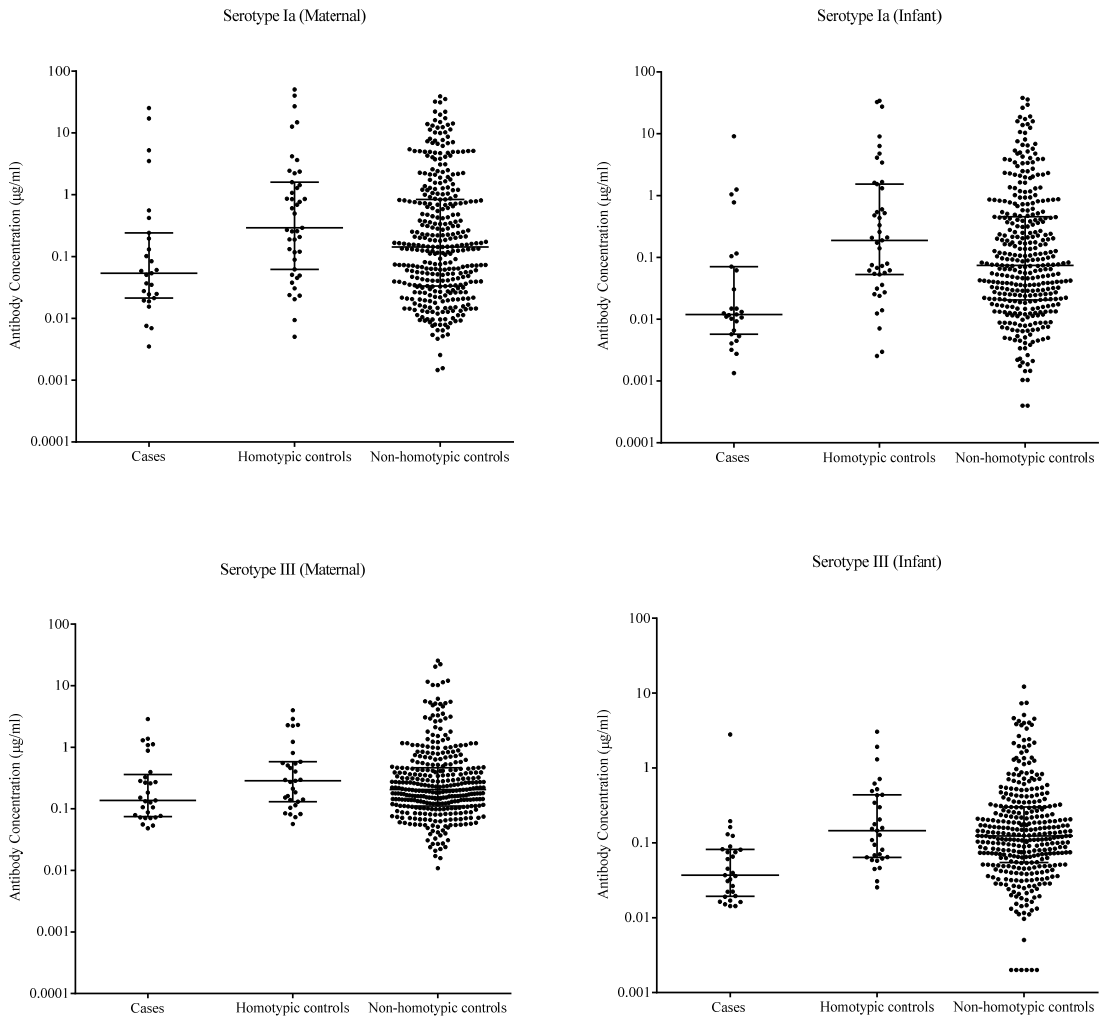


Figure 5.2: Scatter plots comparing maternal serotype Ia, infant serotype Ia, maternal serotype III and infant serotype III antibody concentrations between cases, homotypic controls and non-homotypic controls

Footnote: The centre line represents the median and the upper and lower whiskers represent the 75th and 25th quartile, respectively. y-axis is $\log_{10}$ scale.

Table 5.2: Maternal antibody concentrations (μg/mL) of cases and controls ≥34 weeks of age

	Cases[1]	Homotypic controls[2]	Non-homotypic controls[3]
	Median(IQR)[4] [n=][5]	Median(IQR) [n=]	Median(IQR) [n=]
Serotype Ia			
Overall	0.05 (0.02-0.24) [n=27]	0.29 (0.06-1.60) [n=43]	0.14 (0.03-0.84) [n=360]
EOD[6]	0.06 (0.03-0.42) [n=15]	0.28 (0.09-2.23) [n=30]	0.13 (0.03-0.81) [n=235]
LOD[7]	0.04 (0.02-0.14) [n=12]	0.50 (0.06-1.43) [n=13]	0.15 (0.03-0.92) [n=125]
HIV-infected	0.06 (0.03-0.20) [n=9]	0.12 (0.06-0.50) [n=13]	0.14 (0.04-0.83) [n=164]
HIV-uninfected	0.05 (0.02-0.24) [n=18]	0.77 (0.13-2.44) [n=30]	0.13 (0.03-0.94) [n=196]
Serotype III			
Overall	0.14 (0.08-0.33) [n=29]	0.29 (0.13-0.58) [n=31]	0.21 (0.11-0.47) [n=351]
EOD	0.13 (0.06-1.38) [n=7]	0.25 (0.09-0.91) [n=16]	0.19 (0.11-0.47) [n=198]
LOD	0.14 (0.08-0.28) [n=22]	0.30 (0.14-0.55) [n=15]	0.23 (0.12-0.49) [n=153]
HIV-infected	0.11 (0.07-0.26) [n=17]	0.30 (0.15-0.55) [n=16]	0.22 (0.11-0.54) [n=148]
HIV-uninfected	0.27 (0.11-0.60) [n=12]	0.21 (0.11-2.24) [n=15]	0.20 (0.11-0.46) [n=203]

[1]Mother of infants with invasive GBS disease, [2]Mother was colonized with the same serotype that caused the disease in cases, [3]Mother was either non-colonized or colonized with GBS serotypes that did not cause disease in cases, [4]Median (interquartile range), [5]Number of cases, [6]EOD- Early-onset disease, [7]LOD- Late-onset disease.

Table 5.3: Infant antibody concentrations (μg/mL) of cases and controls ≥34 weeks of age

	Cases[1]	Homotypic controls[2]	Non-homotypic controls[3]
	Median(IQR)[4] [n=][5]	Median(IQR) [n=]	Median(IQR) [n=]
Serotype Ia			
Overall	0.01 (0.01-0.07) [n=27]	0.19 (0.05-1.54) [n=43]	0.08 (0.02-0.45) [n=360]
EOD[6]	0.02 (0.01-0.12) [n=15]	0.21 (0.05-1.66) [n=30]	0.09 (0.02-0.61) [n=235]
LOD[7]	0.01 (0.01-0.01) [n=12]	0.06 (0.04-0.43) [n=13]	0.05 (0.01-0.25) [n=125]
HIV-infected	0.01 (0.01-0.01) [n=9]	0.06 (0.03-0.08) [n=13]	0.06 (0.02-0.30) [n=164]
HIV-uninfected	0.02 (0.01-0.07) [n=18]	0.37 (0.07-3.43) [n=30]	0.09 (0.02-0.60) [n=196]
Serotype III			
Overall	0.04 (0.02-0.08) [n=29]	0.15 (0.06-0.44) [n=31]	0.12 (0.05-0.30) [n=351]
EOD	0.08 (0.03-0.16) [n=7]	0.24 (0.11-0.56) [n=16]	0.16 (0.08-0.42) [n=198]
LOD	0.03 (0.02-0.08) [n=22]	0.07 (0.06-0.21) [n=15]	0.07 (0.03-0.17) [n=153]
HIV-infected	0.03 (0.02-0.05) [n=17]	0.08 (0.06-0.32) [n=16]	0.10 (0.04-0.23) [n=148]
HIV-uninfected	0.07 (0.04-0.09) [n=12]	0.18 (0.09-0.52) [n=15]	0.14 (0.07-0.35) [n=203]

[1]Infants with invasive GBS disease, [2]Infants in which the mother was colonized with the same serotype that caused the disease in cases, [3]Infants in which the mother was either non-colonized or colonized with GBS serotypes that did not cause disease in cases, [4]Median (interquartile range), [5]Number of cases, [6]EOD- Early-onset disease, [7]LOD- Late-onset disease.

A lower proportion of mothers of cases (5/27; 18.5%) as compared to mothers of homotypic controls (20/43; 46.5%) had a serotype Ia antibody concentration ≥0.5 µg/mL (Figure 5.3, Table 5.4). The adjusted odds ratio was 0.18 (95% CI: 0.04-0.73) in cases compared to homotypic controls when the maternal antibody concentration was ≥0.5 µg/mL (Table 5.4). A maternal serotype III antibody concentration ≥0.5 µg/mL was also less prevalent but not significant in cases (20.7%) than homotypic controls (35.5%) with an adjusted odds ratio of 0.27 (95% CI: 0.05-1.56; Figure 5.3, Table 5.4). Similarly, there was a lower proportion of serotype Ia and III antibody concentration ≥0.5 µg/mL in infants with invasive GBS disease than homotypic controls (Table 5.4).

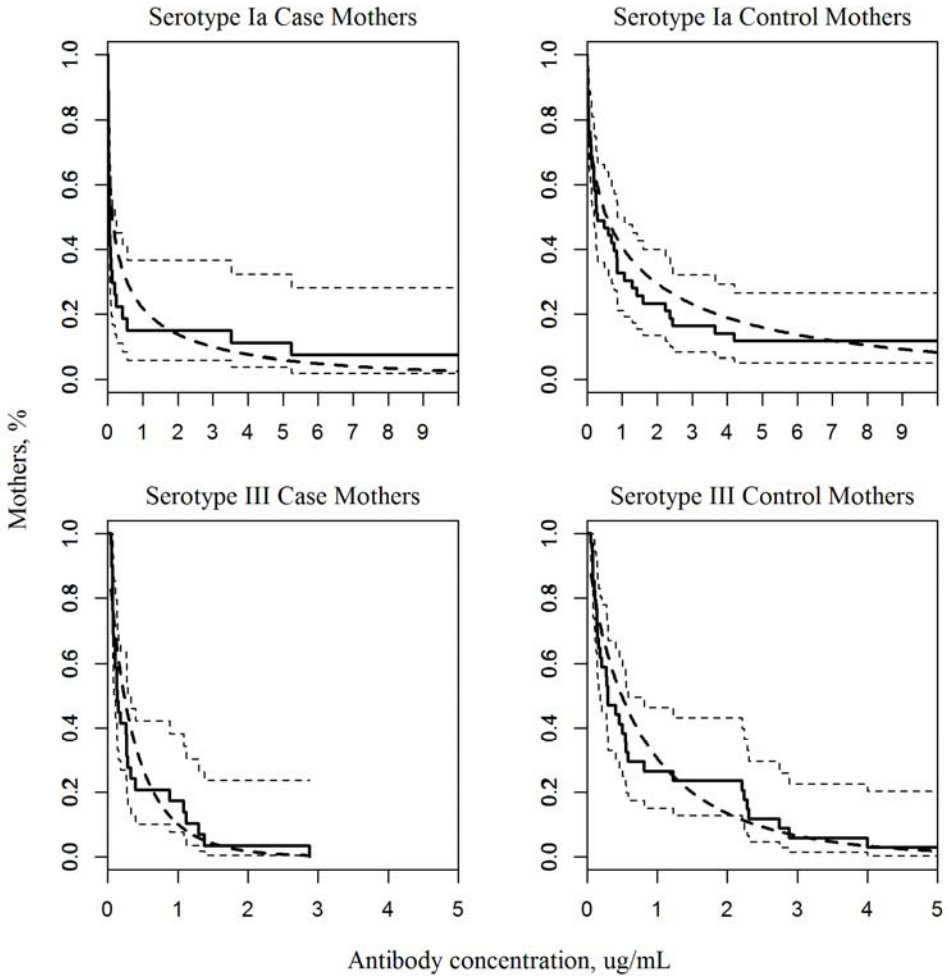


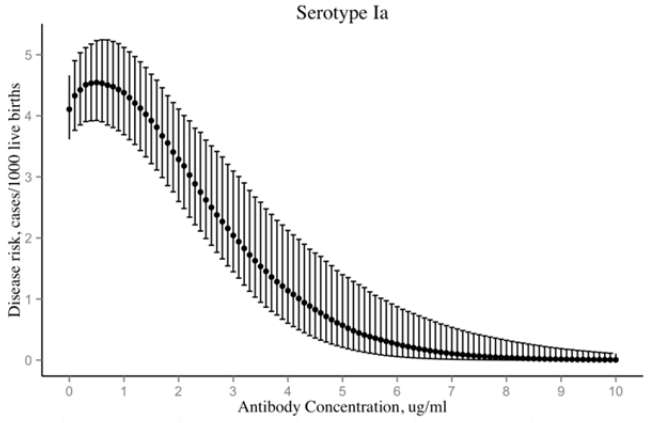
Figure 5.3: Reverse cumulative plots demonstrating the proportion of mothers of cases and homotypic controls at antibody thresholds for serotypes Ia and III.
Footnote: The solid line represents the proportion of mothers at various antibody thresholds. The upper and lower dash lines are the 95% confidence intervals. The bold dash line is the estimated fitted line.

Table 5.4: Comparing antibody (µg/mL) thresholds between matched cases and homotypic controls

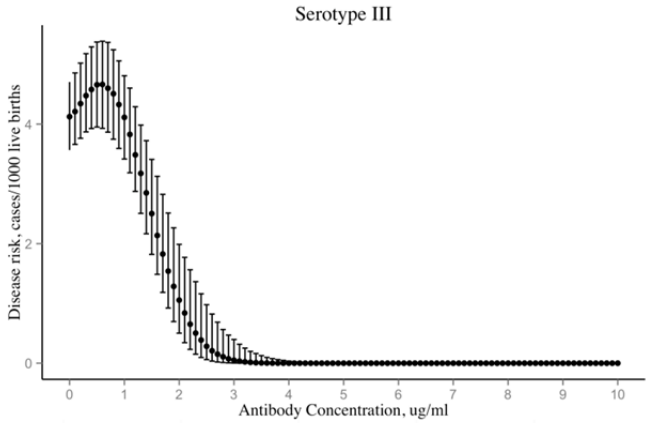
	Cases[1]	Homotypic controls[2]	OR (95% CI)[3]	p-value	aOR (95% CI)[4]	p-value
Serotype Ia	**n=27 (%)**	**n=43 (%)**				
Maternal						
<0.1	17 (63.0)	12 (27.9)				
0.1-<0.5	5 (18.5)	11 (25.6)	0.31 (0.07-1.42)	0.131	0.26 (0.05-1.32)	0.104
≥0.5	5 (18.5)	20 (46.5)	0.18 (0.05-0.68)	0.011	0.18 (0.04-0.73)	0.017
Infant						
<0.1	21 (77.8)	19 (44.2)				
0.1-<0.5	2 (7.4)	9 (20.9)	0.20 (0.04-1.15)	0.072	0.28 (0.04-1.78)	0.176
≥0.5	4 (14.8)	15 (34.9)	0.20 (0.05-0.90)	0.036	0.18 (0.04-0.85)	0.031
Serotype III	**n=29 (%)**	**n=31 (%)**				
Maternal						
<0.1	10 (34.5)	5 (16.1)				
0.1-<0.5	13 (44.8)	15 (48.4)	0.29 (0.05-1.72)	0.174	0.50 (0.07-3.29)	0.467
≥0.5	6 (20.7)	11 (35.5)	0.21 (0.04-1.16)	0.073	0.27 (0.05-1.56)	0.145
Infant						
<0.1	24 (82.8)	13 (41.9)				
0.1-<0.5	4 (13.8)	12 (38.7)	0.38 (0.10-1.47)	0.160	0.44 (0.10-2.02)	0.293
≥0.5	1 (3.4)	6 (19.4)	0.16 (0.02-1.47)	0.105	0.14 (0.02-1.38)	0.093

[1]Infants with invasive GBS disease, [2]healthy infants in which the mother was colonized with the same serotype that caused the disease in cases, [3]Calculated Odds ratio with 95% confidence using conditional logistic regression, [4]Adjusted odds ratio with 95% confidence using conditional logistic regression (serotype Ia: adjusted for parity and day of life at enrolment; serotype III: adjusted for gestational age and day of life at enrolment)

As a significant proportion (approximately 20%) of cases had a maternal antibody concentration ≥0.5 µg/mL and developed serotype Ia and III disease, we evaluated for a sero-correlate threshold of protection using a Bayesian framework. The risk of invasive GBS disease per 1,000 live births decreased with increasing antibody concentrations. The Bayesian model demonstrated an estimated 50%, 70% and >90% reduction in risk of invasive GBS disease with maternal antibody concentrations ≥3 µg/mL, ≥4 µg/mL and ≥6 µg/mL for serotype Ia (Figure 5.4 A), and an estimated 75% and >90% risk reduction with maternal antibody concentrations ≥2 µg/mL and ≥3 µg/mL for serotype III (Figure 5.4 B).



(A)

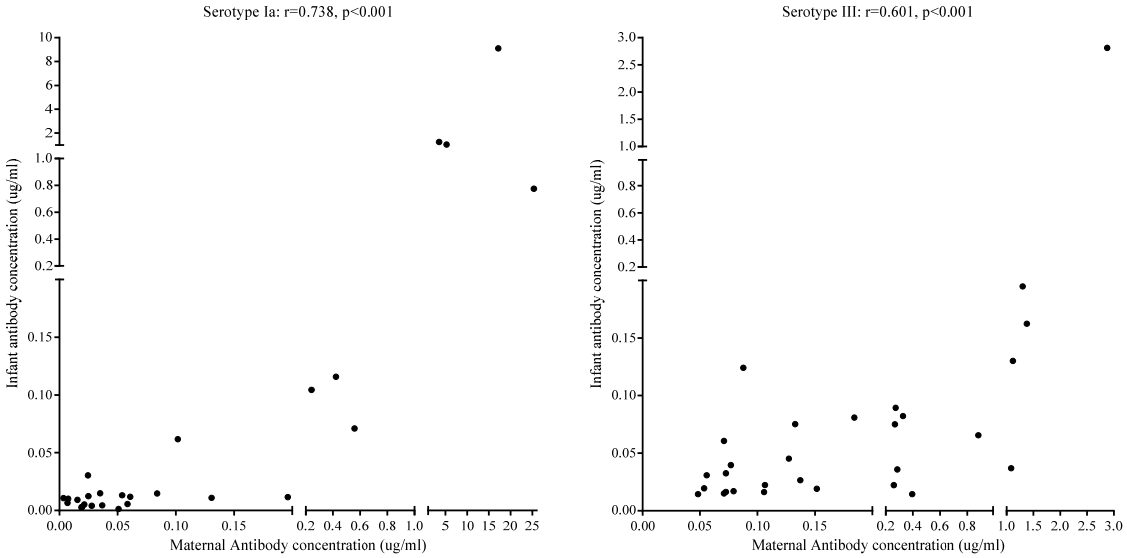


(B)

Figure 5.4: Probability of invasive Group B *Streptococcus* (GBS) disease risk to serotype Ia (A) and serotype III (B) at varying maternal antibody concentrations using a Bayesian model

Footnote: The circles represent the posterior mode (i.e. the most likely value) and vertical lines represent the 50% credible interval.

Maternal and infant antibody concentrations correlated well in cases for serotypes Ia and III (Figure 5.5). In 3/27 (11.1%) infants that developed serotype Ia disease, however, their respective mothers had antibody concentrations above 5 μg/mL (Figure 5.5 A). None of the serotype III mothers of cases had an antibody concentration ≥3 μg/mL (Figure 5.5 B).



(A) (B)

Figure 5.5: Correlation between maternal and infant serotype Ia (A) and III (B) antibody concentrations in infants that developed invasive Group B *Streptococcus* (GBS) disease
Footnote: The Spearman's test was used to measure the correlation between maternal and infant antibody concentrations in infants with disease.

The infant to maternal serotype Ia antibody ratio was significantly lower in cases (0.43; IQR: 0.20-0.61) with EOD as compared to homotypic controls (0.71; IQR: 0.59-1.14; p=0.012; Table 5.5). The infant: maternal serotype III antibody ratio was not significantly lower in cases with EOD (0.55; IQR: 0.12-0.98) as compared to homotypic controls (0.90; IQR: 0.58-1.17; p=0.071; Table 5.5).

Table 5.5: Infant to maternal antibody ratio of early-onset mother-newborn pairs

	Median (IQR)[1] infant/cord to maternal ratio	p-value[2]
Serotype Ia		
Cases (n=15)	0.43 (0.20-0.61)	
Homotypic controls (n=30)	0.71 (0.59-1.14)	0.012
Non-homotypic controls (n=235)	0.67 (0.45-1.00)	0.013
Serotype III		
Cases (n=7)	0.55 (0.12-0.98)	
Homotypic controls (n=16)	0.90 (0.58-1.17)	0.071
Non-homotypic controls (n=198)	0.79 (0.53-1.18)	0.091

[1]Median (interquartile range), [2]p-value- comparing cases with homotypic and non-homotypic controls using Mann-Whitney test.

5.1.3 Comparison of cases to non-homotypic controls

Cases and non-homotypic controls had similar demographic characteristics and infant risk factors for disease (Table 5.6). Maternal and infant serotype Ia and III antibody concentrations were similar between heterotypic and non-colonized controls (data not shown). Non-homotypic controls had lower median maternal and infant antibody concentrations than homotypic controls (Tables 5.2 and 5.3).The median maternal and infant serotype Ia antibody concentrations (in µg/mL) was 0.14 (IQR: 0.03-0.84) and 0.08 (IQR: 0.02-0.45) in non-homotypic controls (Figures 5.2, Tables 5.2 and 5.3). The median maternal and infant serotype III antibody concentrations was 0.21 (IQR: 0.11-0.47) and 0.12 (IQR: 0.05-0.30) in non-homotypic controls (Figures 5.2, Tables 5.2 and 5.3). As with homotypic controls, a higher proportion of non-homotypic controls, as compared to cases, had maternal and infant antibody concentrations ≥0.5 µg/mL (Table 5.7).

Table 5.6: Demographic characteristics and disease risk factors in matched cases and non-homotypic controls ≥34 weeks

	Serotype Ia			Serotype III		
	Cases n=27 [EOD[1]=15, LOD[2]=12]	Controls n=360 [EOD=235, LOD=125]	p-value[3]	Cases n=29 [EOD=7, LOD=22]	Controls n=351 [EOD=198, LOD=153]	p-value
Maternal						
HIV-infected	9 (33.3)	164 (45.6)	0.218	17 (58.6)	148 (42.2)	0.086
HIV-uninfected	18 (66.7)	196 (54.4)		12 (41.4)	203 (57.8)	
Median age in years (IQR[4])	23.9 (19.7-30.0)	24.9 (21.3-30.4)	0.117	26.8 (24.4-31.3)	26.3 (22.3-30.5)	0.207
Median parity (IQR)	0 (0-1)	1 (0-2)	0.100	1 (1-2)	1 (0-2)	0.084
Black-African Race	25 (92.6)	354 (98.3)	0.101	29 (100)	346 (98.6)	0.999
Fever	0/21 (0)	0/339 (0)	0.999	0/17 (0)	0/328 (0)	0.999
PROM[5] (>18 hours)	4/24 (16.7)	40/347 (11.5)	0.508	1/21 (4.8)	30/337 (8.9)	0.999
IAP[6]	3/27 (11.1)	44/354 (12.4)	0.999	0/29 (0)	32/345 (9.3)	0.156
Infant						
Median gestation in weeks (IQR)	39.0 (37.0-40.0)	39.4 (38.0-40.3)	0.214	40.0 (40.0-40.2)	39.5 (38.1-40.3)	0.062
Median birth weight in grams (IQR)	2925 (2720-3275)	3065 (2805-3363)	0.274	3110 (2800-3320)	3110 (2860-3375)	0.677
Male gender	16 (59.3)	187 (51.9)	0.463	15 (51.7)	179 (51.0)	0.940
Day of life at enrolment						
EOD-Median (IQR)	4 (3-5)	1 (1-1)	<0.001	3 (3-5)	1 (1-1)	<0.001
LOD-Median (IQR)	20 (15-29)	19 (14-24)	0.734	17 (11-23)	19 (15-24)	0.210

[1]EOD- Early-onset disease, [2]LOD- Late-onset disease, [3]p-value- using Chi-squared, Fisher exact or Mann-Whitney test, [4]IQR- Interquartile range, [5]PROM- prolonged rupture of membranes, [6]IAP-Intrapartum antibiotic prophylaxis.

Table 5.7: Comparing antibody (μg/mL) thresholds between matched cases and non-homotypic controls

	Cases[1]	Non-homotypic[2] controls	OR (95% CI)[3]	p-value	aOR (95% CI)[4]	p-value
Serotype Ia	**n=27 (%)**	**n=360 (%)**				
Maternal						
<0.1	17 (63.0)	159 (44.2)				
0.1-<0.5	5 (18.5)	88 (24.4)	0.64 (0.22-1.83)	0.403	0.70 (0.24-2.02)	0.504
≥0.5	5 (18.5)	113 (31.4)	0.49 (0.17-1.37)	0.175	0.52 (0.18-1.51)	0.227
Infant						
<0.1	21 (77.8)	200 (55.6)				
0.1-<0.5	2 (7.4)	75 (20.8)	0.28 (0.06-1.23)	0.092	0.31 (0.07-1.40)	0.129
≥0.5	4 (14.8)	85 (23.6)	0.53 (0.18-1.61)	0.263	0.64 (0.20-2.02)	0.448
Serotype III	**n=29 (%)**	**n=351 (%)**				
Maternal						
<0.1	10 (34.5)	74 (21.1)				
0.1-<0.5	13 (44.8)	193 (55.0)	0.47 (0.19-1.14)	0.096	0.50 (0.20-1.24)	0.137
≥0.5	6 (20.7)	84 (23.9)	0.52 (0.18-1.51)	0.227	0.43 (0.14-1.29)	0.132
Infant						
<0.1	24 (82.8)	149 (42.5)				
0.1-<0.5	4 (13.8)	143 (40.7)	0.26 (0.08-0.78)	0.017	0.23 (0.07-0.70)	0.010
≥0.5	1 (3.4)	59 (16.8)	0.13 (0.02-1.06)	0.057	0.10 (0.01-0.84)	0.034

[1]Infants with invasive GBS disease, [2]healthy infants in which the mothers were either non-colonized or colonized with GBS serotypes that did not cause disease in cases, [3]Calculated Odds ratio with 95% confidence using conditional logistic regression, [4]Adjusted odds ratio with 95% confidence using conditional logistic regression (serotype Ia: adjusted for maternal age, parity, black-African race and day of life at enrolment; serotype III: adjusted for maternal HIV-status, parity, intrapartum antibiotic prophylaxis, gestational age and day of life at enrolment)

5.2 Discussion

This study describes a positive association between low capsular antibody concentrations and invasive GBS disease in South African infants from a low-middle income setting. Mothers whose infants developed invasive serotype Ia or III GBS disease had reduced antibody concentrations compared to homotypic controls, and we demonstrated that increased maternal antibody concentrations were associated with a reduced risk of invasive serotype Ia or III disease in the infant. A maternal antibody threshold concentration ≥6 μg/mL and ≥3 μg/mL would provide a >90% reduction in the risk of serotype Ia and III disease.

Identifying a sero-correlate of protection by measuring naturally acquired maternal antibody concentrations in infants with invasive GBS disease as compared to healthy controls could facilitate licensure of the GBS polysaccharide-protein conjugate vaccine in which phase II trials have been concluded (Madhi et al., 2013). Using previous studies to identify a specific putative threshold of protection against serotype-specific invasive GBS disease is, however, difficult because of differences in assay methods, lack of standardized reference sera and differences in participant selection (Dangor et al., 2015). Baker et al. proposed that a maternal antibody concentration ≥1 μg/mL be used as a correlate of protection against invasive serotype Ia and III GBS disease in North American infants (Baker et al., 2014). However, Lin et al., using data derived from a much larger cohort study, suggested that maternal antibody threshold concentrations of ≥5 μg/mL and ≥10 μg/mL protect against serotype I and III disease respectively (Lin et al., 2001, Lin et al., 2004). It is likely that the threshold of protection exists between 1 μg/mL and 10 μg/mL, as supported by our study, and further studies using standardized antibody assays are likely warranted to further define a specific correlate of protection.

In addition to comparing cases with homotypic controls, where maternal colonization status induce antibody responses (Dangor et al., 2015), we specifically compared cases to non-homotypic controls. We did this analysis because, in our setting, we have previously reported variable associations between maternal capsular antibody levels and colonization status (Kwatra et al., 2015). Non-colonized controls may have been colonized with GBS previously which could have induced the higher median antibody concentrations and subsequently resulted in the loss of GBS carriage, or conversely lower antibody levels could have increased susceptibility to the acquisition of GBS colonization (Kwatra et al., 2015).

We report lower infant to maternal antibody ratios, mainly in cases with serotype Ia disease although a similar trend was observed in serotype III disease. This contrasts with a previous study that showed similar infant to maternal antibody ratios between cases and controls (Lin et al., 2001, Lin et al., 2004). Our findings of a lower infant to maternal ratio in cases might represent adsorption of maternal derived homotypic capsular antibody in the infant following the onset of invasive GBS disease.

Although we used a different assay method than in other studies, we used reference serum provided by Dr Carol J Baker to create standard antibody concentrations for our assay. We found lower median maternal antibody concentrations in homotypic controls in our setting (Ia-0.29, IQR: 0.06-1.60 and III-0.29, IQR: 0.13-0.58) compared to controls in the USA (Ia-1.83, IQR: 0.20-5.54 and III-1.64, IQR: 0.14-5.51) (Baker et al., 2014). The lower circulating antibody concentrations in colonized mothers of healthy infants may in part relate to the high prevalence of maternal HIV-infection in our setting.

One of the strengths of our study was that we closely matched cases and controls for variables known to influence infant antibody concentrations (Christensen et al., 1984, Anthony et al., 1994), but our study has some limitations. The association observed with maternal capsular antibody and risk for invasive GBS disease in their infants observed by us, whilst corroborating that of others (Lin et al., 2001, Baker et al., 2014), nevertheless needs to be interpreted in the context that rather than being the effector for protection, it could be a proxy marker of some other immune mediator of protection which is also transferred to the foetus and was not measured for in our study. Furthermore, we were unable to analyse EOD and LOD cases independently due to the relatively small numbers of cases and therefore focused the analysis on maternal antibody concentrations where levels were comparable for EOD and LOD. A further limitation is that antibody levels measured in the infant after confirmation of invasive GBS disease may have been low as a result of consumption during the immune response. These limitations may be overcome by conducting a large resource-intensive study, such as conducted by Lin et al. (Lin et al., 2001), where antibodies are measured at birth in all infants who are prospectively monitored for invasive GBS disease.

In conclusion, we show that maternal GBS capsular antibody levels are associated with protection against invasive GBS disease in infants in a low-middle income setting. Vaccine-induced antibody levels ≥6 μg/mL and ≥3 μg/mL, as measured by our assay, would likely protect the majority of infants against invasive GBS disease caused by serotypes Ia and III in our setting. This work may potentially be useful in the licensure pathway for GBS polysaccharide-protein conjugate vaccine.

6.0 Association between maternal Group B *Streptococcus* (GBS) surface-protein antibody concentrations and invasive disease in their infants

Correlates of protection for maternal GBS serotype-specific capsular antibody levels in protecting their young infants against invasive GBS disease has been proposed in a few studies (Chapter 1.10.1). A drawback of serotype-specific GBS polysaccharide-protein conjugate vaccine, e.g. the trivalent vaccine currently under development, is the possibility for replacement disease if vaccine formulations are limited to select serotypes, even though the majority (79%) of disease are currently caused by the selected serotypes (Edmond et al., 2012, Madhi et al., 2013). This could be overcome by targeting non-serotype-specific GBS epitopes that contribute to the virulence of organism, are genetically conserved between GBS strains, and are immunogenic. Such potential vaccine epitopes include immunogenic surface-proteins (Lindahl et al., 2005, Meinke et al., 2010). In this chapter, the association between maternal IgG antibodies to select GBS surface-proteins and invasive GBS disease in their infants was determined (the published paper is attached as Appendix 4).

6.1 Results

6.1.1 Participant selection and demographic characteristics

In infants born at ≥34 weeks gestational age, serum was available on 70 mother-infant pairs with invasive GBS disease and 487 controls. After stratum matching, the final FbsA and BibA paired analysis included 69 cases, 128 GBS colonized controls and 332 non-colonized controls. Risk factors for invasive GBS disease and demographic characteristics were similar between cases and matched controls, except for history of PROM during labour being more common in cases (19.3%) than matched colonized controls (4.9%, p=0.002), and infants with EOD being older (median: 3 days) at the time of enrolment than matched colonized and non-colonized controls (median: 1 day, p<0.001 for both; Table 6.1 and 6.2).

Table 6.1: Demographic characteristics of matched cases and **colonized** controls ≥34 weeks of age for FbsA and BibA

	FbsA/ BibA		
	Cases n=69 [EOD[1]=34, LOD[2]=35]	Controls n=128 [EOD=75, LOD=53]	p-value[3]
Maternal			
HIV-infected	29 (42.0)	54 (42.2)	0.983
HIV-uninfected	40 (58.0)	74 (57.8)	
Median age in years (IQR[4])	25.4 (21.7-30.4)	25.2 (22.7-30.9)	0.430
Median parity (IQR)	1 (0-2)	1 (0-2)	0.567
Black-African Race	66 (95.7)	126 (98.4)	0.346
Fever	0/50 (0)	0/118 (0)	0.999
PROM (>18 hours)[5]	11/57 (19.3)	6/123 (4.9)	0.002
IAP[6]	4/69 (5.8)	9/124 (7.3)	0.774
Infant			
Median gestation in weeks (IQR)	40.0 (38.3-40.3)	39.3 (38.0-40.4)	0.255
Median birth weight in grams (IQR)	2995 (2800-3250)	3085 (2800-3410)	0.257
Male gender	39 (56.5)	59 (46.1)	0.163
Day of life at enrolment			
EOD-Median (IQR)	4 (3-5)	1 (1-1)	<0.001
LOD-Median (IQR)	17 (12-25)	20 (15-24)	0.265

[1]EOD- Early-onset disease, [2]LOD- Late-onset disease, [3]p-value- using Chi-squared, Fischer exact or Wilcoxon rank-sum (Mann-Whitney) test, [4]IQR-Interquartile range, [5]Prolonged (>18 hours) rupture of membranes [6]IAP-Intrapartum antibiotic prophylaxis.

Table 6.2: Demographic characteristics of cases and **non-colonized** controls ≥34 weeks of age for FbsA and BibA

	FbsA/ BibA		
	Cases n=69 [EOD[1]=34, LOD[2]=35]	Controls n=332 [EOD=206, LOD=126]	p-value[3]
Maternal			
HIV-infected	29 (42.0)	142 (42.8)	0.908
HIV-uninfected	40 (58.0)	190 (57.4)	
Median age in years (IQR[4])	25.4 (21.7-30.4)	25.4 (21.7-30.3)	0.860
Median parity (IQR)	1 (0-2)	1 (0-2)	0.946
Black-African Race	66 (95.7)	327 (98.5)	0.143
Fever	0/50 (0)	0/315 (0)	0.999
PROM (>18 hours)[5]	11/57 (19.3)	39/321 (12.2)	0.142
IAP[6]	4/69 (5.8)	47/329 (14.3)	0.073
Infant			
Median gestation in weeks (IQR)	40.0 (38.3-40.3)	39.3 (38.0-40.2)	0.164
Median birth weight in grams (IQR)	2995 (2800-3250)	3063 (2790-3360)	0.426
Male gender	39 (56.5)	176 (53.0)	0.595
Day of life at enrolment			
EOD-Median (IQR)	4 (3-5)	1 (1-1)	<0.001
LOD-Median (IQR)	17 (12-25)	19 (15-24)	0.314

[1]EOD- Early-onset disease, [2]LOD- Late-onset disease, [3]p-value- using Chi-squared, Fischer exact or Wilcoxon rank-sum (Mann-Whitney) test, [4]IQR-Interquartile range, [5]Prolonged (>18 hours) rupture of membranes [6]IAP-Intrapartum antibiotic prophylaxis.

After strata matching, including specific pilus island matching, the final paired analysis was conducted on 29 invasive GBS cases with PI-1 containing strains and correspondingly 64 PI-1 colonized and 289 non-GBS colonized controls, 37 invasive GBS cases with PI-2a containing strains and correspondingly 77 PI-2a colonized and 319 non-colonized controls, and 29 invasive GBS cases with PI-2b containing strains and correspondingly 29 PI-2b colonized and 279 non-colonized controls. Maternal and infant demographic characteristics and risk factors for disease were similar between cases and PI-specific controls; apart from gestational age (40.2 vs 39.4 weeks, respectively; p=0.014) in PI-1, infant gender (64.9 vs 44.6% males; p=0.038) and the occurrence of prolonged rupture of membranes (21.9% vs 2.7%, p=0.003) in cases for PI-2a (Table 6.3). When comparing cases to non-colonized controls, gestational age differed for PI-1 and PI-2b (Table 6.4). The timing of enrolment for EOD cases differed (median: 3 or 4 days) compared to PI-specific controls (median: 1 day) and non-colonized controls (median: 1 day; Tables 6.3 and 6.4).

Table 6.3: Demographic characteristics of pilus-specific cases and **colonized** controls ≥34 weeks of age

	PI-1			PI-2a			PI-2b		
	Cases n=29 [EOD[1]=14, LOD[2]=15]	Controls n=64 [EOD=39, LOD=25]	p-value[3]	Cases n=37 [EOD=21, LOD=16]	Controls n=77 [EOD=48, LOD=29]	p-value[3]	Cases n=29 [EOD=11, LOD=18]	Controls n=29 [EOD=14, LOD=15]	p-value[3]
Maternal									
HIV-infected	12 (41.4)	23 (35.9)	0.616	14 (37.8)	31 (40.3)	0.804	14 (48.3)	14 (48.3)	0.999
HIV-uninfected	17 (58.6)	41 (64.1)		23 (62.2)	46 (59.7)		15 (51.7)	15 (51.7)	
Median age in years (IQR[4])	25.4 (22.4-31.5)	25.5 (22.1-31.7)	0.772	24.4 (20.9-30.0)	25.2 (22.6-30.5)	0.211	25.4 (22.7-30.3)	28.7 (22.8-31.2)	0.397
Median parity (IQR)	1 (0-2)	1 (0-1)	0.506	0 (0-1)	1 (0-2)	0.121	1 (1-2)	1 (0-2)	0.435
Black-African Race	29 (100.0)	63 (98.5)	0.999	35 (94.6)	76 (98.7)	0.246	29 (100.0)	28 (96.6)	0.999
Fever	0/21 (0)	0/62 (0)	0.999	0/28 (0)	0/73 (0)	0.999	0/19 (0)	0/29 (0)	0.999
PROM (>18 hours)[5]	4/24 (16.7)	3/63 (4.8)	0.088	7/32 (21.9)	2/74 (2.7)	0.003	3/22 (13.6)	2/29 (6.9)	0.641
IAP[6]	1/29 (3.5)	6/64 (9.4)	0.428	4/37 (10.8)	5/77 (6.5)	0.469	0/29 (0)	2/29 (6.9)	0.491
Infant									
Median gestation in weeks (IQR)	40.2 (40.0-40.6)	39.4 (38.1-40.4)	0.014	40.0 (38.0-40.2)	39.4 (38.0-40.3)	0.599	40.0 (40.0-40.6)	39.3 (38.2-40.4)	0.058
Median birth weight in grams (IQR)	3100 (2835-3200)	3123 (2805-3405)	0.438	2960 (2770-3270)	3090 (2860-3370)	0.185	3110 (2835-3210)	3150 (2760-3450)	0.367
Male gender	14 (48.3)	33 (51.6)	0.769	24 (64.9)	34 (44.6)	0.038	14 (48.3)	17 (58.6)	0.430
Day of life at enrolment									
EOD-Median (IQR)	3 (3-5)	1 (1-1)	<0.001	4 (3-5)	1 (1-1)	<0.001	3 (3-5)	1 (1-1)	<0.001
LOD-Median (IQR)	17 (11-27)	20 (15-24)	0.334	20 (13-24)	20 (16-23)	0.669	17 (10-27)	23 (13-24)	0.574

[1]EOD- Early-onset disease, [2]LOD- Late-onset disease, [3]p-value- using Chi-squared, Fischer exact or Wilcoxon rank-sum (Mann-Whitney) test, [4]IQR-Interquartile range, [5]Prolonged (>18 hours) rupture of membranes [6]IAP-Intrapartum antibiotic prophylaxis.

Table 6.4: Demographic characteristics of pilus-specific cases and **non-colonized** controls ≥34 weeks of age

	PI-1			PI-2a			PI-2b		
	Cases n=29 [EOD[1]=14, LOD[2]=15]	Controls n=289 [EOD=173, LOD=116]	p-value[3]	Cases n=37 [EOD=21, LOD=16]	Controls n=319 [EOD=194, LOD=125]	p-value[3]	Cases n=29 [EOD=11, LOD=18]	Controls n=279 [EOD=157, LOD=122]	p-value[3]
Maternal									
HIV-infected	12 (41.4)	114 (39.5)	0.839	14 (37.8)	133 (41.7)	0.652	14 (48.3)	114 (40.9)	0.441
HIV-uninfected	17 (58.6)	175 (60.6)		23 (62.2)	186 (58.3)		15 (51.7)	165 (59.1)	
Median age in years (IQR[4])	25.4 (22.4-31.5)	26.0 (21.6-30.3)	0.711	24.4 (20.9-30.0)	25.2 (21.5-29.7)	0.378	25.4 (22.7-30.3)	26.1 (21.6-30.3)	0.953
Median parity (IQR)	1 (0-2)	1 (0-2)	0.732	0 (0-1)	1 (0-2)	0.196	1 (1-2)	1 (0-2)	0.298
Black-African Race	29 (100.0)	284 (98.3)	0.999	35 (94.6)	314 (98.4)	0.158	29 (100.0)	274 (98.2)	0.999
Fever	0/21 (0)	0/272 (0)	0.999	0/28 (0)	0/302 (0)	0.999	0/19 (0)	0/262 (0)	0.999
PROM (>18 hours)[5]	4/24 (16.7)	34/279 (12.2)	0.520	7/32 (21.9)	39/308 (12.7)	0.147	3/22 (13.6)	30/269 (11.2)	0.725
IAP[6]	1/29 (3.5)	36/286 (12.6)	0.224	4/37 (10.8)	42/316 (13.3)	0.801	0/29 (0)	32/276 (11.6)	0.055
Infant									
Median gestation in weeks (IQR)	40.2 (40.0-40.6)	39.4 (38.0-40.3)	0.003	40.0 (38.0-40.2)	39.3 (38.0-40.2)	0.595	40.0 (40.0-40.6)	39.4 (38.1-40.3)	0.025
Median birth weight in grams (IQR)	3100 (2835-3200)	3095 (2845-3370)	0.605	2960 (2770-3270)	3085 (2835-3365)	0.280	3110 (2835-3210)	3105 (2850-3375)	0.579
Male gender	14 (48.3)	150 (51.9)	0.709	24 (64.9)	170 (53.3)	0.181	14 (48.3)	148 (53.1)	0.698
Day of life at enrolment									
EOD-Median (IQR)	3 (3-5)	1 (1-1)	<0.001	4 (3-5)	1 (1-1)	<0.001	3 (3-5)	1 (1-1)	<0.001
LOD-Median (IQR)	17 (11-27)	19 (15-24)	0.474	20 (13-24)	19 (15-24)	0.795	17 (10-27)	19 (15-24)	0.292

[1]EOD- Early-onset disease, [2]LOD- Late-onset disease, [3]p-value- using Chi-squared, Fischer exact or Wilcoxon rank-sum (Mann-Whitney) test, [4]IQR-Interquartile range, [5]Prolonged (>18 hours) rupture of membranes [6]IAP-Intrapartum antibiotic prophylaxis. (*one mother had twins with GBS, **Parity was unknown in 2 cases)

6.1.2 Antibody levels to FbsA

There was a larger proportion of colonized controls than cases at higher antibody thresholds; the adjusted odds ratio for disease decreased from 0.40 (95% CI: 0.16-1.04) to 0.22 (95% CI: 0.05-0.02) and 0.04 (95% CI: 0.01-0.69) with antibody threshold ≥2000, ≥5000 and ≥10 000AU/mL, respectively (Figure 6.1 and Table 6.5). The odds ratio for disease also decreased with increasing antibody concentrations when comparing cases to non-colonized controls (Table 6.6). The median maternal FbsA antibody concentrations (in AU/mL) was 1942 (IQR: 1120-3688) in cases as compared to colonized controls (2752; IQR: 1620-5108) and non-colonized controls (2296; IQR: 1408-4627; Table 6.7). The median infant FbsA antibody concentrations was 1131 (IQR: 679-2104) in cases as compared to infants of colonized (1744; IQR: 775-3303) and non-colonized controls (1696; IQR: 859-3486, Table 6.8).

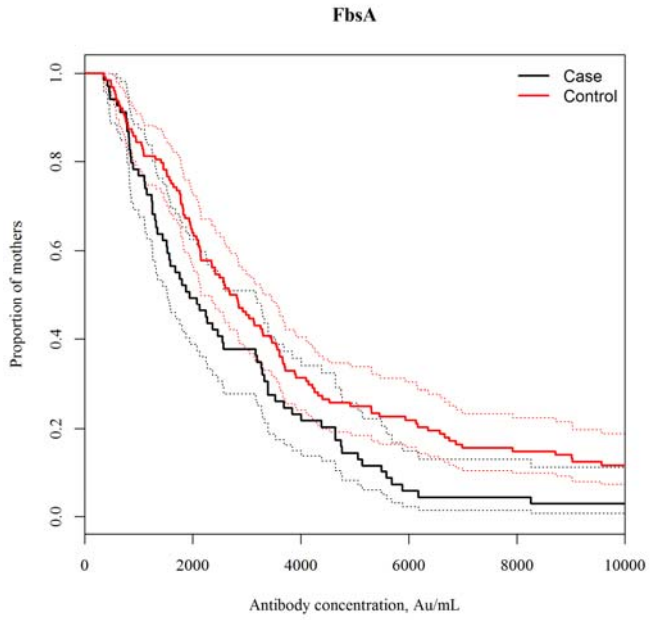


Figure 6.1: Reverse cumulative plots demonstrating the proportion of mothers of cases and colonized controls to antibody concentrations for FbsA

6.1.3 Antibody levels to BibA

The proportion of cases and controls (colonized and non-colonized) with antibody concentrations at various thresholds were similar and the adjusted odds ratios were not significant (Figure 6.2, Table 6.5 and 6.6). The median BibA maternal antibody concentrations (in AU/mL) was 4512 (IQR: 2587-9774) in cases as compared to 5727 (IQR: 2560-9913) in colonized controls and 5243 (IQR: 2420-9871) in non-colonized controls (Table 6.7). The median infant BibA antibody concentrations was 1866 (IQR: 787-3919) in cases as compared to 2901 (IQR: 1554-6593) in infants of colonized and 3063 (IQR: 1397-6447) in non-colonized controls (Table 6.8).

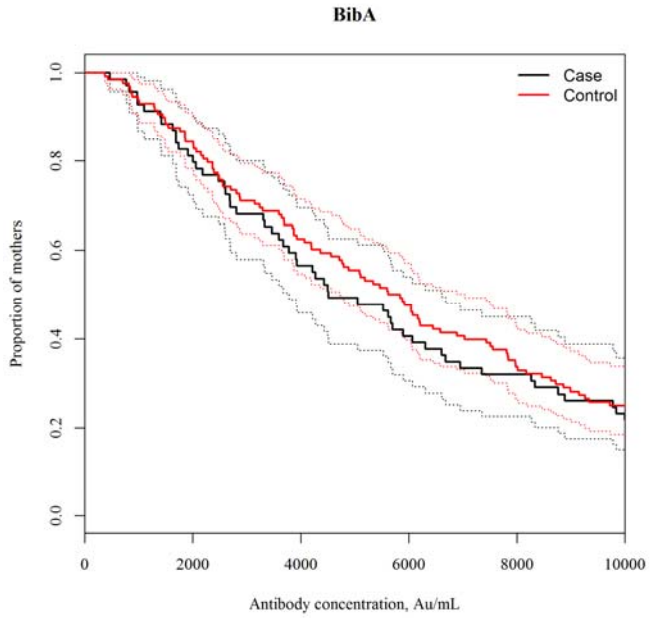


Figure 6.2: Reverse cumulative plots demonstrating the proportion of mothers of cases and colonized controls to antibody concentrations for BibA

Table 6.5: Maternal antibody (AU/mL) thresholds to FbsA and BibA surface-protein epitopes in mothers of cases and **colonized** controls

	Cases	Controls	OR (95% CI)[1]	p-value	aOR (95% CI)[2]	p-value
FbsA	**n=69 (%)**	**n=128 (%)**				
<1000	16 (23.2)	20 (15.6)	Ref			
≥1000	53 (76.8)	108 (84.4)	0.55 (0.26-1.18)	0.124	0.56 (0.24-1.32)	0.182
≥2000	34 (49.3)	82 (64.1)	0.41 (0.18-0.94)	0.035	0.40 (0.16-1.04)	0.061
≥5000	10 (14.5)	32 (25.0)	0.37 (0.12-1.33)	0.082	0.22 (0.05-1.02)	0.053
≥10000	2 (2.9)	15 (11.7)	0.20 (0.03-1.26)	0.086	0.04 (0.01-0.69)	0.027
BibA	**n=69 (%)**	**n=128 (%)**				
<2000	13 (18.8)	20 (15.6)	Ref			
≥2000	56 (81.2)	108 (84.4)	0.62 (0.28-1.38)	0.237	0.54 (0.22-1.36)	0.191
≥5000	34 (49.3)	71 (55.5)	0.66 (0.26-1.50)	0.293	0.53 (0.19-1.48)	0.214
≥10000	15 (21.7)	32 (25.0)	0.39 (0.13-1.16)	0.092	0.30 (0.08-1.17)	0.083
≥15000	11 (15.9)	19 (14.8)	0.47 (0.14-1.56)	0.218	0.43 (0.11-1.71)	0.231

[1]calculated Odds ratio with 95% confidence using conditional logistic regression, [2]Adjusted odds ratio with 95% confidence using conditional logistic regression (BibA and FbsA: adjusted for prolonged rupture of membranes, infant gender, day of life at enrolment)

Table 6.6: Maternal antibody (AU/mL) thresholds to FbsA and BibA surface-protein epitopes in mothers of cases and **non-colonized** controls

	Cases	Non-colonized controls	OR (95% CI)[1]	p-value	aOR (95% CI)[2]	p-value
FbsA	**n=69 (%)**	**n=332 (%)**				
<1000	16 (23.2)	49 (14.8)	Ref			
≥1000	53 (76.8)	283 (85.2)	0.53 (0.28-1.02)	0.057	0.51 (0.24-1.05)	0.067
≥2000	34 (49.3)	191 (57.5)	0.47 (0.23-0.94)	0.034	0.41 (0.18-0.91)	0.028
≥5000	10 (14.5)	76 (22.9)	0.38 (0.15-0.96)	0.040	0.36 (0.12-1.07)	0.066
≥10000	2 (2.9)	39 (11.5)	0.12 (0.02-0.62)	0.011	0.12 (0.02-0.71)	0.019
BibA	**n=69 (%)**	**n=332 (%)**				
<2000	13 (18.8)	66 (19.9)	Ref			
≥2000	56 (81.2)	266 (80.1)	1.00 (0.51-1.95)	0.998	1.10 (0.52-2.33)	0.805
≥5000	34 (49.3)	171 (51.5)	0.90 (0.44-1.83)	0.769	0.93 (0.42-2.07)	0.854
≥10000	15 (21.7)	82 (24.7)	0.84 (0.36-1.96)	0.683	0.94 (0.37-2.37)	0.894
≥15000	11 (15.9)	45 (13.6)	1.36 (0.51-3.67)	0.543	1.52 (0.50-4.58)	0.457

[1]calculated Odds ratio with 95% confidence using conditional logistic regression, [2]Adjusted odds ratio with 95% confidence using conditional logistic regression (BibA and FbsA: adjusted for Black-African Race, prolonged rupture of membranes, IAP, gestational age, day of life at enrolment)

Table 6.7: Maternal antibody concentrations (AU/mL) in cases and controls ≥34 weeks of age

	Cases	Colonized controls (protein/pilus-specific)	Non-colonized controls
	Median(IQR)[1] [n=][2]	Median(IQR) [n=]	Median(IQR) [n=]
FbsA			
Overall	**1942 (1120-3688) [69]**	**2752 (1620-5108) [128]**	**2296 (1408-4627) [332]**
EOD[3]	1741 (863-3529) [34]	2152 (1074-3703) [75]	2139 (1304-4408) [206]
LOD[4]	2465 (1244-4007) [35]	3456 (1954-7920) [53]	2675 (1509-5012) [126]
HIV-infected	1758 (901-3392) [29]	2630 (1312-5934) [54]	1986 (1105-4109) [142]
HIV-uninfected	2012 (1199-4512) [40]	2827 (1826-4323) [74]	2478 (1592-5185) [190]
BibA			
Overall	**4512 (2587-9774) [69]**	**5727 (2560-9913) [128]**	**5243 (2420-9871) [332]**
EOD	5289 (2058-9835) [34]	4072 (2009-7808) [75]	4638 (2309-8949) [206]
LOD	4498 (2607-8884) [35]	8459 (5108-14524) [53]	6007 (2930-13882) [126]
HIV-infected	3926 (2690-6604) [29]	6260 (2391-12477) [54]	5044 (2136-9708) [142]
HIV-uninfected	5756 (2387-10884) [40]	4989 (2811-8987) [74]	5572 (2761-10113) [190]
PI-1			
Overall	**432 (203-3391) [29]**	**1052 (301-6463) [64]**	**789 (317-2419) [289]**
EOD	674 (154-5041) [14]	2650 (487-7888) [39]	821 (329-2926) [173]
LOD	432 (251-2142) [15]	497 (245-2036) [25]	659 (301-2054) [116]
HIV-infected	419 (183-1090) [12]	449 (201-3628) [23]	632 (280-1988) [114]
HIV-uninfected	666 (251-5041) [17]	1569 (497-8121) [41]	886 (327-3195) [175]
PI-2a			
Overall	**2352 (1133-9522) [37]**	**1944 (728-7269) [77]**	**2123 (868-5914) [319]**
EOD	4198 (1239-9522) [21]	2033 (662-6583) [48]	1982 (845-5577) [194]
LOD	2038 (620-8816) [16]	1860 (757-8844) [29]	2197 (1034-8223) [125]
HIV-infected	1625 (616-3622) [14]	1591 (532-5807) [31]	1586 (745-4203) [133]
HIV-uninfected	4405 (1580-12842) [23]	2935 (903-10910) [46]	2563 (1136-8114) [186]
PI-2b			
Overall	**2633 (410-4242) [29]**	**709 (436 -1432) [29]**	**844 (405-2199) [279]**
EOD	2633 (271-10986) [11]	983 (428-6094) [14]	723 (332-1853) [157]
LOD	2423 (436-4242) [18]	705 (436-1127) [15]	892 (509-2443) [122]
HIV-infected	2156 (410-16249) [14]	622 (249-998) [14]	785 (380-2524) [114]
HIV-uninfected	2633 (305-3640) [15]	1127 (614-5964) [15]	903 (409-1895) [165]

[1]Median (interquartile range), [2]number of cases, [3]EOD- Early-onset disease, [4]LOD- Late-onset disease.

Table 6.8: Infant antibody concentrations (AU/mL) of cases and controls ≥34 weeks of age

	Cases	Colonized controls (protein/pilus-specific)	Non-colonized controls
	Median(IQR)[1] [n=][2]	Median(IQR) [n=]	Median(IQR) [n=]
FbsA			
Overall	**1131 (679-2104) [69]**	**1744 (775-3303) [128]**	**1696 (859-3486) [332]**
EOD[3]	1721 (798-2421) [34]	2433 (961-3711) [75]	2260 (1086-4479) [206]
LOD[4]	873 (604-1554) [35]	1264 (588-2103) [53]	1109 (568-1893) [126]
HIV-infected	809 (436-1523) [29]	1283 (513-2710) [54]	1142 (619-2352) [142]
HIV-uninfected	1623 (795-2466) [40]	2135 (956-3653) [74]	2192 (1113-4390) [190]
BibA			
Overall	**1866 (787-3919) [69]**	**2901 (1554-6593) [128]**	**3063 (1397-6447) [332]**
EOD	3326 (1770-7262) [34]	4028 (1644-6613) [75]	3746 (1602-7093) [206]
LOD	982 (547-2237) [35]	2600 (1417-6456) [53]	2287 (1205-4923) [126]
HIV-infected	1288 (773-2176) [29]	2134 (1187-6328) [54]	2485 (1240-5977) [142]
HIV-uninfected	3326 (1029-7267) [40]	4463 (1919-6775) [74]	3653 (1602-7093) [190]
PI-1			
Overall	**408 (76-1452) [29]**	**901 (215-5534) [64]**	**595 (196-1852) [289]**
EOD	839 (256-2721) [14]	1920 (613-7111) [39]	883 (264-2839) [173]
LOD	101 (67-667) [15]	413 (97-955) [25]	297 (110-1041) [116]
HIV-infected	87 (45-420) [12]	636 (94-1920) [23]	331 (128-1323) [114]
HIV-uninfected	697 (183-2615) [17]	1127 (453-7001) [41]	800 (264-2483) [175]
PI-2a			
Overall	**887 (187-2151) [37]**	**1573 (413-5747) [77]**	**1336 (462-5453) [319]**
EOD	928 (548-2225) [21]	2617 (540-6262) [48]	1921 (581-7179) [194]
LOD	730 (102-1659) [16]	744 (302-3422) [29]	713 (364-3390) [125]
HIV-infected	291 (55-903) [14]	1009 (302-4559) [31]	750 (372-3940) [133]
HIV-uninfected	1167 (693-2225) [23]	2087 (515-7306) [46]	1907 (649-5883) [186]
PI-2b			
Overall	**480 (113-2055) [29]**	**510 (253-1749) [29]**	**591 (245-1500) [279]**
EOD	1286 (480-2650) [11]	1677 (311-5073) [14]	716 (301-2206) [157]
LOD	196 (91-1045) [18]	423 (182-602) [15]	478 (182-1145) [122]
HIV-infected	573 (73-2620) [14]	219 (154-712) [14]	400 (143-1218) [114]
HIV-uninfected	480 (128-2055) [15]	1064 (378-3092) [15]	716 (301-1695) [165]

[1]Median (interquartile range), [2]number of cases, [3]EOD- Early-onset disease, [4]LOD- Late-onset disease.

6.1.4 Antibody levels to pilus island proteins

A greater proportion of PI-1 colonized maternal controls had antibody concentrations at higher thresholds than mothers of cases resulting in a decreased odds ratio for disease, however, the adjusted odds ratio did not differ significantly (Figure 6.3 and Table 6.9). The median PI-1 antibody concentrations (in AU/mL) was 432 (IQR: 203-3391) in mothers of invasive GBS cases compared to controls with PI-1 colonization (1052; IQR: 301-6463) and those not colonized by GBS (789; IQR: 317-2419; Table 6.7). The median infant PI-1 antibody concentrations was 408 (IQR: 76-1452) in cases, 901 (IQR: 215-5534) in infants of women colonized with PI-I strains and 595 (IQR: 196-1852) in control infants whose mothers were not-colonized by GBS (Table 6.8).

The proportions of mothers with PI-2a and PI-2b antibodies were similar between cases and controls (Figures 6.4 and 6.5). Similarly median maternal antibody concentrations did not differ between cases than controls (Table 6.7). Comparing maternal PI2a and PI2b antibody concentrations between cases and controls at varying thresholds did not demonstrate differences in the adjusted odds ratio for disease for PI-2a and PI-2b antibodies (Table 6.9 and 6.10).

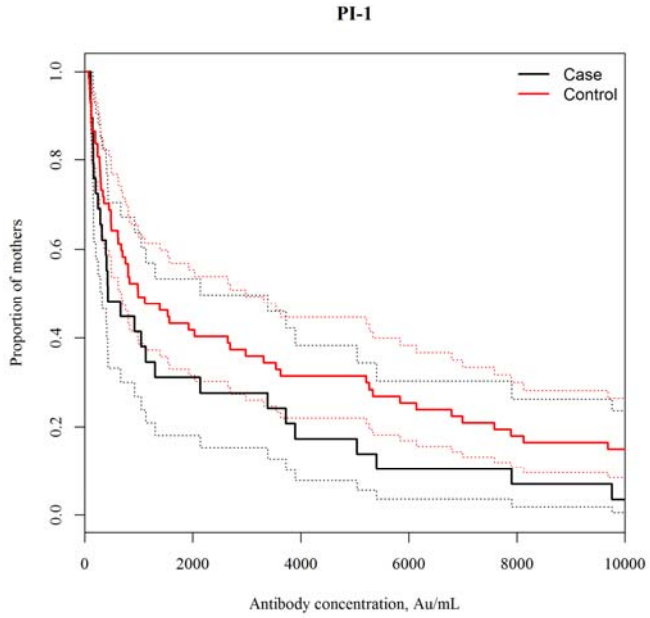


Figure 6.3: Reverse cumulative plots demonstrating the proportion of mothers of cases and colonized controls to antibody concentrations for pilus island-1 (PI-1)

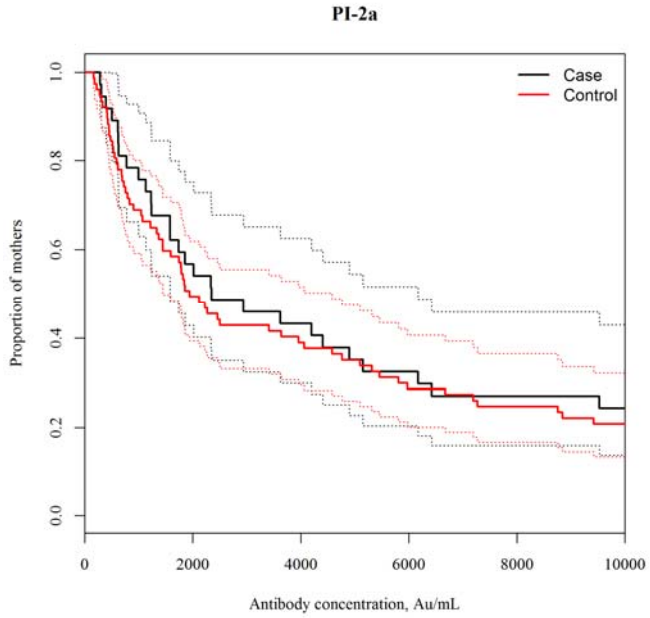


Figure 6.4: Reverse cumulative plots demonstrating the proportion of mothers of cases and colonized controls to antibody concentrations for pilus island-2a (PI-2a)

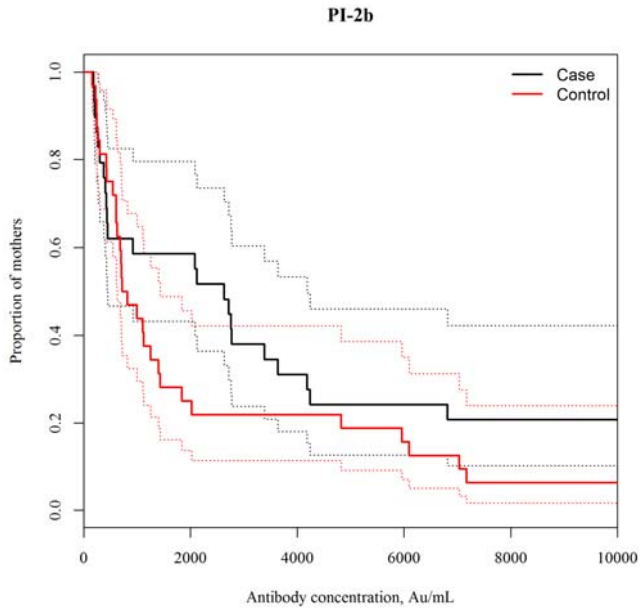


Figure 6.5: Reverse cumulative plots demonstrating the proportion of mothers of cases and colonized controls to antibody concentrations for pilus island-2b (PI-2b)

Footnote: The solid line represents the proportion of mothers at various antibody thresholds. The upper and lower dash lines are the 95% confidence intervals. The bold dash line is the estimated fitted line.

Table 6.9: Maternal antibody (AU/mL) thresholds to pilus island surface-protein epitopes in mothers of cases and **colonized** controls

	Cases	Controls	OR (95% CI)[1]	p-value	aOR (95% CI)[2]	p-value
PI-1	**n=29 (%)**	**n=64 (%)**				
<500	15 (51.7)	23 (35.9)	Ref			
≥500	14 (48.3)	41 (64.1)	0.57 (0.23-1.42)	0.226	0.64 (0.20-2.03)	0.446
≥1000	12 (41.4)	32 (50.0)	0.55 (0.21-1.47)	0.236	0.59 (0.17-2.06)	0.408
≥2000	9 (31.0)	27 (42.2)	0.47 (0.17-1.33)	0.156	0.39 (0.10-1.58)	0.189
≥5 000	5 (17.2)	20 (31.3)	0.28 (0.07-1.11)	0.070	0.29 (0.06-1.43)	0.130
≥10000	1 (3.5)	10 (15.6)	0.15 (0.02-1.31)	0.086	0.10 (0.01-1.31)	0.079
PI-2a	**n=37 (%)**	**n=77 (%)**				
<1000	9 (24.3)	24 (31.2)	Ref			
≥1000	28 (75.7)	53 (68.8)	1.48 (0.57-3.81)	0.417	1.44 (0.45-4.59)	0.533
≥2000	21 (56.8)	38 (49.4)	1.54 (0.57-4.16)	0.392	1.14 (0.33-3.97)	0.834
≥5000	13 (35.1)	27 (35.1)	1.12 (0.37-3.35)	0.844	0.58 (0.14-2.50)	0.466
≥10000	9 (24.3)	16 (20.8)	1.04 (0.31-3.46)	0.945	0.83 (0.18-3.88)	0.812
PI-2b	**n=29 (%)**	**n=29 (%)**				
<1000	12 (41.4)	17 (58.6)	Ref			
≥1000	17 (58.6)	12 (41.4)	1.99 (0.69-5.72)	0.202	1.72 (0.56-5.26)	0.342
≥2000	17 (58.6)	6 (20.7)	4.15 (1.15-14.96)	0.030	3.32 (0.88-12.45)	0.076
≥5000	7 (24.1)	6 (20.7)	1.87 (0.46-7.63)	0.383	1.65 (0.40-6.87)	0.490
≥10 000	6 (20.7)	2 (6.9)	3.21 (0.52-19.95)	0.210	3.03 (0.46-19.76)	0.248

[1]calculated Odds ratio with 95% confidence using conditional logistic regression, [2]Adjusted odds ratio with 95% confidence using conditional logistic regression (PI-1: adjusted for prolonged rupture of membranes, gestational age and day of life at enrolment; PI-2a: adjusted for parity, prolonged rupture of membranes, birth weight, infant gender and day of life at enrolment; PI-2b: adjusted for gestational age and day of life at enrolment)

Table 6.10: Maternal antibody (AU/mL) thresholds to pilus island surface-protein epitopes in mothers of cases and **non-colonized** controls

	Cases	Non-colonized controls	OR (95% CI)[1]	p-value	aOR (95% CI)[2]	p-value
PI-1	**n=29 (%)**	**n=289 (%)**				
<500	15 (51.7)	113 (39.1)	Ref			
≥500	14 (48.3)	176 (60.9)	0.59 (0.27-1.26)	0.173	0.63 (0.29-1.38)	0.248
≥1000	12 (41.4)	125 (43.3)	0.69 (0.31-1.54)	0.364	0.75 (0.33-1.71)	0.495
≥2000	9 (31.0)	81 (28.0)	0.80 (0.33-1.92)	0.614	0.84 (0.34-2.06)	0.699
≥5 000	5 (17.2)	43 (14.9)	0.78 (0.26-2.36)	0.665	0.75 (0.24-2.38)	0.627
≥10000	1 (3.5)	22 (7.6)	0.35 (0.04-2.87)	0.329	0.38 (0.05-3.21)	0.376
PI-2a	**n=37 (%)**	**n=319 (%)**				
<1000	9 (24.3)	87 (27.3)	Ref			
≥1000	28 (75.7)	232 (72.7)	1.10 (0.49-2.46)	0.824	1.35 (0.54-3.37)	0.522
≥2000	21 (56.8)	162 (50.8)	1.27 (0.54-2.95)	0.585	1.55 (0.59-4.09)	0.377
≥5000	13 (35.1)	89 (27.9)	1.39 (0.54-3.60)	0.492	1.71 (0.57-5.19)	0.342
≥10000	9 (24.3)	61 (19.1)	1.25 (0.45-3.47)	0.665	1.66 (0.50-5.45)	0.406
PI-2b	**n=29 (%)**	**n=279 (%)**				
<1000	12 (41.4)	160 (57.3)	Ref			
≥1000	17 (58.6)	119 (42.7)	1.98 (0.90-4.33)	0.088	1.95 (0.88-4.31)	0.101
≥2000	17 (58.6)	72 (25.8)	3.19 (1.44-7.04)	0.004	3.09 (1.37-6.85)	0.006
≥5000	7 (24.1)	35 (12.5)	2.50 (0.91-6.83)	0.074	2.37 (0.84-6.68)	0.101
≥10 000	6 (20.7)	24 (8.6)	2.97 (1.00-8.81)	0.050	2.66 (0.87-8.15)	0.087

[1]calculated Odds ratio with 95% confidence using conditional logistic regression, [2]Adjusted odds ratio with 95% confidence using conditional logistic regression (PI-1: adjusted for gestational age and day of life at enrolment; PI-2a: adjusted for parity, Black-African Race, prolonged rupture of membranes, infant gender and day of life at enrolment; PI-2b: adjusted for IAP, gestational age and day of life at enrolment)

6.1.5 Absolute risk of invasive GBS disease and surface-protein antibodies

Using Bayesian modelling, we analysed the risk of invasive GBS disease in relation to surface-protein antibodies. None of the studied surface-protein antibodies demonstrated a protective threshold against invasive GBS disease, nor were there any significant reductions in the risk of disease with increasing antibody concentrations (Figures 6.6 A-E). Although the adjusted odds ratio had shown significant difference between mothers of cases and colonized controls for FbsA antibodies at thresholds above 10 000 Au/mL and a similar trend for PI-1, no threshold was identified to being associated with a reduced risk of invasive GBS disease for either of these proteins.

Furthermore, in an exploratory analysis, we measured whether there were any correlations between the select surface-protein antibody concentrations and serotypes I and III antibodies in cases and homotypic controls, of which there was none (Figure 6.7).

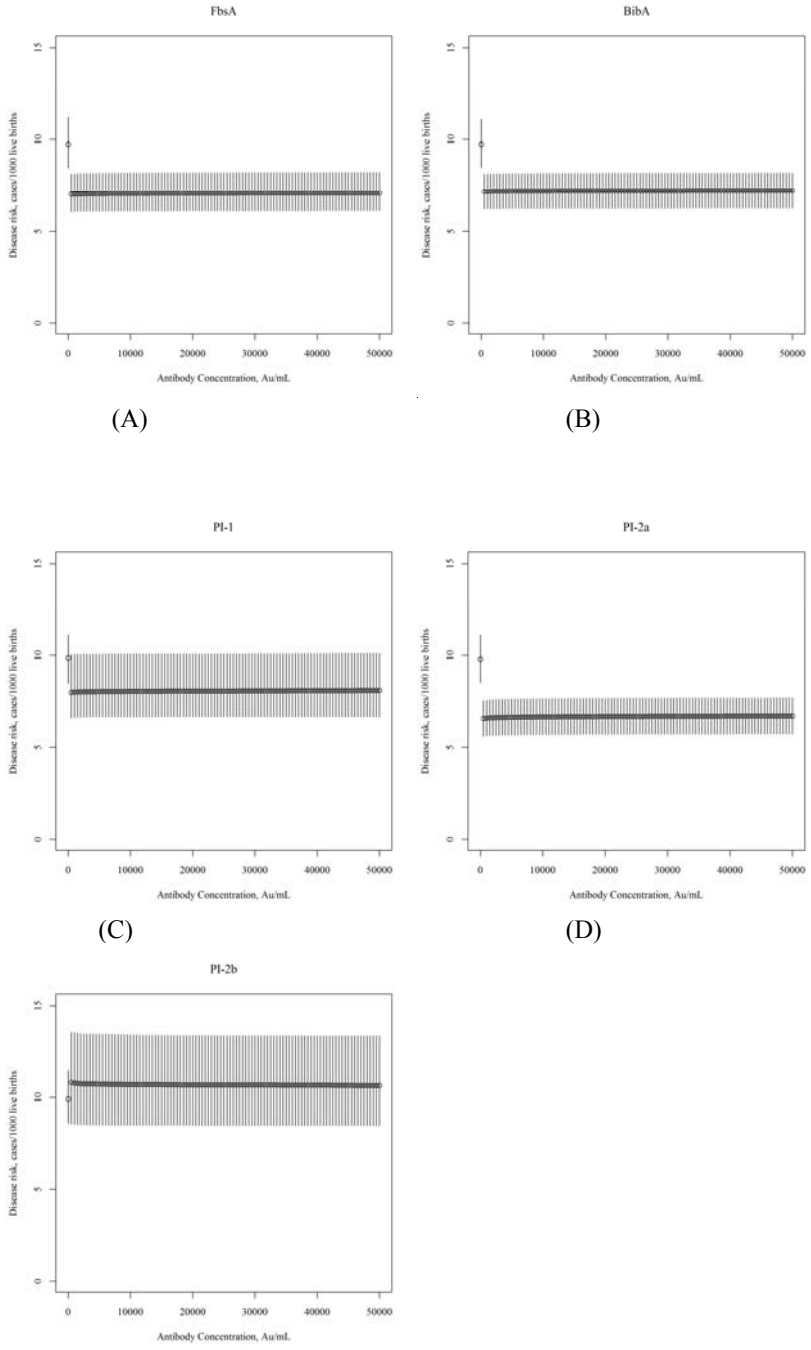


Figure 6.6: Probability of invasive GBS disease risk to FbsA (A), BibA (B), PI-1 (C), PI-2a (D) and PI-2b (E) at varying maternal antibody concentrations using a Bayesian model.

Footnote: The circles represent the posterior mode (i.e. the most likely value) and vertical lines represent the 50% credible interval.

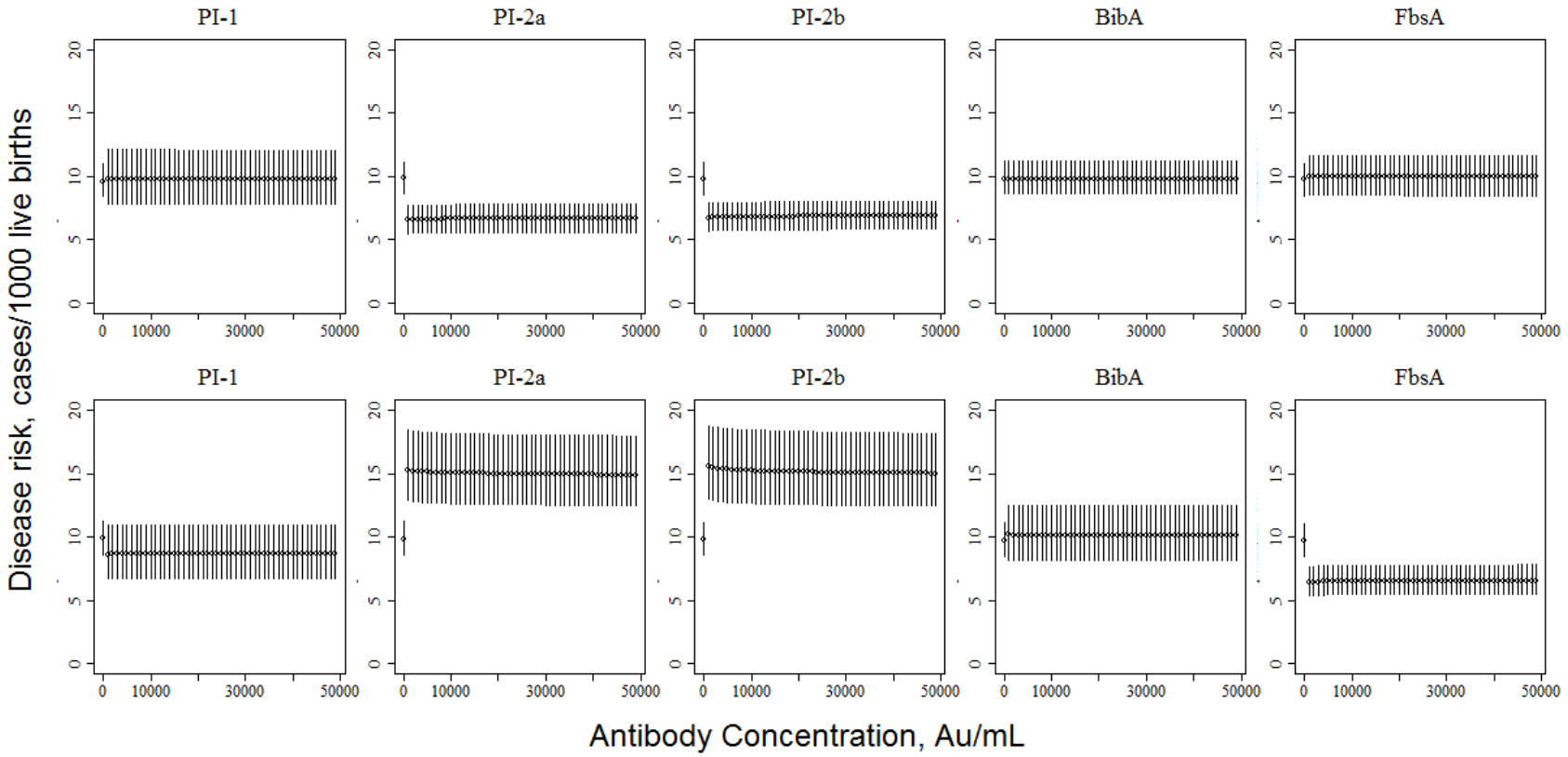


Figure 6.7: Probability of invasive Group B *Streptococcus* (GBS) disease risk to PI-1, PI-2a, PI-2b, BibA and FbsA maternal antibody concentrations in serotype-specific Ia (Top row) and III (Bottom row) cases and homotypic controls.

Footnote: The circles represent the posterior mode (i.e. the most likely value) and vertical lines represent the 50% credible interval.

6.2 Discussion

To our knowledge, this is the first study to report on the association between maternal FbsA, BibA and PI surface-protein antibody concentrations and the risk of invasive GBS disease in infants. We report a relative association between maternal FbsA antibody concentrations and a similar trend for PI-1 antibody concentrations and invasive GBS disease in their infants. However, using Bayesian modelling, there was no absolute association between FbsA and PI-1 antibody concentrations and invasive GBS disease and a correlate of protection could not be defined. Furthermore, no association was identified between maternal or infant BibA and the PI surface-protein antibodies and the risk of invasive GBS disease in infants. Our findings of lack of associations between maternal antibodies to these proteins and protection against invasive GBS disease in their infants are in contrast to the potential of these antigens being developed into vaccines as was suggested in animal model challenge studies.

Studies addressing the role of antibodies to various other GBS surface-proteins in infants with invasive GBS disease or their mothers include Sip, Rib, αC protein and βC protein (Lachenauer et al., 2002, Larsson et al., 2006, Manning et al., 2006, Pannaraj et al., 2007, Pannaraj et al., 2008). Except for one study that measured antibody to the Rib surface protein (Larsson et al., 2006), no association between surface-protein antibodies and the risk of invasive GBS disease has been reported in humans.

In an exploratory analysis, we attempted to identify whether the association between serotype Ia and III antibody concentrations and invasive GBS disease (as demonstrated in chapter 5) could have been as a result of antibodies to these tested surface-proteins being the effector for

protection rather than antibody to serotype Ia and III. We found no correlation between invasive GBS disease and any of the studied surface-protein antibody in serotype Ia or III disease cases and homotypic controls (Figure 6.7).

A limitation of our study was that we did not measure whether GBS cultured isolates expressed FbsA and BibA in cases and controls so as to compare protein antibody concentration by type-specificity. It is however thought that BibA may be expressed more universally (>90%) in GBS strains, and approximately half of strains express FbsA (Santi et al., 2009, Meinke et al., 2010). A further limitation is that we measured antibody concentrations using our own in-house references as no reference sera are currently available.

In conclusion, our study failed to identify a definitive association for higher maternal antibodies to the five studied GBS surface-proteins and risk for invasive GBS disease in young infants, suggesting a low likelihood that these proteins have potential for being developed into successful vaccine candidates on their own.

7.0 Integrated Discussion and Conclusion

In this thesis, there were several major findings. The high incidence of invasive GBS disease in black-Africans from South Africa is coupled with a high case fatality ratio and significant neurological sequelae among survivors. This persistently high incidence of EOD is partly due to the failure of implementation of preventative IAP strategies for EOD and the heightened risk for LOD in infants born to HIV-infected women. In addition, this work further corroborated the association between maternal serotype-specific capsular antibody levels and the risk of invasive GBS disease among young infants in a low-middle income country. Also, our results suggest that maternal HIV-infection would need to be considered in the evaluation of GBS vaccines in settings with a high prevalence of HIV.

The prevalence of GBS colonization in pregnant women in this setting is high and warrants particular attention because maternal colonization is a pre-requisite for EOD. The risk-based strategy of identifying pregnant women to be targeted with IAP has not been effectively executed in our setting, which is likely to be typical of other low-middle income countries. In Soweto, only 23 % of women who fulfil the criteria of intervention outlined by the CDC, actually received antibiotics. In our setting, in contrast to other low income countries where a large proportion of deliveries occur at home, approximately 99% of deliveries occur in a health care setting. Furthermore, in Soweto, 70% of the deliveries in this region occurred at CHBAH, a tertiary academic centre. Thus, even when women deliver at health care facilities, IAP cannot be practically implemented. This may be partly due to a lack of recognition of risk-factors or failure to administer IAP by birth-attendants or under-staffing at the delivery facility, all of which result in an oversight for effective implementation of this strategy. Even though the CDC recommended universal screening and IAP has further reduced EOD, this

approach involves logistical challenges including being expensive and a resource intensive strategy for its success. This includes the need for pregnant women being screened for GBS recto-vaginal colonization at 35-37 weeks of gestational age, their results made available to health workers in a timely manner and IAP provided for all colonized women at least 4 hours prior to delivery. Therefore, in low-middle income settings, an effective GBS vaccine targeting pregnant women is more likely to be feasible in preventing invasive GBS disease than IAP to screened women.

The high incidence of LOD in our setting is partly due to a high prevalence of maternal HIV-infection (29%) amongst pregnant women. Although an improvement in the PMTCT program has led to a decrease in the number of HIV-infected newborns, immunological susceptibilities have been identified in infants that are HIV-exposed-but-uninfected. These vulnerable infants are at a heightened risk of invasive GBS disease partly because of lower maternal GBS antibody concentrations and reduced transplacental antibody transfer in HIV-infected women during pregnancy. This could possibly be mitigated by modifications, such as using a higher dose or more frequent dosing, of the trivalent GBS polysaccharide-protein conjugate vaccine.

A trivalent GBS polysaccharide-protein conjugate vaccine composed of capsular epitopes from serotypes Ia, Ib and III for vaccination of pregnant women to protect their young infants against invasive GBS disease has completed phase-II evaluation. These serotypes cause 70%-80% of all invasive GBS disease in early-infancy. The further clinical evaluation of this vaccine is however challenged by the declining incidence of EOD in high income countries. In order to license this vaccine, a large phase III efficacy trial will be required. This will require a sample size of over 60 000 pregnant women to be conducted in high incidence settings of invasive GBS disease, which will incur tremendous logistical challenges. An alternate

pathway to licensure of some new-vaccines is based on immunologic endpoints for those diseases for which immunological correlates of protection have been established from previous vaccine-studies or through sero-epidemiological studies. This will then be followed by phase IV studies to establish vaccine effectiveness. Consequently, the licensure of the GBS vaccine might be based on immunological parameters should correlates of protection be established in a diversity of settings, similar to that used for the meningococcal vaccine.

In this thesis, I have shown that mothers whose infants developed serotype Ia or III invasive GBS disease had lower antibody concentrations compared to women who were colonized by homotypic serotypes and whose infants remained free of disease. These findings are the first from a low-middle income country that establish a sero-correlate of protection, and corroborate findings from two studies in the USA. The concordance of the data on association of maternal serotype-specific capsular antibody levels and risk of invasive GBS disease in their infants from different settings, lend further credence to licensure of polysaccharide based GBS vaccines using a correlate of protection. Based on the assay employed in our laboratory, we propose that maternal antibody concentrations of ≥6 μg/mL and ≥3 μg/mL are sero-correlates of protection against serotypes Ia and III invasive disease.

In addition to examining the role of maternal capsular antibody and invasive GBS disease in young infants, we performed the first clinical study investigating antibodies to selected GBS surface-proteins that have been shown to be immunogenic in animal models. We were unable to identify a definitive association for higher maternal antibodies to these GBS surface-proteins and risk for invasive GBS disease in young infants, suggesting that these proteins may not be suitable vaccine candidates.

This thesis has identified several areas for further research that may help to further understand invasive GBS disease and develop better therapies. This includes addressing the observation in which some mothers with high antibody levels have infants that develop invasive GBS disease, whereas in contrast, a large proportion of colonised mothers have low antibody levels and their infants remain free of invasive disease. The possible reason for this may be related to virulence potential of the organism and my recommendation is that correlate studies be undertaken against various genotypic strains, including the more virulent ST 17 strains. Additionally, these studies should measure the functionality of antibodies using opsonophagocytic assays. The value of these qualitative assays is still, however, debatable as they require exogenous components which are often also deficient in the newborn (i.e. complement). Consequently, newer microbiological or molecular techniques may explain why some infants born to women with low antibody levels do not develop invasive GBS disease. A further recommendation is that studies be undertaken to address the pathogenesis of LOD, which to date, the burden has remained unchanged even in high-income countries. In addition, breastmilk antibodies and the risk of invasive GBS disease need further evaluation.

In conclusion, the high burden of invasive GBS disease in this low-middle income setting is related to lower maternal GBS capsular antibody levels that may be further deficient in HIV-exposed infants. Vaccine induced antibody levels ≥6 μg/mL and ≥3 μg/mL, as measured by our assay, would likely protect the majority of infants against invasive GBS disease caused by serotypes Ia and III in our setting. This work may potentially be useful in future GBS polysaccharide-protein conjugate vaccine efficacy studies.

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9.0 Appendices

Appendix 1: [PDF] Dangor, Z., Kwatra, G., Izu, A., Lala, S. G. & Madhi, S. A. 2015. Review on the association of Group B *Streptococcus* capsular antibody and protection against invasive disease in infants. Expert Rev Vaccines, 14, 135-49.

Appendix 2: [PDF] Dangor, Z., Lala, S. G., Cutland, C. L., Koen, A., Jose, L., Nakwa, F., Ramdin, T., Fredericks, J., Wadula, J. & Madhi, S. A. 2015. Burden of invasive group B *Streptococcus* disease and early neurological sequelae in South African infants. PLoS One, 10, e0123014.

Appendix 3: [PDF] Dangor, Z., Kwatra, G., Izu, A., Adrian, P., Van Niekerk, N., Cutland, C. L., Adam, Y., Velaphi, S., Lala, S. G. & Madhi, S. A. 2015. HIV-1 Is Associated With Lower Group B *Streptococcus* Capsular and Surface-Protein IgG Antibody Levels and Reduced Transplacental Antibody Transfer in Pregnant Women. J Infect Dis, 212, 453-62.

Appendix 4: [PDF] Dangor, Z., Kwatra, G., Izu, A., Adrian, P., Cutland, C. L., Velaphi, S., Ballot, D., Reubenson, G., Zell, E. R., Lala, S. G. & Madhi, S. A. 2015. Association between maternal Group B Streptococcus surface-protein antibody concentrations and invasive disease in their infants. Expert Rev Vaccines, 1-10.

Appendix 5: Map outlining the six districts of Gauteng Province, South Africa.

Appendix 6: Map outlining the sub-districts/regions of the greater Johannesburg metropolitan area.

Appendix 7: The Denver Developmental Screening Test II (Denver-II).

Appendix 8: Certificate of approval granted by the University of Witwatersrand Human Research Ethics Committee on the 28th September 2013 (HREC number: M120963)

Appendix 9: Certificate of approval granted by the University of Witwatersrand Human Research Ethics Committee on the 7th November 2012 (HREC number: M120905)

Review

EXPERT REVIEWS

Review on the association of Group B *Streptococcus* capsular antibody and protection against invasive disease in infants

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A trivalent Group B *streptococcus* (GBS) polysaccharide-protein conjugate vaccine for vaccination of pregnant women is under development to protect their newborns against invasive GBS disease. Establishing sero-correlates of protection against invasive GBS disease in infants could expedite the licensure pathway of polysaccharide-protein conjugate vaccine. A systematic review of studies reporting on the association of capsular antibodies and invasive GBS disease in infants and colonization in women or newborns was undertaken. Most studies that described maternal and/or infant capsular antibody levels in infants with invasive GBS disease identified an association between low capsular antibody levels in invasive GBS cases compared to controls. Different assay methods and the lack of standardized reference ranges for serotype-specific antibody levels makes it difficult to select an antibody level that may be used as a reliable sero-correlate of protection. Further studies using standardized methods are warranted.

KEYWORDS: capsular antibodies • GBS • infants • sero-correlates • vaccine

Group B *Streptococcus* (GBS) remains the leading cause of neonatal sepsis and meningitis in the USA [1,2], where the burden of disease during the first six days of life (early onset disease [EOD]) has declined by 80% [3]. The major preventative intervention to reduce invasive GBS disease has been maternal recto-vaginal screening for GBS colonization at 35–37 weeks of pregnancy, coupled with intrapartum antibiotics prophylaxis (IAP) to colonized parturient women [3]. However, IAP is expensive and not feasible in settings with limited laboratory facilities or in developing countries where a high proportion of births occur outside of healthcare facilities [4,5]. Furthermore, the incidence of GBS disease in infants 7 to 90 days of age (i.e., late onset disease [LOD]) and the incidence of EOD among prematurely born babies has remained high in the USA despite the provision of IAP [3,6].

Vaccination of pregnant women with a GBS vaccine may offer an alternate strategy for the prevention of invasive GBS disease in young infants. Additionally, maternal GBS vaccination may protect pregnant women against GBS disease and reduce their risk of premature birth and/or stillbirth [6]. A trivalent GBS polysaccharide-protein conjugate vaccine (GBS-CV) composed of capsular serotypes Ia, Ib and III has recently completed Phase I and II trials [7]. Challenges in the licensure of a GBS vaccine targeted at pregnant women include the enrollment of a large number of pregnant women (estimated 60,000) and limited localities where such a study could be undertaken to measure vaccine efficacy against the clinical endpoint of invasive GBS disease [7]. Therefore, alternate strategies that may enable licensure of a GBS-CV include immunogenicity studies that demonstrate whether serotype-specific capsular antibody levels, measured in either the mother and/or neonate, confer protection against invasive GBS disease in the newborn. These sero-correlates of protection, coupled with

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supporting safety data, have been accepted licensure-pathway strategies for meningococcal-, inactivated influenza- and pneumococcal conjugate vaccines [8–10].

To determine whether sero-correlates of protection could be established for invasive GBS disease, we therefore reviewed studies that examined the association between maternal or newborn serotype-specific capsular antibody levels and invasive GBS disease among newborns and/or young infants, Furthermore, we reviewed the association between capsular antibody levels and GBS recto/vaginal colonization among pregnant women or skin surface (or other site) colonization among newborns.

Methods

A literature search was undertaken of PubMed, Medline and Scopus databases using the search terms: 'Streptococcus agalactiae'(MESH) OR 'Streptococcus agalactiae' OR 'Group B Streptococcus' OR 'Group B Streptococcal Infection' OR 'Group B Strep' AND 'Antibody'(MESH) OR 'Antibody' OR 'Immunoglobulin' OR 'IgG' OR 'Anti-GBS' OR 'Immunology' OR 'Immunity'. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist and flow diagram were used to identify, screen and exclude studies. Two authors (ZD and GK) independently carried out searches and abstracted data with a third author (SAM) adjudicating on conflicting results. The minimum inclusion criteria were studies reporting on capsular GBS antibodies in mothers or infants. We did not add age limits to the searches; however, we subsequently excluded studies reporting on invasive disease in adults. Furthermore, we also screened for references of the reviewed manuscripts for any other reports on GBS capsular antibodies and invasive disease or colonization (Figure 1).

Data extraction

We abstracted the following data: study region and population, study design, period of recruitment, methods of identifying cases and controls, clinical presentation of cases, timing of presentation of cases, age range of cases and controls, serotype distribution, serological assay used and type of antibody determination, the availability of reference serum, standardization of assay method and quantitative capsular antibody levels in maternal, cord or infant sera. We also determined whether the study authors proposed threshold capsular antibody levels, either in the mother or young infant, which conferred protection against invasive GBS disease in the neonate.

Other confounding factors such as IAP, and risk factors for disease such as prematurity and prolonged rupture of membranes were reported inconsistently in the studies. Similarly, only a few studies matched or adjusted for factors that may influence antibody levels, including ethnicity, maternal and gestational age.

An exploratory analysis, using exact conditional logistic regression, [11], was undertaken to compare the proportions of cases and controls with antibody levels ≥2 μg/ml that were reported in various studies. In one study, the raw numerical data were not reported; hence, we derived the number of cases and controls from the reverse cumulative plots. We reported the odds ratio (95% CI) for invasive GBS disease in infants with an antibody level <2 μg/ml. A meta-analysis was conducted and the pooled odds ratios (95% CI) reported [12]. Data were analyzed using STATA version 13.0 (College Station, TX, USA) and SAS version 9.2 (Cary, NC, USA). Two-tailed p-values <0.05 were considered statistically significant.

Findings

Out of 144 published articles, we identified 18 studies [13–30] that reported on capsular antibody levels in infants with invasive GBS disease and 29 studies that reported on maternal or newborn colonization with GBS [29,31–58]. Six studies were excluded because they were unavailable in English [38,42,44–46,58]. Furthermore, we identified 17 animal model studies reporting natural antibody responses or survival following inoculum of lethal doses of GBS strains after passive immunization of antiserum [21,24,59–73]. These animal model studies were not part of the main review.

Association between GBS capsular antibody levels & invasive GBS disease in young infants

The association between serotype-specific capsular antibody levels and invasive GBS disease in newborns was initially characterized in 1976 by Baker and colleagues [13], who subsequently extended their work in two larger studies [14,19]. Using radioactive antigen binding assays (RABA), Baker and colleagues suggested that a capsular serotype III antibody level >2 μg/ml conferred protection against early-onset GBS III disease based on a study of 111 mothers of infants with serotype III GBS disease (including 32 cases of EOD) and 45 control women colonized with serotype III who delivered healthy newborns [19]. None of the 111 infants with invasive GBS disease had an antibody level >1.6 μg/ml, while 29 (64.4%) of healthy newborns had antibody levels >2 μg/ml. Thirty-three (73.3%) of 45 colonized control mothers, but only 6 (18.8%) of 32 mothers of infants with EOD had antibody level >2 μg/ml (Table 1).

Subsequent studies conducted by Gotoff and colleagues [21,22,24] and Gray and colleagues [23,26] measured capsular IgG levels (using ELISA) against serotypes Ia, Ib, II and III. Although these authors did not suggest a putative antibody level of protection, lower serotype-specific capsular antibodies in infants with EOD and LOD were found in cases compared with controls, as were the finding in most of the other studies.

A sero-correlate of protection against invasive GBS disease was demonstrated in a prospective cohort study compromising 138,740 newborns [27,28]. Serotype-specific colonized healthy newborn controls were observed to have higher serotype-specific capsular antibody than the matched infant cases with serotypes Ia and III invasive GBS disease. The odds of invasive GBS disease was 0.12 (95% CI: 0.02–0.93) at a maternal antibody threshold of ≥5 μg/ml for serotype Ia and 0.09 (95% CI: 0.01–0.78) at a threshold of ≥10 μg/ml for

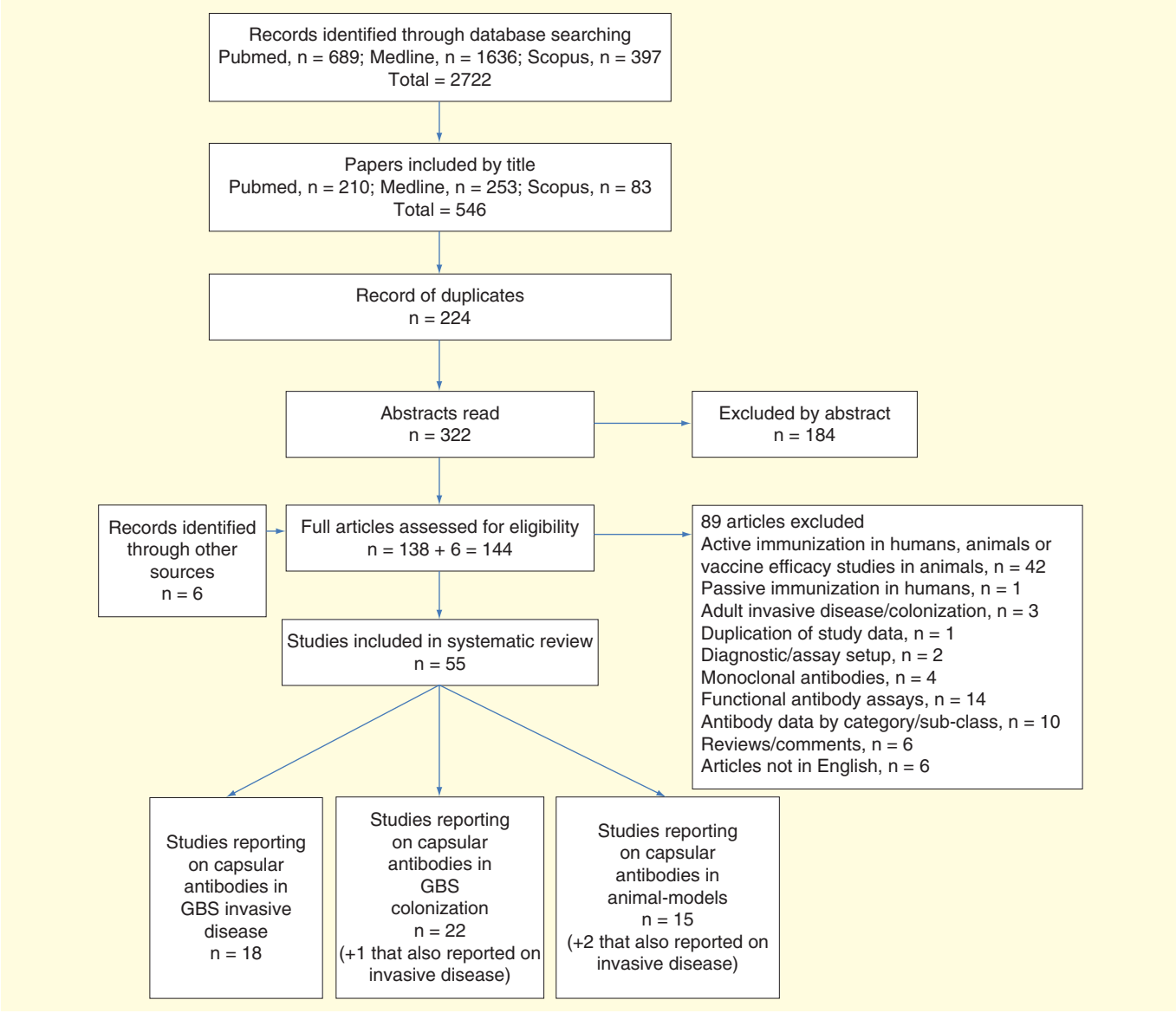

Figure 1. Flow diagram of selected studies reporting on capsular Group B *Streptococcus* antibodies.
GBS: Group B *Streptococcus*.

serotype III, indicating possible serotype-specific differences in the antibody concentration required to protect against invasive GBS disease [27,28].

More recently, a reanalysis of sera from a matched case–control study conducted by Baker and colleagues reported lower median serotypes Ia, III and V antibody concentrations in EOD cases compared with controls [30]. Using logistic regression, the relative risk of developing invasive GBS disease with an antibody level of ≥0.5 μg/ml was 0.11 (95% CI: 0.01–0.74) for serotype Ia, 0.09 (95% CI: 0.00–0.72) for serotype III and 0.29 (95% CI: 0.01–3.10) for serotype V. Also, using a Bayesian model, it was suggested that the risk of serotype III GBS disease decreased substantially with antibody concentrations ≥0.45 μg/ml. Consequently, a threshold of ≥1 μg/ml in the mother at birth was proposed as a putative measure of protection against invasive GBS disease in the newborn for serotypes Ia, III and V [30].

Figure 2A & 2B compares the proportion of invasive GBS disease cases and controls with an antibody level ≥2 μg/ml to serotypes Ia and III. An antibody level of ≥2 μg/ml was chosen as this was initially suggested by Baker and colleagues to protect against serotype III GBS disease [19]. Although these studies are not directly comparable due to differences in methodology and absence of standardized immunological assay, in a meta-analysis, the proportion of invasive GBS disease cases with a serotype-specific capsular antibody ≥2 μg/ml was generally lower than in controls. The odds of invasive GBS disease was 6.56 (95% CI: 2.10–20.55) and 2.38 (95% CI: 1.20–4.70) times greater in infants whose mothers had antibody levels <2 μg/ml for serotypes III and Ia, respectively.

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Table 1. Studies describing capsular antibody concentrations in mothers of infants with and without invasive group B *Streptococcus* disease.

Study (year) Country Study design	Case[†] serotyp (n)	EOD	LOD	Control[‡] serotype (n)	Assay used	GBS antibodies (total Ig or IgG)	Antibody levels (μg/ml) in cases with invasive GBS disease		Antibody levels (μg/ml) in healthy infant controls		Ref.
							Maternal antibody level (n)[¶]	*Newborn/infant antibody level (n)*	*Maternal antibody level (n)*	*Newborn/infant antibody level (n)*	
Baker *et al.* (1976) USA CC	III (7)	3	4	III (29)	RABA	III (Total Ig)	0 of 7 had detectable levels	0 of 5 had detectable levels	22 of 29 (76%) had detectable levels	3 of 3 (100%) had detectable levels	[13]
Baker *et al.* (1977)[#] USA CC	III (31)	9	22	III (43)	RABA	III (Total Ig)	1 (<1–26)[††] [n = 29]	Sepsis 0.65 (0.34–1.52)[††] [n = 8] Meningitis 0.45 (0.32–1.52)[††] [n = 16]	12 (<1–>40)[††] [n = 43]	NR	[14]
Baker *et al.* (1977) USA CC	III (17)	8	9	III (43)	RABA	III (Total Ig)	2 of 15 had detectable levels	0 of 17 had detectable levels	31 of 43 (72%) had detectable levels	NR	[15]
Wilkinson *et al.* (1978) USA Cohort	III (10)	NI	NI	III (4)[‡‡]	RI	III (Total Ig)	31.7[§§] [n = 4]	9.3[§§] [n = 8]	12.1[§§] [n = 2]	11.0[§§] [n = 4]	[16]
Christensen *et al.* (1980) Sweden CC	Ia (1) Ib (3) III (3)	4	3	Ia (4) Ib (3) II (1) III (8)	RPA	Ia, Ib, III (IgG)	1/7 (14%) had higher levels than controls	NR	12/13 (92%) had higher levels than cases	NR	[17]
Vogel *et al.* (1980) USA Cohort	Ia (2) Ib (8) II (4) III (40)	NI	NI	Ia (22) Ib (28) II (22) III (36)	IF	Ia, Ib, II, III (IgG)	Ia: 0%; Ib: 13%; II: 0%; III: 20% had detectable levels	NR	Ia: 59%; I b: 57%; II: 6%; III: 50% had detectable levels	NR	[18]
	III (111)	32	79	III (45)	RABA						[19]

Note: Lin 2001, and Lin 2004 used reference serum from Nabi Biopharmaceuticals, Feldman 1990 used reference serum from Carol Baker. Baker 1981 reported similar median antibody concentrations (0.4, range 0.3–1.6) for infants with LOD but the comparison was done only for EOD.
[†]Case, number of infants with invasive GBS disease stratified by disease serotype.
[‡]Control, number of GBS rectal/vaginal/cervical colonized mothers of healthy infant stratified by colonizing serotype.
[§]Cord/mother, maternal or cord blood samples were analyzed but not differentiated.
[¶][n =] indicates the number of samples analyzed.
[#]Denotes that some concentrations were derived from figures.
[††]Median (range).
[‡‡]Indicates GBS skin surface/throat colonization in the infant.
[##]Median (interquartile range).
[§§]Mean.
[¶¶]Geometric mean concentration.
CC: Case–control; ELISA: Enzyme linked immunosorbent assay; EOD: Early onset disease (0–6 days from birth); LOD: Late onset disease (7–90 days from birth); IF: Indirect immunofluorescent assay; Ig: Immunoglobulin; NI: Not indicated; NR: Not reported; RABA: Radioactive antigen binding assay; RI: Radioimmunoassay; RPA: Radiolabeled protein A.

Table 1. Studies describing capsular antibody concentrations in mothers of infants with and without invasive group B *Streptococcus* disease (cont.).

Study (year) Country Study design	Case[†] serotyp (n)	EOD	LOD	Control[‡] serotype (n)	Assay used	GBS antibodies (total Ig or IgG)	Antibody levels (μg/ml) in cases with invasive GBS disease		Antibody levels (μg/ml) in healthy infant controls		Ref.
							Maternal antibody level (n)[¶]	*Newborn/infant antibody level (n)*	*Maternal antibody level (n)*	*Newborn/infant antibody level (n)*	
Baker *et al.* (1981) USA CC						III (total Ig)	Sepsis 0.6 (0.3–40.3)[††] [n = 18] Meningitis 0.6 (0.3–1.55)[††] [n = 14]	Sepsis 0.4 (0.3–1.3)[††] [n = 18] Meningitis 0.3 (0.3–1.1)[††] [n = 14]	12.6 (0.3–40.3)[††] [n = 45]	5.8 (0.3–40.3)[††] [n = 45]	
Christensen *et al.* (1982) Sweden CC	Ia (2) Ib (4) II (2) III (8)	12	3	Ia (10) Ib (5) II (5) III (9)	RPA	Ia, Ib, II, III (IgG)	2/16 (13%) had higher levels than controls	NR	27/29 (93%) had higher levels than cases	NR	[20]
Klegerman *et al.* (1983) USA Cohort	Ia (11)	8	3	Ia (25)	ELISA	Ia (IgG)	(cord/mother)[§] 0.04 (<0.03–0.16)[††] [n = 11] 0/11 had levels ≥0.17μg/ml		9/25 (36%) had levels ≥1μg/ml	NR	[21]
Gotoff *et al.* (1984) USA Cohort	Ib (9)	5	4	Ib (25)	ELISA	Ib (IgG)	(cord/mother) 0.06 (<0.03–0.09)[††] [n = 9] 0/9 had levels ≥0.2μg/ml		0.15 (<0.02–4.7)[††] [n = 25] 11/25 (44%) had levels ≥0.2μg/ml	NR	[22]
Gray *et al.* (1985)[#] USA Cohort	II (15)	13	2	II (70)[‡‡]	ELISA	II (IgG)	(cord/mother) 1.77[§§] [n = 15] (range:0–4.5)		4.8[§§] [n = 70]	4.7[§§] [n = 70]	[23]
Gotoff *et al.* (1986) USA Cohort	III (42)	NI	NI	III (25)	ELISA	III (IgG)	(cord/mother) 0.05 (<0.02–0.3)[††] [n = 42]		0.78 (0.1–10.7)[††] [n = 25]	NR	[24]

Note: Lin 2001, and Lin 2004 used reference serum from Nabi Biopharmaceuticals, Feldman 1990 used reference serum from Carol Baker. Baker 1981 reported similar median antibody concentrations (0.4, range 0.3–1.6) for infants with LOD but the comparison was done only for EOD.
[†]Case, number of infants with invasive GBS disease stratified by disease serotype.
[‡]Control, number of GBS rectal/vaginal/cervical colonized mothers of healthy infant stratified by colonizing serotype.
[§]Cord/mother, maternal or cord blood samples were analyzed but not differentiated.
[¶][n =] indicates the number of samples analyzed.
[#]Denotes that some concentrations were derived from figures.
[††]Median (range).
[‡‡]Indicates GBS skin surface/throat colonization in the infant.
[##]Median (interquartile range).
[§§]Mean.
[¶¶]Geometric mean concentration.
CC: Case–control; ELISA: Enzyme linked immunosorbent assay; EOD: Early onset disease (0–6 days from birth); LOD: Late onset disease (7–90 days from birth); IF: Indirect immunofluorescent assay; Ig: Immunoglobulin; NI: Not indicated; NR: Not reported; RABA: Radioactive antigen binding assay; RI: Radioimmunoassay; RPA: Radiolabeled protein A.

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Table 1. Studies describing capsular antibody concentrations in mothers of infants with and without invasive group B *Streptococcus* disease (cont.).

Study (year) Country Study design	Case[†] serotyp (n)	EOD	LOD	Control[‡] serotype (n)	Assay used	GBS antibodies (total Ig or IgG)	Antibody levels (μg/ml) in cases with invasive GBS disease		Antibody levels (μg/ml) in healthy infant controls		Ref.
							Maternal antibody level (n)[¶]	*Newborn/infant antibody level (n)*	*Maternal antibody level (n)*	*Newborn/infant antibody level (n)*	
Feldman *et al.* (1990)[#] UK Cohort	III (19)	NI	NI	III (2)[‡‡] Other (8)[‡‡]	ELISA	III (IgG)	0.6[§§] [n = 19] (range: <0.5–4.3)	NR	15[§§] [n = 10] (range: <0.5–>100)	NR	[25]
Gray *et al.* (1990)[#] USA Cohort	Ia (8)	NI	NI	Ia/c (94)[‡‡]	ELISA	Ia (IgG)	(cord/mother) 1.0[§§] [n = 8]		9.1(8.3)[§§] [n = 94]	6.5 (5.1)[§§] [n = 94]	[26]
Lin *et al.* (2001) USA CC	Ia (53)	53	0	Ia (336)[‡‡]	ELISA	Ia (IgG)	0.32[¶¶] [n = 49]	0.22[¶¶] [n = 49]	0.65[¶¶] [n = 326]	0.52[¶¶] [n = 323]	[27]
Lin *et al.* (2004) USA CC	III (29)	29	0	III (330)[‡‡]	ELISA	III (IgG)	2.73[¶¶] [n = 28]	2.03[¶¶] [n = 27]	4.27[¶¶] [n = 306]	3.29[¶¶] [n = 312]	[28]
Matsubara *et al.* (2002) Japan Cohort	VIII (4)	4	0	VIII (13)	ELISA	VIII (IgG)	0.41[¶¶] [n = 4]	0.49[¶¶] [n = 4]	5.53[¶¶] [n = 13]	NR	[29]
Baker *et al.* (2013) USA CC	Ia (17) III (9) V (7)	33	0	Ia (51) III (27) V (21)	ELISA	Ia, III, V (IgG)	0.20 (0.06–1.68)[##] [n = 17] 0.06 (0.02–0.12)[##] [n = 9] 0.09 (0.04–0.80)[##] [n = 7]	NR	1.83 (0.20–5.54)[##] [n = 51] 1.64 (0.14–5.51)[##] [n = 27] 0.53 (0.07–1.0)[##] [n = 21]	NR	[30]

Note: Lin 2001, and Lin 2004 used reference serum from Nabi Biopharmaceuticals, Feldman 1990 used reference serum from Carol Baker. Baker 1981 reported similar median antibody concentrations (0.4, range 0.3–1.6) for infants with LOD but the comparison was done only for EOD.
[†]Case, number of infants with invasive GBS disease stratified by disease serotype.
[‡]Control, number of GBS rectal/vaginal/cervical colonized mothers of healthy infant stratified by colonizing serotype.
[§]Cord/mother, maternal or cord blood samples were analyzed but not differentiated.
[¶][n =] indicates the number of samples analyzed.
[#]Denotes that some concentrations were derived from figures.
[††]Median (range).
[‡‡]Indicates GBS skin surface/throat colonization in the infant.
[##]Median (interquartile range).
[§§]Mean.
[¶¶]Geometric mean concentration.
CC: Case–control; ELISA: Enzyme linked immunosorbent assay; EOD: Early onset disease (0–6 days from birth); LOD: Late onset disease (7–90 days from birth); IF: Indirect immunofluorescent assay; Ig: Immunoglobulin; NI: Not indicated; NR: Not reported; RABA: Radioactive antigen binding assay; RI: Radioimmunoassay; RPA: Radiolabeled protein A.

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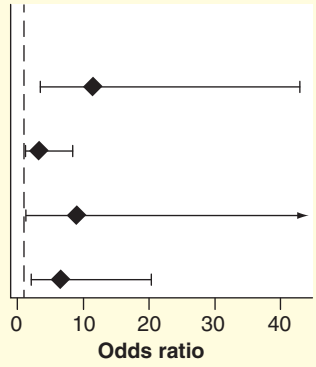
Study (year)	Cases	Controls	OR (95% CI)	p-value
Baker, (1981) [19]	6/32 (19%)	33/45 (73%)	11.45 (3.54, 43.02)	<0.001
Lin, *et al.* (2004) [28]	15/26 (58%)	116/143 (81%)	3.16 (1.16, 8.26)	0.023
Baker, *et al.* (2013) [30]	0/9 (0%)	~12/27 (45%)	9.00 (1.23. Inf)	0.023
Combined	21/67 (31%)	161/215 (75%)	6.56 (2.10, 20.55)	0.001



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Study (year)	Cases	Controls	OR (95% CI)	p-value
Klegerman, (1983) [21]	0/8 (0%)	5/25 (20%)	2.45 (0.29, Inf)	0.448
Lin, *et al.* (2001) [27]	7/45 (16%)	88/319 (28%)	2.06 (0.87, 5.69)	0.151
Baker, *et al.* (2013) [30]	~4/17 (24%)	~24/51 (47%)	2.85 (0.75, 13.63)	0.151
Combined	11/70 (16%)	117/395 (30%)	2.38 (1.20, 4.70)	0.013

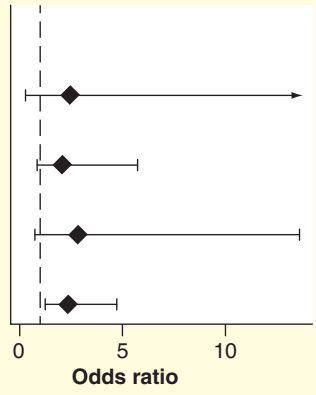

Figure 2. Proportion of mothers of infants with a capsular antibody concentration (A) ≥2 μg/ml for serotype III, a meta-analysis; and (B) ≥2 μg/ml for serotype Ia, a meta-analysis.
Using exact conditional logistic regression, OR were used to compare the proportion of mothers of infants with invasive GBS disease (cases) versus mothers of wellbaby controls with an antibody concentration ≥2 μg/ml.
~ In the study by Baker 2013, we derived the number of cases and controls from the reverse cumulative plots.
GBS: Group B *Streptococcus*; OR: Odds ratio.

Association between GBS capsular antibody levels in GBS colonized & noncolonized mothers & infants

Linden and colleagues suggested that urogenital carriage may induce antibody production [35]. All studies, except one, were cross-sectional and measured antibody levels at delivery or during pregnancy [33]. In general, serotype-specific antibody levels were higher in colonized compared with noncolonized pregnant women [29,31–33,35–37,43,49,53,55–57] and colonized compared with noncolonized newborns [40,41]. In a few studies, however, antibody levels were similar between colonized and noncolonized women, including for serotype III [35,53,56], Ib [35,55] and Ia [53] (Table 2).

Discussion

An association between low serotype-specific antibody levels in mothers and the occurrence of invasive GBS disease in infants is reported in most studies. The different antibody assays and the lack of standardized reference ranges for capsular-specific IgG, despite the existence of reference serum (available from Dr Carol Baker and Nabi Biopharmaceuticals), makes it difficult to select a specific antibody level that may be used as a reliable sero-correlate of protection. In addition, studies have not independently explored the association between antibody levels and LOD and were mainly focused on serotypes Ia and III. Further prospective cohort studies, in diverse settings, are likely needed to establish sero-correlate of protection for the five most prevalent GBS serotypes. Such studies should also measure functional antibody, using opsonophagocytic activity assays (OPA), to improve the elucidation of the sero-correlate of protection against invasive GBS disease. This could contribute to the licensure pathway of a GBS vaccine without needing to undertake large-scale efficacy trials in pregnant women.

In 1981, Baker and colleagues suggested an antibody threshold of >2 μg/ml against serotype III as a correlate of protection [13]. This antibody threshold had subsequently been supported in other studies using ELISA [24,25]. Although RABA and ELISA demonstrated significant correlation [74,75], ELISA has the advantages of being able to detect immunoglobulin subclasses and has a lower detection limit for measuring antibodies compared with RABA [74]. More recently, a threshold of ≥1 μg/ml in women at birth has been proposed as a correlate for protection in newborns against serotypes Ia and III invasive GBS disease for EOD [30]. This is, however, in contrast to the

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Table 2. Studies describing capsular antibody concentrations in colonized women or newborns with group B *Streptococcus* and noncolonized.

Study (year) Country Study design	GBS colonization		Assay used	GBS antibodies (total Ig or IgG)	Antibody levels (μg/ml) in colonized		Antibody levels (μg/ml) in non-colonized		Ref.
	Colonized serotype (n)	Non-colonized[†] (n)			*Maternal antibody level*	*Newborn/ infant antibody level*	*Maternal antibody level*	*Newborn/ Infant antibody level*	
Comparison between colonized[‡] and noncolonized pregnant women									
Beachler *et al.* (1979) USA Cohort	III (13) Other (18)	65	RABA	III (Total Ig)	All: 1.1 (0.4–>40.3)[§] III: 4.8 (0.6–>40.3)[§]	NR	1 (0.4–>40.3)[§]	NR	[31]
Cleat *et al.* (1980) UK CC	Not specified (10)	10	ELISA	Ia, Ib, Ic, II, III (IgG)	Ia: 1.600[#]; Ib:1.533[#]; Ic: 1.076[#]; II:1.449[#]; III: 1.533[#]	Ia:0.910[#]; Ib:0.972[#]; Ic:0.627[#]; II:0.774[#]; III:0.824[#]	Ia: 1.062[#]; Ib:0.976[#]; Ic: 0.714[#]; II:0.894[#]; III: 1.032[#]	Ia: 0.497[#]; Ib: 0.521[#]; Ic: 0.374[#]; II: 0.461[#]; III: 0.516[#]	[32]
Baker *et al.* (1980) USA Cohort	III (12) Other (9)	70	RABA	III (Total Ig)	III: 5.5 (0.4–40.3)[§] Other: 0.6 (0.3–22.2)[§]	NR	0.7 (0.4–40.3)[§]	NR	[33]
Linden *et al.* (1982) Sweden CC	Ia(20) II (10) III (23)	20	RPA	Ia, Ib,II, III (IgG)	Had higher levels for Ia and II ($p<0.05$) than non-colonized	NR	NR	NR	[35]
Anthony *et al.* (1984) USA Cohort	III (15)	13	ELISA	III (IgG)	0.21[††] (range: <0.05–1.07)	0.25[††] (range: 0.10–0.88)	0.08[††] (range: <0.05–0.20)	0.08[††] (range: <0.05–0.26)	[36]
Skidmore *et al.* (1985) Canada Cohort	Ia(7); Ib(8); Ic(9); II (9); III (14); NT (10)	349	IF	Ia, Ib, Ic, II, III (IgG)	Ia: 100%; Ib: 75%; Ic: 78%; II: 89%; III: 100% had detectable levels [n = 47][‡‡]	NR	Ia: 71%; Ib:36%; Ic: 51%; II: 66%; III: 60% had detectable levels [n = 358]	NR	[37]

[†]Noncolonized, no microbiological evidence of GBS colonization.
[‡]Colonized women, GBS rectal/vaginal/cervical colonization peri-partum or postdelivery in a women.
[§]Median (range).
[¶]Denotes that concentrations were derived from figures.
[#]Mean (SD).
[††]Geometric mean concentration.
[‡‡][n =] indicates the number of samples analyzed as they differ from numbers reported for GBS colonization.
[§§]kU/ml, a reference value of 640 arbitrary kU was assigned to each milliliter of GBS IgG.
[¶¶]Colonized newborn, GBS skin surface/throat colonization in a healthy newborn.
CC: Case–control; ELISA: Enzyme linked immunosorbent assay; IF: Indirect immunofluorescent assay; NR: Not reported; NT: Not typable; RABA: Radioactive antigen binding assay; RPA: Radiolabeled protein A.

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Table 2. Studies describing capsular antibody concentrations in colonized women or newborns with group B *Streptococcus* and noncolonized (cont.).

Study (year) Country Study design	GBS colonization		Assay used	GBS antibodies (total Ig or IgG)	Antibody levels (μg/ml) in colonized		Antibody levels (μg/ml) in non-colonized		Ref.
	Colonized serotype (n)	Non-colonized[†] (n)			*Maternal antibody level*	*Newborn/ infant antibody level*	*Maternal antibody level*	*Newborn/ Infant antibody level*	
Comparison between colonized[‡] and noncolonized pregnant women (cont.)									
Ratei *et al.* (1990) Germany Cohort	Not specified (23)	21	Indirect ELISA	Ia, Ib, II, III	Ia:0.74 (0.32)[#] Ib:1.02 (0.38)[#] II:0.17 (0.14)[#] III:0.63 (0.25)[#]	NR	Ia: 0.53 (0.18)[#] Ib: 0.67 (0.32)[#] II: 0.12 (0.10)[#] III: 0.40 (0.17)[#]	NR	[43]
Hordnes *et al.* (1996) Norway Cohort	Not specified (34)	166	ELISA	Ia, II, III (IgG)	Ia:83.7 (24.2–252.6)[§] II:99.2 (36.9–828.5)[§] III:118.3 (40.8–246.9)[§] (in kU/ml)[§§] [n = 18]	NR	Ia: 58.8(9.7–608.2)[§] II: 98.2(12.3–249.8)[§] III: 85.0(12.3–249.8)[§] (in kU/ml) [n = 67]	NR	[49]
Suara *et al.* (1998) Gambia Cohort	Ia/c (5); Ib (1); II (7); III (2); IV (1); V (10); NT (1)	109	RABA	Ia, III (Total Ig)	III:1.1 (0.50)[††] [n = 27]	III: 0.89 (0.52)[††] [n = 27]	III: 1.0 (0.50)[††] [n = 97]	III:0.75 (0.39)[††] [n = 97]	[53]
Campbell *et al.* (2000)[¶] USA Cohort	Ia (74); Ib (25); II (67); III (71); V (54)	193	ELISA	Ia, Ib, II, III, V (IgG)	Ia: 2.80[§] Ib: 0.25[§] II: 0.50[§] III: 1.00[§] V: 0.20[§]	NR	Ia: 0.20[§] Ib: 0.15[§] II: 0.30[§] III: 0.15[§] V: 0.10[§]	NR	[55]
Davies *et al.* (2001) Canada Cohort	Ia (17);Ib(8); II (13); III (14); IV (3); V (15); NT (5)	153	ELISA	Ia, Ib, II, III, V (IgG)	Ia: 2.41 (0.03–134.32)[§] Ib: 0.98 (0.05–18.23)[§] II: 4.63 (0.09–34.61)[§] III: 0.07 (0.03–49.51)[§] V: 0.96 (0.08–48.72)[§]	NR	Ia: 0.20(0.03–70.75)[§] Ib: 0.20(0.05–28.78)[§] II: 0.17(0.03–19.88)[§] III: 0.08(0.03–17.91)[§] V: 0.06(0.01–44.66)[§]	NR	[56]

[†]Noncolonized, no microbiological evidence of GBS colonization.
[‡]Colonized women, GBS rectal/vaginal/cervical colonization peri-partum or postdelivery in a women.
[§]Median (range).
[¶]Denotes that concentrations were derived from figures.
[#]Mean (SD).
[††]Geometric mean concentration.
[‡‡][n =] indicates the number of samples analyzed as they differ from numbers reported for GBS colonization.
[§§]kU/ml, a reference value of 640 arbitrary kU was assigned to each milliliter of GBS IgG.
[¶¶]Colonized newborn, GBS skin surface/throat colonization in a healthy newborn.
CC: Case–control; ELISA: Enzyme linked immunosorbent assay; IF: Indirect immunofluorescent assay; NR: Not reported; NT: Not typable; RABA: Radioactive antigen binding assay; RPA: Radiolabeled protein A.

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Table 2. Studies describing capsular antibody concentrations in colonized women or newborns with group B *Streptococcus* and noncolonized (cont.).

Study (year) Country Study design	GBS colonization		Assay used	GBS antibodies (total Ig or IgG)	Antibody levels (μg/ml) in colonized		Antibody levels (μg/ml) in non-colonized		Ref.
	Colonized serotype (n)	Non-colonized[†] (n)			*Maternal antibody level*	*Newborn/ infant antibody level*	*Maternal antibody level*	*Newborn/ Infant antibody level*	
Comparison between colonized[‡] and noncolonized pregnant women (cont.)									
Matsubara *et al.* (2002/3) Japan Cohort	Ia (4); Ib(6); II (4); III(5); V (3); VI (9); VIII (13); NT (4)	535	ELISA	VI, VIII (IgG)	VI: 1.80 (8.63)[††] VIII: 5.53 (2.79)[††]	NR	VI: 0.41 (0.23)[††] VIII: 1.53 (0.32[††]	NR	[29,57]
Comparison between colonized[¶¶] and noncolonized newborns									
Gray *et al.* (1988) USA Cohort	II (29) Other (31)	30	ELISA	II (Total Ig)	II: 3.3 (2.2)[#] Other: 4.7 (5.7)[#]	II: 2.0 (1.9)[#] Other 1.8 (1.7)[#]	2.2 (1.5)[#]	0.9 (1.0)[#]	[40]
Gray *et al.* (1989) USA Cohort	III (114)	54	ELISA	III (IgG)	NR	14/114 (12%) had levels >2μg/ml	NR	2/54 (4%) had levels >2μg/ml	[41]

[†]Noncolonized, no microbiological evidence of GBS colonization.
[‡]Colonized women, GBS rectal/vaginal/cervical colonization peri-partum or postdelivery in a women.
[§]Median (range).
[¶]Denotes that concentrations were derived from figures.
[#]Mean (SD).
[††]Geometric mean concentration.
[‡‡][n =] indicates the number of samples analyzed as they differ from numbers reported for GBS colonization.
[§§]kU/ml, a reference value of 640 arbitrary kU was assigned to each milliliter of GBS IgG.
[¶¶]Colonized newborn, GBS skin surface/throat colonization in a healthy newborn.
CC: Case–control; ELISA: Enzyme linked immunosorbent assay; IF: Indirect immunofluorescent assay; NR: Not reported; NT: Not typable; RABA: Radioactive antigen binding assay; RPA: Radiolabeled protein A.

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10-fold higher antibody concentration proposed by Lin and colleagues [27,28]. Such differences may be due to differences in control selection and serological assays [30] and therefore, highlight the need to establish a standardized assay to measure GBS capsular antibodies.

Quantitative determination of IgG capsular antibodies transferred from mother to infant is unlikely to be the only determinant of the correlate of protection; and this should probably be supplemented with OPA to measure antibody functionality [76]. The functionality of naturally occurring capsular antibodies in infants with invasive GBS disease and in pregnant women has been evaluated by some [76–79]; however, whether *in vitro* evaluation of opsonophagocytosis (i.e., phagocytosis initiated by an opsonin) in newborn serum will translate into protection is unclear, due to the OPA assay including exogenous components such as complement that may be physiologically deficient in the newborn [80]. Although opsonization of serotype III by naturally occurring antibodies in colonized and noncolonized women was similar [76], natural acquired capsular antibody to serotype III in infants with invasive disease due to the homotypic serotype did not demonstrate opsonophagocytosis activity [78]. An association between higher antibody concentrations in healthy newborns and donor sera have however been shown to be associated with opsonophagocytosis [79,81], and OPA activity was greater post-GBS vaccination than prevaccination [82,83].

A small proportion of infants developed invasive GBS disease despite high antibody levels at birth in their mothers [14,19]. This paradox may be due to maternal acquisition of GBS occurring just prior to delivery, resulting in a rapid increase of poorly functional antibodies being transferred to the fetus, which are inadequate to protect the newborn against invasive disease. The other possibility is that studies using RABA may have measured elevated IgM, rather than IgG, in the sera of mother's blood with recently acquired GBS infection/colonization. Furthermore, antibody concentrations required to protect against different serotypes may vary, as demonstrated in experimental animal model and *in vitro* studies [21,76].

Due to various confounding factors, especially the timing of recto-vaginal acquisition of GBS, it is difficult to gauge the significance of an association between GBS antibody levels and colonization. Although studies reported higher serotype-specific capsular antibody levels in pregnant women colonized by the homotypic serotype compared with noncolonized women, these studies were cross-sectional and unable to address the effect that the timing of GBS acquisition had on capsular antibody levels. Further longitudinal cohort studies in pregnant women are required to evaluate whether high serotype-specific capsular antibody level (and OPA titers) during early stages of pregnancy is able to reduce the risk of later recto-vaginal acquisition of homotypic serotype colonization. Also, these studies could address the effect that capsular antibody has on the duration of colonization and the antibody kinetics associated with new colonization episodes. Such studies could inform whether capsular antibody induced through vaccination can impact on recto-vaginal colonization in the women, which would also be important for protection of premature children against invasive GBS disease in the absence of them benefiting from transplacental antibody acquisition that mainly occurs beyond 34 weeks of gestational age.

Conclusion

The potential of preventing invasive GBS disease among young infants through maternal immunization with multivalent serotype-specific GBS-CV is supported by the observation of an inverse association between maternal and/or infant serotype-specific capsular antibody levels and the risk of invasive GBS disease. Further studies, using standardized methods, which measure antibody concentrations as well as OPA in the women and their infants, are warranted to establish serological correlates of protection against EOD and LOD. Furthermore, the association between maternal capsular antibody and risk of subsequent GBS colonization during pregnancy also warrants further investigation, both in terms of natural and vaccine-induced capsular antibody.

Expert commentary

GBS is currently the leading cause of sepsis and meningitis in neonates despite adequate preventative strategies in developed countries. IAP to screened GBS colonized women between 35 and 37 weeks' gestation has been recommended by the CDC. This preventative strategy has resulted in a more than 80% decline in the incidence of early-onset GBS disease EOD in the USA since 1990. In developing countries, however, such strategies have not been utilized due to logistical and resource limitations. Furthermore, a large proportion of deliveries in developing countries tend to occur outside the hospital setting. These limitations in developing countries, coupled with the failure of IAP in reducing the incidence of LOD, have favored the need for an alternate strategy in the prevention of invasive GBS disease.

Vaccinating pregnant women against GBS infection may protect infants from invasive GBS disease. Maternal antibodies transferred from mother to fetus have been proposed to be protective against GBS disease, and deficiencies in maternal antibody have been associated with disease in cases compared with controls. A trivalent GBS-CV composed of capsular epitopes from serotypes Ia, Ib and III has completed Phase II evaluation among pregnant women in Europe, North America and Africa. These serotypes cause 70–80% of all invasive GBS disease in early infancy. In order to license this vaccine, a large Phase III efficacy trial will be required. This would require a sample size of over 60,000 pregnant women to be conducted in a GBS prevention naive setting with a high incidence of disease, which will incur tremendous logistical challenges. An alternate pathway to licensure of some new vaccines is based on immunological endpoints for those diseases for which immunological correlates of protection have been established from previous vaccine studies or through sero-epidemiological studies. This would then be followed by Phase IV studies to establish vaccine effectiveness. This strategy of licensure through a correlate of

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protection is not novel and has been previously adopted in licensure of other vaccines. Primarily, an antibody concentration that would reduce the risk of disease would need to be adequately determined and thus, a systematic review of studies reporting on the association of capsular antibodies and invasive GBS disease in infants and colonization in women or newborns was undertaken.

This review has highlighted some of the deficiencies in the methods used to determine sero-correlates of protection. Only a few studies have addressed the association between capsular GBS antibody levels and invasive disease, focusing mainly on serotypes Ia and III. Furthermore, differences in study design, age range of invasive disease cases and antibody assay methods (including absence of an internationally standardized reference serum) limited the comparability of studies as well as the interpretation of the serologic outputs proposed as putative measures of protection.

Five-year view

Licensure of a trivalent GBS-CV could be achieved in the next few years. Sero-epidemiological studies describing putative levels of protection to various serotypes can expedite this process. We recommend that further studies be carried out in a diversity of settings using standardized methods and assays, including the use of standardized reference serum.

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Key issues

- Group B *Streptococcus* (GBS) is the leading cause of neonatal sepsis and meningitis, despite current preventative measures.
- Vaccinating pregnant women against GBS infection may protect infants from invasive GBS disease and may be an alternative strategy.
- Establishing sero-correlates of protection against specific capsular epitopes causing disease may favor the licensure of the GBS polysaccharide-protein conjugate vaccine.
- Limited studies using nonstandardized methods have identified an association between low capsular GBS antibodies and the risk of developing invasive disease; however, no established measure of protection can be drawn from these.
- Further studies using standardized methods are warranted in a diversity of settings to establish a sero-correlate of protection against early- and late-onset disease.

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RESEARCH ARTICLE

Burden of Invasive Group B *Streptococcus* Disease and Early Neurological Sequelae in South African Infants

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Abstract

Introduction

Group B *Streptococcus* (GBS) is a leading cause of neonatal sepsis and meningitis. We aimed to evaluate the burden of invasive early-onset (0–6 days of life, EOD) and late-onset (7–89 days, LOD) GBS disease and subsequent neurological sequelae in infants from a setting with a high prevalence (29.5%) of HIV among pregnant women.

Methods

A case-control study was undertaken at three secondary-tertiary care public hospitals in Johannesburg. Invasive cases in infants <3 months age were identified by surveillance of laboratories from November 2012 to February 2014. Neurodevelopmental screening was done in surviving cases and controls at 3 and 6 months of age.

Results

We identified 122 cases of invasive GBS disease over a 12 month period. Although the incidence (per 1,000 live births) of EOD was similar between HIV-exposed and HIV-unexposed infants (1.13 vs. 1.46; p = 0.487), there was a 4.67-fold (95%CI: 2.24–9.74) greater risk for LOD in HIV-exposed infants (2.27 vs. 0.49; p<0.001). Overall, serotypes Ia, Ib and III constituted 75.8% and 92.5% of EOD and LOD, respectively. Risk factors for EOD included offensive draining liquor (adjusted Odds Ratio: 27.37; 95%CI: 1.94–386.50) and maternal GBS bacteriuria (aOR: 8.41; 95%CI: 1.44–49.15), which was also a risk-factor for LOD (aOR: 3.49; 95%CI: 1.17–10.40). The overall case fatality rate among cases was 18.0%.

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The adjusted odds for neurological sequelae at 6 months age was 13.18-fold (95%CI: 1.44–120.95) greater in cases (13.2%) than controls (0.4%).

Discussion

The high burden of invasive GBS disease in South Africa, which is also associated with high case fatality rates and significant neurological sequelae among survivors, is partly due to the heightened risk for LOD in infants born to HIV-infected women. An effective trivalent GBS conjugate vaccine targeted at pregnant women could prevent invasive GBS disease in this setting.

Introduction

There has been slow progress in the decline of neonatal mortality rates in developing countries where severe bacterial infections accounted for an estimated 680 000 neonatal deaths in 2012 [1, 2]. Group B *streptococcus* (GBS) has been recognized as a leading contributor of neonatal sepsis and meningitis in developed countries, even though intra-partum antibiotic prophylaxis (IAP) is routinely administered to GBS-colonized pregnant women [3–5]. Additionally, neurodevelopmental problems are seen in about 22–50% of infants surviving GBS meningitis [6–10]. In developing countries, such as South Africa, where GBS screening and IAP during pregnancy is not standard-of-care, the mortality rate from invasive GBS disease is higher than in developed countries (10–60% compared to 7–11%) [11–13]. Furthermore, in South Africa, the high prevalence of maternal HIV-infection (29.5%) [14] is likely to aggravate the burden of invasive GBS disease [15]. We therefore prospectively determined the incidence of invasive GBS disease, including the effect of maternal HIV-infection on disease burden in infants born in Johannesburg. Furthermore, we evaluated risk factors for invasive GBS disease and assessed early neurodevelopmental sequelae in GBS-affected infants and healthy controls.

Methods

Between November 2012 and February 2014, we undertook a case-control study at the three largest academic hospitals in Johannesburg; namely Chris Hani Baragwanath Academic Hospital (CHBAH), Charlotte Maxeke Johannesburg Academic Hospital and Rahima Moosa Mother and Child Hospital. The standard-of-antenatal care for the prevention of invasive GBS in neonates does not include universal screening for recto-vaginal GBS colonization during pregnancy although IAP is provided to women who have risk factors such as maternal fever and prolonged rupture of membranes ($\geq$18 hours prior to delivery). Blood and cerebrospinal fluid (CSF) cultures are routinely performed in infants admitted with suspected sepsis or meningitis. HIV infection testing is routinely performed in pregnant women and confirmed using two independent rapid antibody screening tests [16]. Pregnant women with a CD4+ lymphocyte count >350 cells/mm^3 and WHO stage 1 and 2 received antiretroviral prophylaxis with zidovudine (AZT); whilst those with CD4+ lymphocyte count $\leq$350 cells/mm^3 or WHO stage 3 or 4 were initiated on triple antiretroviral therapy (ART). From April 2013, all pregnant women irrespective of CD4+ lymphocyte count were initiated on ART [16, 17].

Invasive GBS disease (cases) were defined as an infant <90 days of age in whom GBS was cultured from blood, CSF or other normally sterile sites; or when GBS was identified in CSF by latex agglutination. Cases were identified by ZD through daily surveillance of the pediatric

wards and microbiology services at the three hospitals. Early-onset disease (EOD) was defined when GBS was isolated in infants younger than seven days of life, and infants between 7–89 days of age with GBS disease were regarded as having late-onset disease (LOD).

Control subjects were matched for: (i) gestational age to term, or within 2 weeks for cases born <37 weeks gestation, (ii) maternal HIV-infection status, (iii) maternal age (within 2.5 years of the case mother's age), and (iv) enrollment within 0–6 days after birth for EOD cases and within 14 days (but >7 days of life) of chronological age for LOD cases. Controls for EOD were selected from admission and labor wards at CHBAH, whereas controls for LOD were identified through the birth registries and contacted telephonically for possible study-enrolment. For cases born at ≥34 weeks gestational age, at least 5 controls (mean: 7; range: 5–14) were matched for EOD and 3 controls (mean: 5; range: 3–7) for LOD. For cases born at <34 weeks gestational age, at least one control (mean: 2; range: 1–5) was matched for EOD and at least one control (mean: 2, range: 1–4) for LOD. All controls were clinically well at enrolment, and followed up to confirm they did not develop invasive GBS disease.

Cases and controls were followed up at 3 and 6 months of the infant's chronological age. These visits were carried out by either one of three trained research assistants or by ZD. At these visits, the infant's underwent neurological and development examinations and were screened using the Denver Developmental Screening Test II (Denver-II). The Denver-II makes a valuable screening tool (83% sensitivity) with a high degree of test-retest and inter-examiner reliability [18, 19]. The Denver-II tests 4 domains; gross-motor, fine-motor, language and personal-social. Each test item is represented horizontally as a percentile age range (25–90%) for which it is normally estimated that the item can be achieved. A "fail" or "refusal" by the infant in an item to the left of the age line is classified as a "delay", whilst a "fail" or "refusal" by the infant in an item through the 75–90% age percentile is classified as a "caution". The final result was then scored as "normal" (no delays or 1 caution) or "suspect/abnormal" (≥2 cautions or ≥1 delay) in each of the four domains. We defined neurological sequelae as an abnormal Denver-II developmental screening test for any of the four domains or hypertonia and/or hyperreflexia detected on examination. Infants with developmental delay were referred to occupational, physical and/or speech therapists. Visual and hearing assessments were not routinely tested on participants.

Laboratory methods

GBS was isolated from blood samples using the Bact/Alert microbial system (Organon Teknika, Durham, NC). Positive specimens were subsequently plated on blood or chocolate agar incubated both aerobically and at 35 degrees under 5–10% CO2, and observed for colony growth for 72 hours. Gram-staining was performed on CSF samples, which were also plated onto blood or chocolate agar plates, inoculated into an enrichment broth (Brain Heart Infusion, Diagnostics Media Production) and observed for colony growth for 72 hours. Specimens were also analyzed by a GBS antigen agglutination test if the CSF cell counts were suggestive of bacterial meningitis. Positive GBS isolates were serotyped and stored.

Although screening for maternal GBS colonization is not a routine investigation in Johannesburg, maternal colonization status was determined for participants enrolled in the study by separately swabbing the lower vagina and rectum using Rayon tipped swabs and charcoal-free Amies transport medium (Medical Wire Equipment Co. Ltd. Cat: MW170). In addition, a mid-stream urine specimen was also cultured. Mothers of cases and controls were swabbed at the time of enrolment, while controls matched to EOD were swabbed immediately after delivery. Swabs were plated onto CHROMAgar StrepB plates (Media Mage Cat: M10155) which were incubated at 37°C for 18–24 hours in aerobic conditions and examined for growth of

mauve GBS-like colony morphologies. Identified colonies were subjected to further confirmatory tests, such as the catalase test, growth on bile esculin agar, inability to hydrolyze esculin, Christie Atkinson Munch-Petersen (CAMP) test and B antigen latex agglutination test [20]. Serotyping for GBS types Ia, Ib, II to IX was performed using latex agglutination (Statens Serum Institute, SSI, Sweden) [21]. Non-typeable and discordant isolates were further characterized by a single-plex PCR method for serotypes Ia, Ib, II, III, IV and V using primer sequences described by Poyart et al. [22].

Statistical analysis

The incidence (per 1,000 live births) of invasive GBS disease over a twelve month period was calculated as the number of cases (EOD or LOD) in black-African infants that specifically resided in regions D and G of the Johannesburg metropolitan area. We only included black African infants with GBS disease residing in these specified regions because the care-givers of these infants predominantly access health care at either CHBAH or RMMCH. We did not undertake incidence calculation for non-black African infants or black-African infants not residing in regions D and G because these infants were likely to utilize other health care facilities not under surveillance in the study. There were 31504 live births over 12 months in regions D and G; 8827 (28%) infants were born to HIV-infected women [23].

For proportions, Chi-square or Fischer's exact test were used to compare demographic and clinical characteristics between cases of EOD and LOD. Medians were reported for non-parametric variables and compared using the Wilcoxon rank-sum (Mann-Whitney) test. Serotype distributions were reported as proportions of the total number of cases serotyped and stratified by EOD and LOD.

Univariate analysis was used to identify risk factors for invasive GBS disease, predictors of infant mortality and to compare neurological sequelae. For the multivariate analysis, adjusted odds ratios (aOR) using conditional logistic regression was used to adjust for variables with p-values <0.15 detected by univariate analysis. For the identification of risk factors predisposing to invasive GBS disease, we also included gestational age, maternal age and HIV status. For neurological sequelae, we adjusted for factors that may impact on neurodevelopment; including, gender, gestational age, birth weight <2500 grams, perinatal asphyxia, mechanical ventilation, infant HIV-exposure status and previous non-GBS-related hospitalizations. Data was analyzed using STATA version 13.1 (College Station, Texas, USA). Two-tailed p-values <0.05 were considered statistically significant. The study was approved by the University of Witwatersrand Human Research Ethics Committee (HREC number: M120963). Written informed consent was obtained from mothers of infants at enrolment for participation in the study.

Results

There were 122 infants (<90 days-of-age) with invasive GBS disease over a 12 month period, including 82 (67.2%) at CHBAH, 22 (18.0%) at CMJAH and 18 (14.8%) at RMMCH. Most infants (n = 116; 95.1%) were of black-African descent and 48 (39.4%) of all infants were born to HIV-infected mothers. Sixty six (54.1%) infants had EOD, of which 63 (95.5%) were identified within the first 24 hours of life. The predominant clinical presentation was sepsis (97.0%) and meningitis (58.9%) in infants with EOD and LOD, respectively (Table 1).Overall, 44 (36.1%) cases occurred in infants born before 37 completed gestational weeks; EOD occurred significantly more commonly than LOD in prematurely-born infants (45.4% versus 25.0%; p = 0.019; Table 1). Recurrence of invasive GBS disease occurred in two infants (1.6%), and one case and one control were diagnosed as HIV-infected at 6 weeks of age. Group B Streptococcus was cultured in 119 (97.5%) cases, whilst 3 (2.5%) cases of meningitis were identified on GBS latex

Table 1. Demographic characteristics of infants with invasive Group B *Streptococcal* (GBS) disease.

	All cases, n = 122	EOD[1], n = 66	LOD[2], n = 56	OR(95%CI)[3]	p-value[4]
Gestational Age					
≥37 weeks	78 (63.9)	36 (54.6)	42 (75.0)	0.40 (0.17–0.93)	0.019
<37 - ≥34 weeks	14 (11.5)	8 (12.1)	6 (10.7)	1.15 (0.32–4.31)	0.808
<34 weeks	30 (24.6)	22 (33.3)	8 (14.3)	3.00 (1.13–8.56)	0.015
Birth Weight					
≥2500 grams	77 (63.1)	38 (57.6)	39 (69.6)	0.59 (0.26–1.33)	0.169
1500–2499 grams	27 (22.1)	14 (21.2)	13 (23.2)	0.89 (0.35–2.30)	0.791
1000–1499 grams	10 (8.2)	7 (10.6)	3 (5.4)	2.10 (0.45–13.12)	0.292
≤999 grams	8 (6.6)	7 (10.6)	1 (1.8)	6.53 (0.79–299.28)	0.068
Gender					
Male	68 (55.7)	35 (53.0)	33 (58.9)	0.79 (0.36–1.72)	0.513
Race					
Black	116 (95.1)	62 (93.9)	54 (96.4)	0.57 (0.05–4.20)	0.526
Mixed race	6 (4.9)	4 (6.1)	2 (3.6)		
Maternal HIV status					
HIV-infected	48 (39.4)	17 (25.8)	31 (55.4)	0.27 (0.12–0.64)	<0.001
HIV-uninfected	73 (59.8)	48 (72.7)	25 (44.6)	2.67 (1.15–6.24)	0.012
HIV-unknown	1 (0.8)	1 (1.5)			
Mode of delivery					
Caesarean-section	29 (23.8)	20 (30.3)	9 (16.1)	2.27 (0.87–6.25)	0.066
Vertex delivery	91 (74.6)	45 (68.2)	46 (82.1)	0.47 (0.18–1.18)	0.078
Unknown	2 (1.6)	1 (1.5)	1 (1.8)		
GBS isolation					
Blood only	87 (71.3)	64 (97.0)	23 (41.1)	45.91 (10.04–410.36)	<0.001
CSF[5] only	13 (10.7)		13 (23.2)		<0.001
Blood and CSF	22 (18.0)	2 (3.0)	20 (35.7)	0.06 (0.01–0.26)	<0.001
Infant age at presentation					
Median(range)	0 (0–74)	0 (0–5)	15 (7–74)		
<24hours	63 (51.6)	63 (95.5)			
1–6 days	3 (2.5)	3 (4.5)			
7–28 days	41 (33.6)		41 (73.2)		
>28 days	15 (12.3)		15 (26.8)		

[1]EOD-Early-onset disease.

[2]LOD-Late-onset disease.

[3]OR(95%CI)-calculated odds ratio with 95% confidence comparing EOD to LOD.

[4]p-value-using Chi-squared, Fischer exact or Wilcoxon rank-sum (Mann-Whitney) test.

[5]CSF-Cerebrospinal fluid.

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agglutination of CSF samples, Of the 35 cases of meningitis, 6 (17.1%) had >250 red cells/μl in their CSF.

HIV-exposed infants were 3.50 (95% CI: 1.53–8.09) times more likely to suffer from LOD than EOD. Additionally HIV-exposed infants were 6.85 (95% CI: 2.64–18.31) fold more likely to have GBS meningitis than HIV-unexposed infants. The CSF biochemistry and cytology parameters were similar between HIV-exposed and-unexposed infants: median CSF protein (p = 0.203), glucose (p = 0.364), polys (p = 0.984) and lymphs (p = 0.813).

Incidence and serotype distribution of invasive GBS disease

Of 31 504 live births, there were 75 cases of invasive GBS disease in black-African infants residing in regions D and G; 73 (89.0%) infants presented to CHBAH and 2 (11.1%) to RMMCH. The overall incidence (per 1,000 live births) of invasive GBS disease was 2.38 (95% CI: 1.87–2.98); the incidences of EOD (n = 43) and LOD (n = 32) were 1.37 (95% CI: 0.99–1.84) and 1.02 (95%CI: 0.70–1.43) respectively. The estimated incidence of disease was significantly higher in HIV-exposed than in HIV-unexposed infants [3.40 (95% CI: 2.29–4.85) versus 1.94 (95% CI: 1.41–2.60) respectively; p = 0.016]. The incidence of EOD was similar in HIV-exposed (1.13; 95%CI: 0.54–2.08) and HIV-unexposed (1.46; 95%CI: 1.00–2.04; p = 0.487) infants but the incidence risk ratio of LOD was 4.67 (95% CI: 2.24–9.74) greater in HIV-exposed (2.27; 95% CI: 1.39–3.50) compared to HIV-unexposed infants (0.49; 95%CI: 0.24–0.87; p<0.001). Among the 66 cases of EOD; 32 (48.5%) were caused by serotype Ia, 5 (7.6%) by serotype Ib, 3 (4.5%) by serotype II, 13 (19.7%) by serotype III, 1 (1.5%) by serotype IV and 12 (18.2%) by serotype V. Among the 56 cases of LOD; 15 (26.8%) were caused by serotype Ia, 34 (60.7%) by serotype III, 4 (7.1%) by serotype V and 3 (5.4%) were not typed. Serotype III was the commonest (n = 23; 71.9%) cause of GBS meningitis, followed by serotype Ia (n = 8; 25.0%)

Risk factors for early-onset GBS invasive disease

Offensive draining liquor (aOR: 27.37; 95% CI: 1.94–386.50) was a risk factor for EOD, whereas maternal GBS bacteriuria was a risk factor for EOD (aOR: 8.41; 95% CI: 1.44–49.15) and LOD (aOR: 3.49; 95% CI: 1.17–10.40) (Table 2). Maternal fever (≥38°C) was observed in only one case. Although the occurrence prolonged (>18 hours prior to delivery) rupture of membranes (PROM) was more common in EOD cases than controls, no increased risk was found in the multivariate analysis (p = 0.213) (Table 2). Thirteen (12.8%) cases mothers were not swabbed at enrollment. The prevalence of GBS colonization was higher in EOD cases (74.5%) than controls (25.1%). Maternal risk factors were not different in HIV-infected and -uninfected mothers (S1 Table).

Intra-partum antibiotic prophylaxis (IAP) was not administered to most mothers who had at least one risk factor (per Center for Disease Control risk based criteria for IAP; i.e. gestation <37 weeks, PROM and maternal intra-partum fever) predisposing to neonatal GBS disease [24]. Among EOD cases, 5 (16.1%) of 31 mothers with at least one risk factor received IAP ≥4 hours prior to delivery, two (6.5%) received IAP within 4 hours of delivery and 24 (77.4%) did not receive IAP during labor. Among controls, 36 (34.6%) of 104 mothers with at least one risk factor received IAP ≥4 hours prior to delivery, four (3.9%) received IAP within 4 hours of delivery and 64 (61.5%) did not receive IAP during labor. For infants born to mothers who received IAP at least 4 hours before delivery, the odds of acquiring EOD was 0.36 (95% CI: 0.10–1.08).

Clinical presentation of GBS invasive disease

Infants with EOD presented most frequently with respiratory distress (83.3%), whilst other clinical and laboratory signs of sepsis occurred less frequently (<15%) (S2 Table). Respiratory distress was less common among LOD (35.7%) than EOD cases (p<0.001), but pyrexia occurred more frequently in LOD (39.3% vs 3.0%; p<0.001). As compared to EOD, infants with LOD also had an increased odds of presenting with poor feeding (OR: 20.71; 95% CI: 4.54–187.69), irritability (OR: 16.65; 95% CI: 5.03–69.74) and lethargy (OR: 3.37; 95% CI 1.17–10.51), and were more likely to have CRP >40 mg/l (58.7% vs 30.5%; p = 0.004) and leucopenia (37.5% vs 12.5%; p = 0.001) (S2 Table)

Table 2. Risk factors for invasive Group B *Streptococcal* (GBS) disease in early-onset and late-onset disease cases and matched controls.

	Cases	Controls	Univariate-OR (95%CI)[1]	p-value	Multivariate-OR (95%CI)[2]	p-value
Early-onset disease	n = 56	n = 323				
Maternal GBS colonization	35/47 (74.5)	81/323 (25.1)	8.71 (4.15–19.23)	<0.001	3.38 (0.77–14.83)	0.107
Prolonged ROM (>18hours)[3]	14/49 (28.6)	32/313 (10.2)	3.51 (1.57–7.54)	<0.001	2.08 (0.61–7.08)	0.239
Maternal fever (≥38.0°C)[4]	1/50 (2.0)	0/319 (0)		0.136		
Offensive liquor	10/52 (19.2)	1/317 (0.3)	75.24 (10.05–3274.04)	<0.001	27.37 (1.94–386.50)	0.014
Maternal GBS Bacteriuria	27/47 (57.5)	22/220 (10.0)	12.15 (5.51–26.79)	<0.001	8.41 (1.44–49.15)	0.018
Any IAP[5]	7/31 (22.6)	40/104 (38.5)	0.47 (0.16–1.26)	0.103		
IAP ≥4 hours prior to delivery	5/31 (16.1)	36/104 (34.6)	0.36 (0.10–1.08)	0.074		
No IAP	24/31 (77.4)	64/104 (61.5)	2.14 (0.80–6.41)	0.103		
Late-onset disease	n = 46	n = 212				
Maternal GBS colonization	28/42 (66.7)	64/212 (30.2)	4.63 (2.17–10.11)	<0.001	2.44 (0.88–6.79)	0.088
Prolonged ROM(>18hours)[3]	2/35 (5.7)	18/204 (8.8)	0.63 (0.07–2.83)	0.746		
Offensive liquor	2/38 (5.3)	3/203 (1.5)	3.70 (0.30–33.27)	0.178		
Maternal GBS Bacteriuria	18/42 (42.9)	25/212 (11.8)	5.61 (2.48–12.46)	<0.001	3.49 (1.17–10.40)	0.025
Any IAP	1/12 (8.3)	16/56 (28.6)	0.23 (0.01–1.84)	0.269		
IAP ≥4 hours prior to delivery	1/12 (8.3)	10/56 (17.9)	0.42 (0.01–3.59)	0.674		
No IAP	11/12 (91.7)	40/56 (71.4)	4.40 (0.54–201.01)	0.269		

[1]Univariate-OR(95%CI)-calculated odds ratio with 95% confidence using Fischer exact test comparing cases and controls.

[2] Multivariate-OR(95%CI)-calculated odds ratio with 95% confidence of disease using conditional logistic regression (For early-onset disease: adjusted for HIV-status, maternal age at delivery, gestational age, maternal GBS colonization, prolonged ROM, offensive liquor, maternal temperature>38, GBS bacteriuria and any intra-partum antibiotics. For late-onset disease: adjusted for HIV-status, maternal age at delivery, gestational age, maternal GBS colonization and GBS bacteriuria).

[3] Prolonged ROM (>18 hours)-prolonged rupture of membranes.

[4]Maternal fever during labor.

[5]IAP-Intrapartum antibiotic prophylaxis to pregnant women that met risk-based criteria (gestation <37 weeks, PROM and maternal intra-partum fever).

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Mortality and neurological outcomes of GBS invasive disease

The overall case fatality rate among cases was 18.0% (22/122), including 22.7% (15/66) for EOD and 12.5% (7/56) for LOD. Most deaths (14/22; 63.6%) occurred within 48 hours of hospital admission or birth. Twenty three (18.9%) infants were admitted to intensive care, of whom 19 (10 EOD and 9 LOD) required mechanical ventilation and 8 (5 EOD and 3 LOD) required inotropic support (Table 3). The mortality rate among infants requiring ventilation was 60.0% (n = 6) for EOD and 55.6% (n = 5) for LOD, and seven (87.5%) infants requiring inotropic support demised. Significant infant predictors of mortality were gestational age <34 weeks (aOR: 9.45; 95% CI: 2.11–42.29), apnea at presentation (aOR: 16.54; 95% CI: 1.55–176.33), seizures (aOR: 6.71; 95% CI: 1.07–42.24) or the need for inotropic support (aOR: 281.93; 95% CI: 7.32–10864.64) (Table 3). HIV-exposed infants were not at increased risk of death (aOR: 0.14; 95% CI: 0.02–0.79).

Of the 100 surviving cases discharged from hospital, both the three and six monthly follow-ups were completed for 63 cases and 214 controls; whilst a further 10 cases and 66 controls only attended one of the two visits (S3 Table). Reasons for follow-up data being unavailable in the remaining cases included 6 whose parents declined for study participation, 4 cases born to women considered unable to provide informed consent and 17 cases were lost to follow-up. At 3 months of age, there were concerns about normal neurological development in 9 of 68 (13.2%) infants with invasive GBS disease and 1 of 262 (0.4%) control infants (Table 4). GBS-affected infants were 21.48 (95% CI: 2.58–179.15; p = 0.005) times more likely have

Table 3. Predictors of mortality from invasive Group B streptococcus (GBS) disease.

	Demised, n = 22	Survived, n = 100	Univariate-OR (95%CI)[1]	p-value	Multivariate-OR (95%CI)[2]	p-value
Timing of disease						
Early-onset disease	15 (68.2)	51 (51.0)	2.06 (0.71–6.47)	0.143	1.31 (0.29–5.95)	0.726
Late-onset disease	7 (31.8)	49 (49.0)	0.49 (0.16–1.41)	0.143		
Mode of presentation						
Meningitis	5 (22.7)	30 (30.0)	0.69 (0.18–2.18)	0.608		
Gestational age						
<34 weeks	11 (50.0)	19 (19.0)	4.26 (1.42–12.58)	0.002	9.45 (2.11–42.29)	0.003
HIV-exposure						
HIV-exposed	4 (18.2)	44 (44.0)	0.28 (0.07–0.95)	0.030	0.14 (0.02–0.79)	0.027
HIV-unexposed	17 (77.3)	56 (56.0)	2.67 (0.85–9.92)	0.092		
HIV-unknown	1 (4.5)					
Gender						
Male	11 (50.0)	57 (57.0)	0.75 (0.27–2.12)	0.549		
Clinical features						
Apnea	7 (31.8)	6 (6.0)	7.31 (1.79–29.7)	<0.001	16.54 (1.55–176.33)	0.020
Seizures	5 (22.7)	8 (8.0)	3.38 (0.76–13.34)	0.058	6.71 (1.07–42.24)	0.043
High/intensive care						
Mechanical Ventilation support	11 (50.0)	8 (8.0)	11.5 (3.31–40.06)	<0.001	0.34 (0.03–3.77)	0.376
Inotropic support	7 (31.8)	1 (1.0)	46.2 (5.09–2101.36)	<0.001	281.93 (7.32–10864.64)	0.002
Lab markers						
WCC[3](<5x10^9/l)	6 (27.3)	23 (23.0)	1.26 (0.36–3.88)	0.670		
CRP[4](>40mg/l)	6 (27.3)	39 (39.0)	0.59 (0.17–1.76)	0.302		

[1]OR(95%CI)-calculated odds ratio with 95% confidence comparing infants that demised versus survivors of GBS disease using Chi-squared or Fischer exact test.

[2] Multivariate-OR(95%CI)-calculated odds ratio with 95% confidence using logistic regression (adjusted for timing of disease, HIV-exposure, prematurity (<34 weeks), ventilation, inotropic support, apnea, seizures).

[3]WCC-White cell count.

[4]CRP-C-reactive protein.

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neurological sequelae than controls. Three cases; one with hypertonia and one with an personal-social delay on Denver-II subsequently showed signs of recovery from neurological impairment at 6 months, whilst one case did not attend the visit.

At 6 months of age, four additional cases had an abnormal Denver-II screening test. Amongst the cases; two had fine-motor delay only, one had gross-motor delay only, one had gross and fine-motor delay and one had gross, fine-motor and personal-social delay. Four cases had hypertonia and/or hyper-reflexia on neurological examination with a normal Denver-II assessment. The only control with an abnormal Denver-II screening test had gross motor delay. GBS-affected infants were 13.18 (95% CI: 1.44-120.95; p = 0.023) times more likely have neurological sequelae than controls. Neurological abnormalities were detected in a greater proportion of GBS-affected infants with meningitis (23.5%) than sepsis (9.8%). Hydrocephalus was confirmed in two infants with meningitis.

Discussion

Our study confirms the high incidence of invasive GBS disease (2.38 per 1 000 live births) observed in the last two decades in South Africa [11, 25], which is about twice the overall

Table 4. Neurological sequelae of infants with invasive Group B *Streptococcus* (GBS) disease at 3 and 6 month visits.

	Cases			Controls	Univariate-OR (95%CI) [1]	p-value	Multivariate-OR (95%CI) [2]	p-value
	Sepsis	Meningitis	Overall					
3 months	**n = 49**	**n = 19**	**n = 68**	**n = 262**				
Overall[3]	3 (6.1)	6 (31.6)	9 (13.2)	1 (0.4)	39.81 (5.27–1751.09)	<0.001	21.48 (2.58–179.15)	0.005
Abnormal Denver-II assessment[4]	2 (4.1)	1 (5.3)	3 (4.4)	1 (0.4)				
Hypertonia/hyper-reflexia[5]	1 (2.0)	5 (26.3)	6 (8.9)	0				
6 months	**n = 51**	**n = 17**	**n = 68**	**n = 232**				
Overall	5 (9.8)	4 (23.5)	9 (13.2)	1 (0.4)	35.24 (4.66–1550.57)	<0.001	13.18 (1.44–120.95)	0.023
Abnormal Denver-II assessment	4 (7.8)	1 (5.9)	5 (7.4)	1 (0.4)				
Hypertonia/hyper-reflexia	1 (2.0)	3 (17.6)	4 (5.9)	0				

[1] Univariate-OR(95%CI)- calculated Odds ratio with 95% confidence using Fischer exact test comparing overall cases and controls

[2] Multivariate-OR(95%CI)- calculated Odds ratio with 95% confidence using conditional logistic regression (adjusted for gender. gestational age, birth weight ≥2500, perinatal asphyxia, ventilation at presentation, HIV-status and previous non-GBS admissions).

[3]Number (%) of cases and controls with neurological sequelae based on abnormal Denver-II assessments and hypertonia/hyper-reflexia.

[4]Abnormal Denver-II assessments in four tested domains (Gross Motor, Fine Motor, Language and Personal/Social).

[5]Hypertonia and/or hyper-reflexia on neurological examination of infant with a normal Denver-II assessment.

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incidence in Africa (1.21; 95%CI: 0.50–1.91) and other regions [13]. Furthermore, we observed a five-fold greater risk of LOD in HIV-exposed compared to HIV-unexposed infants. The observed case fatality rate (18.0%) was similar to that previously reported [11]; this rate is lower than rates reported for Kenya (46%) and Malawi (33%) but almost double the rates reported in high income settings (7–11%) [12, 13]. Concerns about neurological development were noted in a significant proportion (13.2%) of infants with invasive GBS disease surviving to 6 months-of-age.

Unlike the declining trend of EOD in the United States (USA), most likely due to the implementation of IAP [26], there has been no significant change in the incidence rates of EOD in South Africa [11]. The lack of recognition of risk-factors for invasive GBS disease by staff, the late presentation of expectant mothers to antenatal facilities, and the severely under-staffed delivery units are likely factors to explain why only a quarter of women eligible for IAP received this therapy timeously even though the majority of births (±99%) occur in health-care facilities.

Maternal GBS bacteriuria, which is a surrogate marker of heavy recto-vaginal colonization, was significantly associated with EOD and LOD. In our study, maternal GBS bacteriuria was identified in 43% of mothers of LOD cases, of which almost 90% were infected with the same serotype that was isolated from maternal urine sample. These finding strongly support that IAP should be provided to mothers with GBS bacteriuria as it may be a risk factor for both EOD and LOD [24].

In keeping with the higher morbidity caused by infectious diseases in HIV-exposed infants in low-middle income countries [27, 28], the high maternal HIV prevalence (29.5%) may account, in part, for the high burden of invasive GBS disease in South Africa. Although the incidence of LOD among HIV-unexposed infants in our setting is similar to that seen in the USA and other countries [5, 13], we found that HIV-exposed infants were at a greater risk of developing LOD compared to their unexposed peers, as reported [15]. The reasons for this are unclear but may be related to perturbations of the infant immune system caused by exposure to HIV virion *in-utero* or maternal ART [29]; or lower levels of transferred maternal antibody predisposing HIV-exposed infants to invasive GBS disease [30]. Notably, no significant

difference was observed when comparing CD4+counts amongst mothers of cases of LOD and controls (data not shown).

Significant predictors for invasive GBS disease related-death in our study included premature birth, apnea and/or seizures; which are indicators of severe illness in neonates [31]. Contrary to previous reports, in our study, HIV-exposure did not predict mortality in infants with invasive GBS disease [28]. Most deaths (63.6%) occurred within 48 hours of hospitalization, highlighting the fulminant nature of invasive GBS disease. Neurological sequelae was noted in a higher proportion of infants surviving GBS meningitis, similar to other reports [6]. The relatively low overall risk of neurological sequalae in our setting may also in part be related to the high mortality in these infants. Furthermore, in the absence of screening for auditory and visual deficits, as well as the early assessments, we are likely to have underestimated the number of infants with neurological sequelae from invasive GBS disease. There have been previous reports of long-term neurological sequelae in 26–50% of GBS meningitis survivors at 3–18 years of age [7–10], and we are continuing follow-up of children in this study to evaluate their long-term neurological outcomes.

Our results show that serotype Ia, instead of serotype III, is now the commonest (48.5%) cause of EOD in South Africa [11, 32]. In keeping with results from high-income countries [33, 34], the proportion of EOD and LOD caused by serotype V is increasing in South Africa [35]. Although there are differences in the invasive potential of different GBS serotypes, with serotype III being most invasive [32], temporal changes in serotype distribution associated with recto-vaginal colonization are mirrored by changes in their relative contribution to EOD as observed with serotype Ia over a twenty-year surveillance period in the United Kingdom [36]. Molecular characterization has however recognized the highly invasive ST-17 clone to be associated with serotype III invasive disease [37]. Nevertheless, the majority of serotypes causing EOD (76%) and LOD (93%) in our study were due to serotypes Ia, Ib and III, which are included in a trivalent polysaccharide protein conjugate vaccine targeted at immunization of pregnant women currently in clinical trials [38]

Limitations of our study include case enrolments over a single year; nevertheless, we identified a large number of invasive GBS cases and report a persistently high incidence of invasive GBS disease. Due to study constraints, we did not blind examiners performing neurodevelopmental screening tests but plan to do so at future visits. Although other developmental screening test are available (i.e. Bailey), we were limited to using the Denver-II screening test which has been shown to be reliable in young infants [19]. Furthermore, we currently only report on neurological sequelae up to 6 months of age, and did not have any follow-up outcomes on 27% of cases discharged from hospital. The short-term follow-up for neurological sequelae could fail to identify mild development delay or learning problems that manifest later in life, or conversely may over-estimate the long-term sequelae as the neurological system matures in children [39]. We were also unable to identify any significant differences in neurodevelopmental outcomes in HIV-exposed and-unexposed infants due to a small sample of infants with neurological sequelae.

Maternal vaccination effectively protects young infants against diseases such as tetanus, influenza and pertussis until 6 months of age [40–42]. Our study emphasizes the need to consider targeted vaccination of pregnant women for the prevention of invasive GBS disease in low-resource settings with a high prevalence of maternal HIV infection and where screening for recto-vaginal GBS colonization and IAP administration is not logistically feasible. An experimental trivalent GBS vaccine has been reported poorly immunogenic in HIV-infected pregnant women [43] and the immunogenicity of newer GBS conjugate vaccines therefore needs to be urgently evaluated in settings with a high prevalence of maternal HIV-infection.

Supporting Information

S1 Table. Risk factors for Group B streptococcus (GBS) invasive disease in HIV-infected and-uninfected mothers of GBS cases.
(DOCX)

S2 Table. Clinical and laboratory features of infants with invasive Group B streptococcal (GBS) disease.
(DOCX)

S3 Table. Baseline demographic characteristics of Group B streptococcus (GBS) cases and matched controls for 3 and 6 month visits.
(DOCX)

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Author Contributions

Conceived and designed the experiments: ZD SGL SAM CLC. Performed the experiments: ZD AK LJ FN TR JF JW. Analyzed the data: ZD SGL SAM. Wrote the paper: ZD SGL SAM.

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MAJOR ARTICLE

HIV-1 Is Associated With Lower Group B *Streptococcus* Capsular and Surface-Protein IgG Antibody Levels and Reduced Transplacental Antibody Transfer in Pregnant Women

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Background. Human immunodeficiency virus (HIV)-exposed infants are at increased risk of invasive Group B *Streptococcus* (GBS) disease; however, the reason for this increased susceptibility has not been characterized.

Methods. We compared GBS capsular and surface-protein maternal immunoglobin G antibody concentrations and cord-maternal ratios between HIV-infected and HIV-uninfected mother-newborn dyads.

Results. Median capsular antibody concentrations (μg/mL) were lower in HIV-infected than HIV-uninfected women for serotypes Ib ($P = .033$) and V ($P = .040$); and for pilus island (PI)–1 ($P = .016$), PI-2a ($P = .015$), PI-2b ($P = .015$), and fibrinogen-binding protein A ($P < .001$). For serotypes Ia and III, cord-maternal ratios were 37.4% ($P < .001$) and 32.5% ($P = .027$) lower in HIV-infected compared to HIV-uninfected mother-newborn dyads. The adjusted odds of having capsular antibody concentration ≥2 μg/mL when comparing HIV-infected to -uninfected women were 0.33 (95% confidence interval [CI], .15–.75) and 0.34 (95% CI, .12–1.00) for serotypes Ia and III, respectively. Antibody levels and cord-maternal ratios were independent of $CD4^+$ lymphocyte counts or HIV-1 viral load.

Conclusions. The lower GBS antibody concentrations and reduced transplacental antibody transfer in HIV-infected women, which likely contribute to their infants being at heightened susceptibility for invasive GBS disease, could possibly be mitigated by vaccination with a GBS conjugate vaccine currently under clinical development.

Keywords. antibody; Group B *Streptococcus*; HIV; immunity; *Streptococcus agalactiae*; transplacental transfer.

Group B *Streptococcus* (GBS) is a leading cause of sepsis and meningitis in newborns and young infants [1, 2]. A meta-analysis of studies undertaken from 2000 to 2010 reported the highest incidence of invasive GBS disease to be in low-middle income countries from Eastern and Southern Africa [3–7]. Maternal and newborn GBS serotype-specific capsular antibody has been associated with protection against homotypic serotype invasive GBS disease in infants [8]. Furthermore, GBS surface proteins which facilitate adherence to host epithelium such as pilus island (PI) PI-1, PI-2a, PI-2b; fibrinogen-binding protein A (FbsA); and GBS immunogenic bacterial adhesin (BibA) have been shown to be immunogenic, and induce antibodies in animal-model studies that improved survival following systemic GBS inoculation challenges [9–11].

Although maternal human immunodeficiency virus (HIV) infection is not associated with higher prevalence of recto-vaginal GBS colonization during pregnancy or at birth [12–16], a greater risk of invasive GBS disease has been reported in HIV-exposed compared

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to HIV-unexposed infants [17, 18]. The basis for the increased susceptibility to invasive GBS disease in HIV-exposed infants remains to be ascertained and could include maternal HIV infection being associated with lower concentrations of protective GBS antibodies or impaired transplacental antibody transfer [19].

The aim of this study was to determine the effect of maternal HIV infection on immunoglobin G (IgG) serotype-specific (Ia, Ib, III, and V) capsular antibody and select GBS surface-protein (PI-1, PI-2a, PI-2b, BibA, and FbsA) antibody concentrations in the mother and transplacental transfer to their newborns.

METHODS

We undertook a cross-sectional study of pregnant women delivering at Chris Hani Baragwanath Academic Hospital from January to July 2013. This tertiary-level care hospital serves the black-African community of Soweto and surrounding areas. Pregnant women in this region deliver either at this hospital (approximately 22 000 births annually) or at the midwife-obstetric units (approximately 9500 births annually) [20].

The HIV-1 sero-prevalence among pregnant women in this setting was 28.4% during the study period [20]. The provision of antiretroviral therapy (ART) to prevent mother-to-child transmission of HIV has been detailed elsewhere [21, 22]. Briefly, following routine confirmation of HIV infection in the pregnant women, a $CD4^+$ lymphocyte count is measured, which at the time if >350 cells/μL, zidovudine (AZT) was provided until delivery. Pregnant women with a $CD4^+$ count ≤350 cells/μL or World Health Organization clinical stage 3 or 4 were initiated on triple ART. From April 2013, all HIV-infected pregnant women irrespective of $CD4^+$ lymphocyte count were initiated on triple ART [22].

The study sample size was calculated based on the assumption that the antibody transfer rate is normally distributed with a standard deviation of approximately 0.5. We also assumed a transplacental antibody transfer ratio of 1.0 in HIV-uninfected mother-newborn dyads [23, 24]. A sample of 79 HIV-infected and 79 HIV-uninfected pregnant women was required to detect at least 20% difference in transplacental transfer ratio between HIV-exposed compared to HIV-unexposed newborns with 80% power and $\alpha < 0.05$.

Study staff enrolled women in the labor and delivery wards during normal working hours from Monday to Friday. Inclusion criteria were: an infant birth weight ≥2500 grams, known maternal HIV status during pregnancy, and willingness to participate in the study. Gestational age was estimated using the following hierarchy of methods: antenatal ultrasound examination before 24 completed gestational weeks, the Ballard score done within 24 hours of birth, a reliable history of the last menstrual period, an antenatal sonar done at ≥24 weeks, or the fundal symphysis height (centimeters) examination during labor. Cord blood was taken at the time of birth and maternal blood within 12 hours of delivery from enrolled participants. Cord blood was withdrawn using a needled syringe from the umbilical vessels. Blood samples were allowed to clot at room temperature and transported to the Respiratory and Meningeal Pathogens Research Unit within 4–6 hours for processing and storage. The blood was stored at 2°C–8°C if not processed immediately for a maximum period of 24 hours. Blood was centrifuged for 5 minutes at a 3220 relative centrifugal force and the serum then aliquoted and stored at −70°C. Serum samples were thawed and analyzed in batches. Newborns were not tested for HIV-1 infection immediately after delivery.

The Luminex fluorescence based microbead immunosorbent assay was used to measure IgG antibodies to capsular serotypes Ia, Ib, III, and V, and to surface-proteins PI-1, PI-2a, PI-2b, BibA, and FbsA. Capsular and PI protein antigens were kindly provided by Novartis Vaccines and Diagnostics (Italy), while BibA and FbsA protein antigens were provided by Valneva Austria GmbH. Capsular polysaccharides were coupled to the microsphere beads (Bio-Rad, Hercules, California) with the crosslinking agent 4-(4,6 dimethoxy[1,3,5]triazin-2-yl)-4-methyl-morpholinium (DMTMM) and protein antigens were coupled to beads with a 2-step carbodiimide reaction [25, 26]. Polygam (purified pooled commercial gammaglobulin; National Bioproducts, South Africa) was used as reference serum and calibrated with standard capsular serotype-specific GBS reference serum kindly provided by Prof Carol J. Baker. For protein-specific antigen antibody determination, reference serum was assigned arbitrary units (AU) of 10 000 AU/mL. Bead fluorescence was read with the Bio-Plex 200 instrument using Bio-Plex Manager 5.0 software (Bio-Rad, Texas). Details are described in the Supplementary Appendix.

Serum capsular IgG was reported in micrograms per milliliter (μg/mL) with a lower limit of detection of 0.0008, 0.002, 0.004, and 0.016 μg/mL for serotypes Ia, Ib, III, and V, respectively; while protein-specific IgG was reported in AU per milliliter (AU/mL) with a lower limit of detection of 41, 110, 46, 6, and 19 per AU/mL for Pil-1, Pil-2a, Pil-2b, BibA, and FbsA, respectively. Samples below these limits were assigned a value of half the lower limit of detection for statistical analysis.

For analytical specificity of each GBS antigen-microsphere set, reference serum was incubated at 1:100 dilutions with each GBS antigen and incubated at 37°C for 2 hours. The specificity was recorded as the difference in reactivity between the absorbed and unabsorbed serum samples in a multiplex assay. Homologous inhibition was >90% for all capsular polysaccharide and protein antigens with the exception of serotype V (88%) and Fbs-A protein (32%). Heterologous inhibition across antigens was <15%; except for serotype Ib, which was inhibited by 31% with serotype Ia, and for serotype V, which was inhibited by 17% with serotype III.

In HIV-infected women, $CD4^+$ lymphocyte counts measured during pregnancy were recorded and maternal blood obtained at the time of delivery was tested for HIV-1 RNA viral load

using the real-time polymerase chain reaction COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, version 2.0 (Roche COBAS; Roche Molecular Systems, Branchburg, New Jersey), which has a lower limit of detection of 20 copies per milliliter, with values below this being assigned an arbitrary value of 20.

Maternal GBS colonization was assessed at delivery by performing separate lower vaginal and rectal swabs. Rayon-tipped swabs were used for sampling, which was placed into Amies transport medium without charcoal (Medical Wire Equipment Co Ltd Cat: MW170, UK) and transported to the laboratory for processing. The laboratory methods of GBS identification and serotyping on vaginal and rectal swabs have been described [27].

Data Analysis

Maternal and cord blood IgG antibody concentrations were measured, and cord-blood-to-maternal ratio calculated to compare the efficiency of transplacental antibody transfer between HIV-exposed and HIV-unexposed newborns. Demographic characteristics were compared between HIV-uninfected and HIV-infected mother-newborn dyads using χ^2 or Fisher's exact test for proportions; while the Mann–Whitney test was used to compare the medians. Antibody concentrations remained nonparametric after log transformation; thus, median concentrations are reported.

Median maternal antibody concentrations were compared between HIV-uninfected and HIV-infected women at delivery and cord blood antibody concentrations between HIV-unexposed and HIV-exposed newborns using the Mann–Whitney test. Using quantile regression, we further compared median maternal antibody concentrations, cord blood antibody concentrations, and cord-maternal ratios between HIV-uninfected and HIV-infected women, and adjusted for overall colonization, colonizing serotype for homotypic capsular antibodies, maternal age, and parity. We also compared the proportions of HIV-infected and -uninfected women with capsular antibody concentrations above various thresholds proposed to be protective against invasive GBS disease in their infants [8]. In HIV-infected women, $CD4^+$ T-lymphocyte counts and HIV-1 viral load was correlated with maternal antibody concentrations and cord-maternal ratios using Spearman's test. Furthermore, we compared maternal antibody concentrations and cord-maternal ratios at varying $CD4^+$ lymphocyte counts and HIV-1 viral load thresholds using the Mann–Whitney test.

Data were analyzed using STATA version 13.1 (College Station, Texas) and GraphPad Prism version 6.05 for Windows (GraphPad Software, La Jolla, California). Two-tailed P values < .05 were considered statistically significant. Written informed consent was obtained from the women at time of study enrollment. The study was approved by the University of Witwatersrand Human Research Ethics Committee (HREC number: M120905) and registered as an observational study on the South African National Clinical Trial Register (DOH-27-0113-4310).

RESULTS

Of the 320 women screened, 70 refused consent and 76 failed to meet the inclusion criteria. We therefore enrolled 174 mother-newborn dyads, 10 of whom were subsequently excluded (including 9 dyads where the newborn gestational age was ≤36 weeks, and 1 dyad in whom maternal blood was taken >12 hours following delivery). Thus, 164 mother-newborn dyads were analyzed, including 81 HIV-uninfected and 83 HIV-infected women, all of whom had singleton births. Except for HIV-infected women being older (median 30.7 vs 26.0 years; $P = .006$), they were otherwise similar in demographic characteristics compared to HIV-uninfected women (Table 1). Among the 83 HIV-infected women at the time of delivery, 36 (43.4%) were on triple ART, 46 (55.4%) on AZT only, and 1 (1.2%) had not received any ART. The median duration on triple ART from initiation to delivery was 13.4 weeks (range, 1.4–>44) and 17.1 weeks (range, 2.4–42.7) for women on AZT only. Overall, 49 (29.9%) of 164 women were colonized with GBS; colonization rates were similar in HIV-uninfected (27.2%) and HIV-infected (32.5%) women (Table 1). The commonest colonizing serotype was Ia (59.1% of all serotypes) in HIV-uninfected women and III (40.7% of serotypes) in HIV-infected women (Table 1).

All women had detectable antibody levels to all 4 GBS serotypes, although cord blood antibody levels were not detected in 2 samples for serotype Ia and in 5 samples each for serotypes Ib, III, and V. Regarding surface-protein antibodies, only 1 woman had undetectable antibody levels to PI-2a. For cord blood samples, antibody levels were undetectable on 2 samples for BibA, 4 samples for FbsA, 5 samples for PI-1 and PI-2b, and 6 samples for PI-2a. The final analysis included all samples, as results were similar when the above samples were excluded from the analysis (data not shown).

Maternal HIV Infection Status and Capsular Antibodies

Median capsular antibody concentrations (μg/mL) were lower in HIV-infected than HIV-uninfected women for serotypes Ib (0.06 vs 0.09; $P = .033$) and V (0.40 vs 0.59; $P = .040$); similar trends were observed for serotype Ia (0.13 vs 0.36; $P = .077$), but this difference was not significant (Figure 1*A*–*D*, Supplementary Table 1). Median cord blood capsular antibody concentrations (for all serotypes) were significantly lower in HIV-exposed than in HIV-unexposed newborns; the respective antibody concentrations (μg/ml) for serotypes Ia, Ib, III, and V were 0.07 versus 0.26 ($P = .005$), 0.07 versus 0.15 ($P = .013$), 0.15 versus 0.25 ($P = .005$), and 0.34 versus 0.57 ($P = .004$) (Figure 1*A*–*D*, Supplementary Table 1).

After adjusting for confounding factors, we compared maternal antibody concentrations between HIV-infected and -uninfected women at multiple percentiles using quantile regression analysis. Significant differences in antibody concentrations for serotypes Ia, III, and V between HIV-infected and -uninfected women were

Table 1. Demographic and Recto-vaginal Colonization Characteristics of HIV-Uninfected and HIV-Infected Mother-Newborn Dyads

	All Mother-newborn Dyads (n = 164)	HIV-uninfected Mother-newborn Dyads (n = 81)	HIV-infected Mother-newborn Dyads (n = 83)	*P* Value[a]
Mother				
Age: Median (range)	28.0 (18.2–42.7)	26.0 (18.2–42.0)	30.7 (18.7–42.7)	.006
Parity: Median (range)	1 (0–5)	1 (0–5)	1 (0–4)	.079
Black-African race	157 (98.1)	78 (96.3)	83 (100.0)	.118
GBS colonization				
Colonized mothers[b]	49 (29.9)	22 (27.2)	27 (32.5)	.453
Ia	21 [42.9][c]	13 [59.1]	8 [29.6]	.131[d]
Ib	2 [4.1]	0 [0]	2 [7.4]	
II	4 [8.2]	2 [9.1]	2 [7.4]	
III	14 [28.6]	3 [13.6]	11 [40.7]	
V	9 [18.4]	5 [22.7]	4 [14.8]	
Newborn				
Male gender	90 (54.9)	45 (55.6)	45 (54.2)	.863
Gestational age: Median (range)	40.0 (36.1–44.0)	40.0 (36.1–44.0)	40.0 (36.4–43.5)	.997
Birth weight: Median (range)	3063 (2500–4415)	3130 (2510–4415)	3034 (2500–3910)	.194

Abbreviations: GBS, Group B *Streptococcus*; HIV, human immunodeficiency virus.

[a] *P*-value after comparing HIV-uninfected and HIV-infected mother-newborn dyads using Chi-square, Fisher's exact or Mann–Whitney test.

[b] Number of GBS rectal/vaginal colonized mothers stratified by colonizing serotype (an HIV-uninfected mother was dual colonized with Ia and V).

[c] Serotype proportion of colonized mothers.

[d] Multiple 2-way comparisons using Fischer's exact test.

found at higher percentiles (above 65th), suggesting that HIV-infected women also tended to have lower antibody concentrations that HIV-uninfected at higher percentiles (Supplementary Table 2). Corroborating this, we demonstrated that a lower proportion of HIV-infected women had capsular antibody concentrations above thresholds of ≥1 μg/mL and ≥2 μg/mL for serotypes Ia, III, and V (Table 2). Using multivariate analysis, with an antibody concentration of <0.5 μg/mL as a referent, the adjusted odds of having capsular antibody concentration ≥2 μg/mL in HIV-infected compared to -uninfected women were 0.33 (95% confidence interval [CI], .15–.75; $P = .008$), 0.34 (95% CI, .12–1.00; $P = .049$), and 0.50 (95% CI, .16–1.54; $P = .228$) for serotypes Ia, III, and V, respectively (Table 2).

Overall, median cord-maternal ratios for capsular antibody ranged between 75% to 119% in HIV-uninfected mother-newborn dyads and 47% to 93% among HIV-infected mother-newborn dyads (Table 3). In the multivariate model, after adjusting for overall colonization, serotype-specific colonization, maternal age, and parity, the cord-maternal ratio was 37.4% ($P < .001$) and 32.5% ($P = .027$) lower for serotypes Ia and III in HIV-infected compared to HIV-uninfected mother-newborn dyads (Table 3). Two infants born to HIV-infected women developed late-onset GBS meningitis from serotypes Ia and III at 19 and 22 days of age, and among whom their mothers antibody concentrations were 0.08 and 0.12 for the homotypic serotypes and the transplacental ratio was 0.14 and 0.69, respectively.

Maternal HIV Infection Status and Surface-Protein Antibodies

As compared to HIV-uninfected women, HIV-infected women had lower median antibody concentrations (AU/mL) against surface-protein PI-1 (549 vs 1020; $P = .016$), PI-2a (1130 vs 1972; $P = .015$), PI-2b (611 vs 1072; $P = .015$), and FbsA (1444 vs 2169; $P < .001$), but not significantly so for BibA (3829 vs 4790; $P = .236$) (Figure 2*A*–*E*, Supplementary Table 1). Cord blood median surface-protein antibody concentrations were lower in HIV-exposed compared to HIV-unexposed newborns for PI-1 (502 vs 1177; $P = .039$), PI-2b (478 vs 865; $P = .024$), and FbsA (1717 vs 2758; $P = .010$) (Figure 2*A*–*E*, Supplementary Table 1). The median cord-maternal ratios (range, 76%–126%) were similar for all antibodies directed against surface-proteins between HIV-uninfected and HIV-infected mother-newborn dyads (Table 3).

Effect of HIV Viral Load and CD4$^+$ Lymphocyte Count on GBS Antibody in HIV-infected Women

In HIV-infected women, 71 of 83 (85.5%) had a CD4$^+$ lymphocyte count measured within 6 months before delivery with a median CD4$^+$ lymphocyte count of 423 cells/μL (range, 46–1268). The median HIV-1 viral load in 79/83 (95.2%) participants was 96 copies/mL (range, 20–146 055) and undetectable

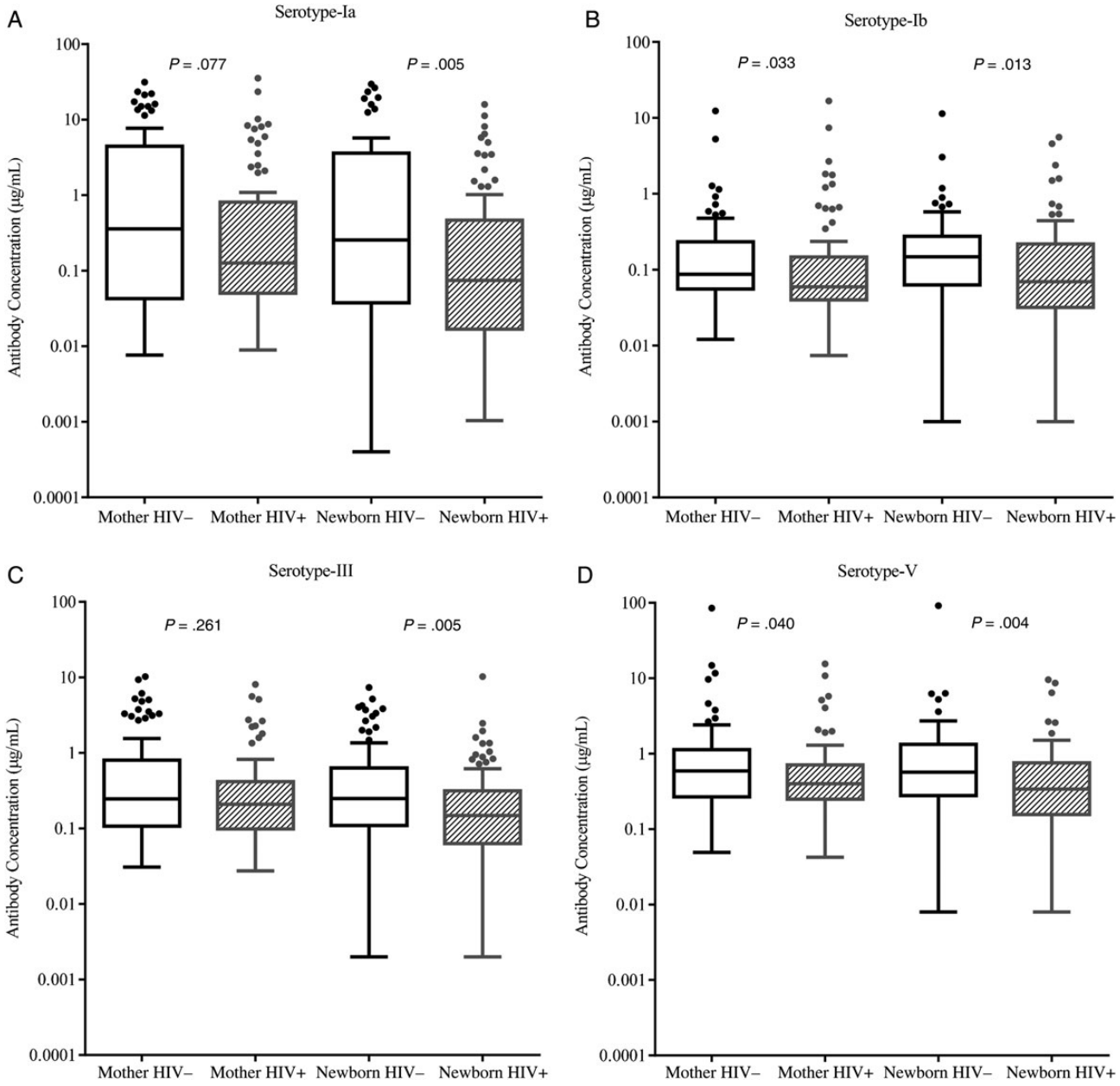

Figure 1. Tukey box-and-whisker plots comparing capsular antibody concentrations of serotype-Ia (*A*), serotype-Ib (*B*), serotype-III (*C*), and serotype-V (*D*) between HIV-uninfected and -infected mothers, and HIV-unexposed and -exposed newborns. The *y*-axis has been $\log_{10}$ scaled. For the box-and whisker-plots, the box represents the distance of the 25th and 75th percentiles with the median represented by the solid line within the box. The upper whisker represents 1.5 times the interquartile distance from the 75th centile, while the lower whisker represents 1.5 times the interquartile distance from the 25th centile. The dot symbols represent outliers above the upper whisker. Abbreviations: HIV, human immunodeficiency virus; Mother HIV+, HIV infected; Mother HIV–, HIV uninfected; Newborn HIV+, HIV exposed; Newborn HIV–, HIV unexposed.

in 28 of the 79 (35.4%) samples. There was no correlation between $CD4^+$ lymphocyte counts and maternal antibody concentrations or between $CD4^+$ lymphocyte counts and cord-maternal ratios for any of the 9 measured antibodies. Furthermore, median maternal antibody concentrations and cord-maternal ratios were similar when stratified by different thresholds of $CD4^+$ lymphocyte counts (Supplementary Tables 3 and 4). Similarly, there was no correlation between maternal HIV-1 viral load and maternal antibody concentration or cord-maternal ratios for any of the 9 measured antibodies (Supplementary Tables 3 and 4).

DISCUSSION

The findings from our study suggest that the possible mechanisms for the increased susceptibility to invasive GBS disease

Table 2. Proportion of HIV-infected and HIV-uninfected Women With Capsular Antibody Concentrations (μg/mL) Above Different Thresholds

Antibody Concentration	HIV-infected n = 83	HIV-uninfected n = 81	aOR (95%CI)[a]	*P* Value
Ia				
<0.5	59 (71.1)	46 (56.8)	Referent	
≥0.5	24 (28.9)	35 (43.2)	0.44 (.22–.89)	.021
≥1	17 (20.5)	30 (37.0)	0.37 (.16–.72)	.005
≥2	14 (16.9)	26 (32.1)	0.33 (.15–.75)	.008
Ib				
<0.5	72 (86.7)	72 (88.9)	Referent	
≥0.5	11 (13.3)	9 (11.1)	1.34 (.51–3.52)	.550
≥1	7 (8.4)	4 (4.9)	2.11 (.57–7.78)	.261
≥2	3 (3.6)	2 (2.5)	1.95 (.30–12.59)	.482
III				
<0.5	64 (77.1)	55 (67.9)	Referent	
≥0.5	19 (22.9)	26 (32.1)	0.48 (.23–1.02)	.058
≥1	10 (12.1)	17 (21.0)	0.37 (.14–.95)	.038
≥2	7 (8.4)	14 (17.3)	0.34 (.12–1.00)	.049
V				
<0.5	49 (59.0)	37 (45.7)	Referent	
≥0.5	34 (41.0)	44 (54.3)	0.58 (.30–1.11)	.099
≥1	14 (16.9)	23 (28.4)	0.46 (.21–1.03)	.059
≥2	6 (7.2)	10 (12.3)	0.50 (.16–1.54)	.228

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus.

[a] Adjusted odds ratio (OR) (95%CI)-calculated OR with 95% confidence of disease using logistic regression (adjusted for parity, maternal age and serotype-specific colonization).

in HIV-exposed infants may relate to lower maternal capsular and surface-protein antibody concentrations, and inefficient transplacental transfer of capsular antibody to the fetus of HIV-infected women [28–30]. HIV-infected women had lower GBS capsular antibody concentrations than their HIV-uninfected counterparts, and notably a lower proportion of

Table 3. Transplacental Antibody Transfer (Cord to Maternal Blood Ratio) Between HIV-uninfected and HIV-infected Mother-newborn Dyads

	HIV-uninfected Mother-newborn Dyads Median CMR[a] (IQR)[b] n = 81	HIV-infected Mother-newborn Dyads Median CMR (IQR) n = 83	Reduction, %[c]	*P* Value[d]
Capsular serotypes				
Ia	0.749 (0.562–1.021)	0.469 (0.322–0.754)	37.4	<.001
Ib	1.187 (0.730–1.959)	0.930 (0.593–1.574)	21.7	.483
III	0.902 (0.605–1.229)	0.609 (0.407–0.976)	32.5	.027
V	0.954 (0.677–1.310)	0.825 (0.543–1.158)	13.5	.084
Surface-proteins				
PI-1	1.056 (0.835–1.453)	0.948 (0.669–1.431)	10.2	.379
PI-2a	0.904 (0.545–1.317)	1.262 (0.613–3.000)	NR[e]	.213
PI-2b	1.006 (0.598–1.588)	0.904 (0.562–1.521)	10.1	.500
BibA	0.860 (0.687–1.139)	0.759 (0.539–1.126)	11.7	.207
FbsA	0.964 (0.601–1.695)	1.159 (0.454–2.347)	NR	.385

Abbreviation: HIV, human immunodeficiency virus.

[a] Cord to maternal ratio (CMR).

[b] Interquartile range (IQR).

[c] Reduction in cord to maternal ratio comparing HIV-infected and HIV-uninfected mother-newborn dyads; calculated as the cord to maternal ratio for HIV-infected/HIV-uninfected women, subtracted from 1.

[d] Using quantile regression (adjusted for overall colonization, colonizing serotype for capsular antibodies, maternal age and parity).

[e] No reduction.

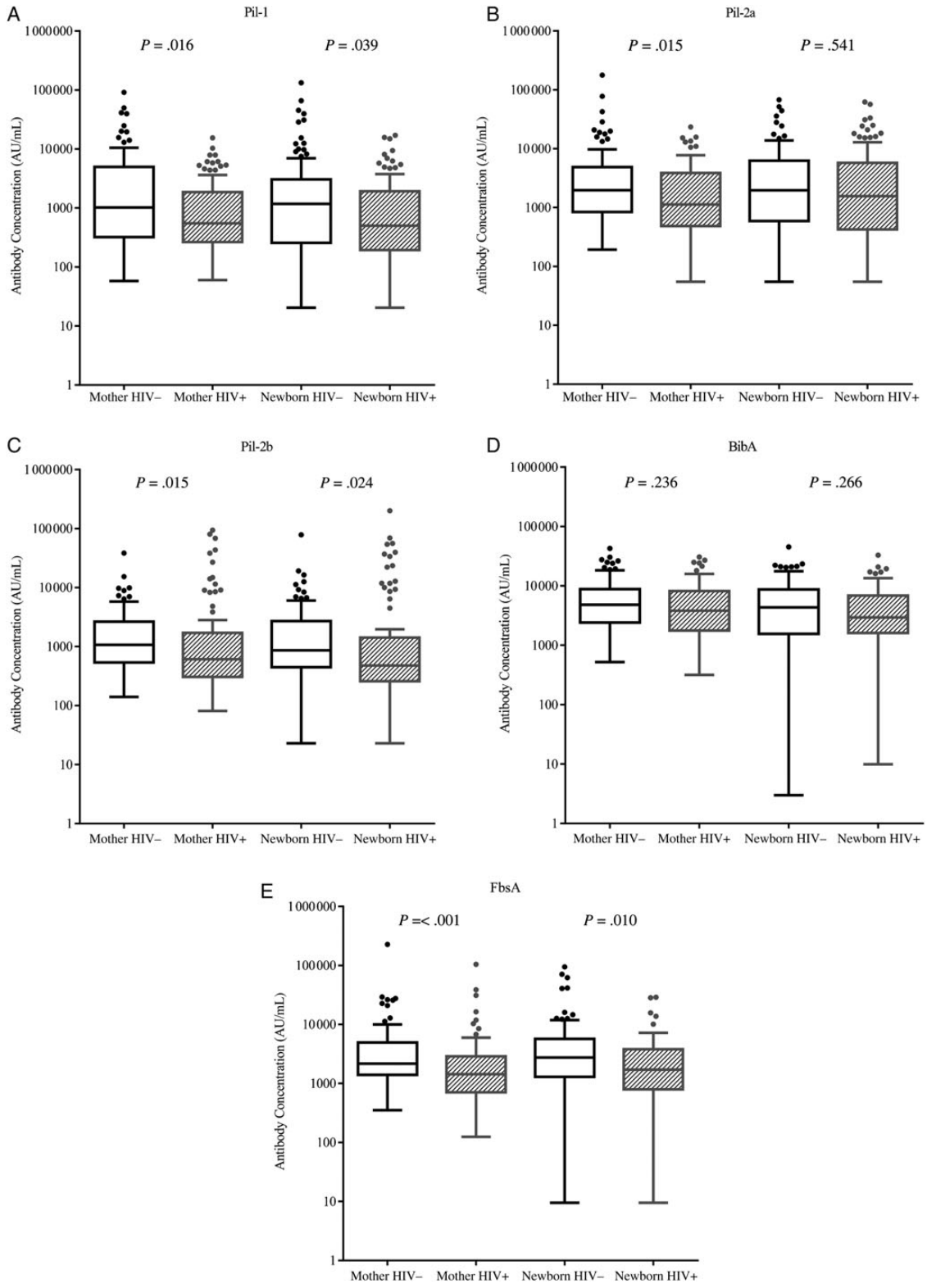

Figure 2. Tukey box-and-whisker plots comparing surface-protein antibody concentrations of Pil-1 (*A*), Pil-2a (*B*), Pil-2b (*C*), BibA (*D*), and FbsA (*E*) between HIV-uninfected and -infected mothers, and HIV-unexposed and -exposed newborns. The *y*-axis has been $\log_{10}$ scaled. For the box-and-whisker plots, the box represents the distance of the 25th and 75th percentiles with the median represented by the solid line within the box. The upper whisker represents 1.5 times the interquartile distance from the 75th centile, while the lower whisker represents 1.5 times the interquartile distance from the 25th centile. The dot symbols represent outliers above the upper whisker. Abbreviations: AU, arbitrary units; HIV, human immunodeficiency virus; Mother HIV+, HIV infected; Mother HIV−, HIV uninfected; Newborn HIV+, HIV exposed; Newborn HIV−, HIV unexposed.

HIV-infected women had capsular antibodies above the putative "protective" thresholds that has been reported to protect against invasive GBS disease in their infants [8]. The lower GBS antibody concentrations in HIV-infected women could represent waning of natural acquired antibody or reduced humoral immune responsiveness to recto-vaginal colonization, which likely induces the antibody responses (personal correspondence Gaurav Kwatra-manuscript under preparation). Additionally, reduced maternal exposure to GBS may also result in lesser antibody production to various serotype-specific epitopes [8]. This is supported by some studies that reported a lower prevalence of GBS colonization in HIV-infected women, including previously in our setting [15, 16], although this was not observed in the current study cohort.

The transplacental transfer of antibodies to serotypes Ia and III, which account for the majority (72%) of invasive GBS disease globally [3], was 37.4% and 32.5% lower in HIV-exposed compared to HIV-unexposed newborns, respectively. Additionally, maternal capsular antibody concentrations were lower in HIV-infected women compared to HIV-uninfected women for serotypes Ib and V, with a trend toward being lower for serotype Ia, but not for serotype III. Serotype III, which has the highest invasive potential, is the least immunogenic of all serotypes [31, 32] and this may explain why concentrations were similar in HIV-infected and -uninfected women. Furthermore, the trend toward higher colonization prevalence of serotype III in HIV-infected compared to HIV-uninfected women in our study may have contributed to similar serotype III antibody concentrations between the women.

We also measured antibody concentrations to select GBS surface-proteins, which induce antibody responses and could be possible vaccine targets. There is, however, a paucity of data on these GBS surface-protein antibody concentrations and no international reference standards exist. Thus, we can only report on the comparisons using in-house reference serum employed consistently across all samples. HIV-infected women had lower median concentrations for all GBS surface-proteins, although antibody differences to BibA were not significant. In addition, we observed that contrary to the capsular antibody transfer, the transfer of surface-protein antibodies from mother to fetus was more efficient, and similar between HIV-infected and HIV-uninfected mother-newborn dyads. This may occur because surface-protein antibodies, which are mainly subclass IgG1, are more efficiently transferred than capsular antibodies, which are predominantly of subclass IgG2 [33].

Our results are consistent with reports showing reduced transplacental transfer of maternal antibodies directed against epitopes of varicella (31% reduction), measles (35% reduction), pneumococcus (24%–30% reduction), *Haemophilus influenzae* type b (23% reduction), pertussis (40% reduction), and tetanus (27%–52% reduction) in HIV-infected compared to HIV-uninfected mother-newborn dyads [28, 29, 34–36]. However, no difference in transplacental antibody transfer between HIV-infected and -uninfected women for pathogens such as herpes, some pneumococcal serotypes, and influenza has also been reported [29, 34, 37]. Transplacental IgG antibody transfer is thought to occur via an active transport mechanism utilizing neonatal Fc receptors found on the placenta [30, 33, 38]. The decrease in transplacental antibody transfer in HIV-infected women is thought to be as a consequence of maternal hypergammaglobulinemia, which saturates the neonatal Fc receptors [39]. Other reasons for the variation in transplacental antibody transfer may relate to differences in IgG subclass and mechanism of transfer of antibody (ie, active or passive transport) [33].

Although our study did not identify a significant association between $CD4^+$ lymphocyte counts and HIV-1 viral loads on maternal antibody and cord-maternal ratios among HIV-infected women, the study was not powered (with a sample size of 79) to detect a significant relationship when the true correlation is between −0.35 and 0.35. Similarly, no association has been observed between maternal $CD4^+$ lymphocyte counts and transplacental transfer of pneumococcal, *H. influenzae* type b, pertussis, and tetanus antibodies in HIV-infected women [28, 29], whereas a positive correlation with $CD4^+$ lymphocyte counts and maternal antibody concentrations was reported to antibodies to pertussis, pneumococcus, and tetanus [28]. More recently, a large European cohort study reported an increased risk of bacterial infections in HIV-exposed infants, particularly in women with low $CD4^+$ lymphocyte counts [40]. Most pregnant women in our setting had undetectable HIV-1 viral load and had immune reconstituted at the time of antibody sampling. A study conducted in Nairobi in HIV-infected women reported a 44% decrease of measles antibody transfer with every $\log_{10}$ increase in viral load, indicating that infants born to women with advanced maternal HIV infection may be at increased risk of disease due to reduced acquisition of maternal antibody concentrations [41].

Limitations of our study include that we did not match for age and colonization status in HIV-infected and -uninfected women; however, we adjusted for these factors in the multivariate analysis and findings remained consistent. Furthermore, we did not quantify the effect that cross-reactivity of serotype Ib with Ia (as previously documented by Brigsten et al [42]) may have had on the absolute antibody concentration for serotype Ib. The assay was, however, applied consistently to both HIV-infected and -uninfected dyads and hence is unlikely to alter the differences observed between HIV-infected and HIV-uninfected women in our study. Also, our study only measured IgG antibodies, while IgA antibodies may also be transplacentally transferred, and have been associated with protection against GBS invasive disease in animal model studies [11, 43]. Additionally, $CD4^+$ lymphocyte counts were measured as part of standard-of-care at any time within 6 months (mean, 2.8 months) of delivery and the study was not specifically powered

to address whether immunological status or HIV-1 viral load were associated with differences in maternal antibody or transplacental antibody transfer.

The lower GBS antibody concentrations and reduced transplacental antibody transfer in HIV-infected women, which places their infants at risk for invasive GBS disease, may be mitigated by maternal GBS vaccination. Furthermore, an investigational trivalent GBS polysaccharide-protein conjugate vaccine was found to be less immunogenic in HIV-infected than HIV-uninfected pregnant women [44]. Therefore, in HIV-burden settings, maternal vaccination may require modified formulations or dosing schedules in HIV-infected women.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Original Research

EXPERT REVIEWS

Association between maternal Group B Streptococcus surface-protein antibody concentrations and invasive disease in their infants

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Objectives: Group B Streptococcus (GBS) surface-proteins have been shown to be immunogenic and potential vaccine candidates. We aim to determine the association between maternal IgG antibodies to select GBS surface-proteins and invasive GBS disease in their infants. Methods: Using a matched case–control study, maternal antibody levels for GBS-immunogenic bacterial adhesin, fibrinogen-binding protein A and pilus-island (PI) PI-1, PI-2a, PI-2b were compared between infants with invasive GBS disease and well-baby controls. Results: The absolute risk of disease did not differ between cases and colonized controls with increasing antibody concentrations for these surface-proteins. There was, however, a relative risk reduction in invasive disease associated with fibrinogen-binding protein A, with an adjusted odds ratio of 0.04 (95% CI: 0.01–0.69) at antibody levels ≥10,000 AU/ml. Conclusion: We have not demonstrated an association between naturally occurring fibrinogen-binding protein A, GBS-immunogenic bacterial adhesin, and PI surface-protein antibodies and the risk of invasive disease in young infants. These surface-proteins may not be suitable GBS vaccine candidates.

KEYWORDS: BibA, FbsA, GBS, Group B Streptococcus, pilus island, Streptococcus agalactiae

Group B Streptococcus (GBS) remains the most frequent cause of sepsis and meningitis in young infants, even in high-income countries where intrapartum antibiotic prophylaxis (IAP) is provided to recto-vaginally colonized pregnant women [1–3]. This highlights the need for vaccines targeted against this pathogen. An association between maternal GBS serotype-specific capsular antibody levels and invasive GBS disease has been determined [4]. A drawback of the current trivalent (serotypes Ia, Ib, and III) GBS polysaccharide–protein conjugate vaccine currently under development is that there exist the possibility for replacement disease if vaccine formulations are limited to select serotypes, even though the majority (79%) of the disease is caused by these three of the 10 known serotypes [5,6]. This could be overcome by targeting non-serotype-specific GBS epitopes that contribute to the virulence of the organism, are genetically conserved between GBS strains, and are immunogenic.

A number of surface-proteins have been studied as potential candidates for vaccine development in animal-model studies [7,8] (**SUPPLEMENTARY TABLE 1**) [supplementary material can be found online at www.informahealthcare.com/suppl/14760584.2015.1085307]. Only a few surface-proteins (surface immunogenic protein [Sip], resistance to proteases immunity group B [Rib], αC protein and βC protein), however, have been studied in infants to determine the association between

antibody levels and invasive GBS disease [9–13], of which, only antibodies to Rib protein have shown promise. The objective of our study is to determine the association between maternal antibodies to GBS immunogenic bacterial adhesin (BibA), fibrinogen-binding protein A (FbsA), and pilus island (PI) proteins of PI-1, PI-2a, and PI-2b and invasive GBS disease in their infants <90 days age. These surface-proteins function primarily in attachment of the bacterium to the host surface [14]. In addition, it is thought that all strains carry at least one PI, whereas the expression of FbsA and BibA is more variable across strains [8,15].

Methods

We undertook a matched case–control study at three secondary-tertiary level hospitals in Johannesburg, South Africa, from November 2012 to February 2014. The study population and standard-of-care practices at these hospitals have been described [16]. In brief, pregnant women at these hospitals are not routinely screened for recto-vaginal GBS colonization, although IAP is recommended for pregnant women with other risk factors for invasive GBS disease in their infants. Young infants admitted to these hospitals with clinical features of sepsis or meningitis are investigated with blood cultures and cerebrospinal fluid cultures to identify causative pathogens.

Through daily laboratory and pediatric ward surveillance at the hospitals, infants <90 days of age with confirmed invasive GBS disease based on culture from blood, cerebrospinal fluid or other normally sterile site, or by bacterial latex agglutination on cerebrospinal fluid were identified. Early-onset disease (EOD) was defined as isolation of GBS in the first 6 days of life, while late-onset disease (LOD) was defined as isolation of GBS from days 7 to 89 of age. Controls (infants who were free of invasive GBS disease) were matched to the timing of disease in the cases; first 6 days of life for EOD cases and within 14 days (but >7 days of age) for LOD cases, to maternal age (±2.5 years of the case), maternal HIV-status and gestational age (≥37 weeks gestation or ±2 weeks of gestational age of the case if the case was preterm). We attempted to match five controls to each case of invasive GBS disease.

At enrolment, the mothers of cases and controls had rectal and lower vaginal swabs collected for GBS culture and serotyping. The laboratory methods for GBS identification and serotyping have been previously described [17]. PI typing of GBS invasive and colonizing isolates was done by real-time PCR using Taqman probes for PI-1, PI-2a, and PI-2b, with primers that target the genomic regions coding for the ancillary protein (AP)-1 of each PI [18]. In brief, frozen GBS isolates were sub-cultured on sheep blood agar supplemented with nalidixic acid and colistin and incubated overnight at 37°C in 5% CO_2. One GBS colony was suspended in the 300 µl nuclease-free water and heated for 10 min at 95°C. The tubes were centrifuged and the resulting supernatant was used in the PCR. The PCRs were performed on an AB 7500 instrument (Applied Bio-systems; Woodlands, Singapore) in a 25 µl reaction volume with TaqMan universal PCR master mix (Applied Bio-systems; Foster city, CA, USA). The detection of PI-2b was performed as a single-plex reaction, and PI-1 and PI-2a were detected in duplex. GBS strains 2603 V/R (PI-1 and PI-2a) and COH1 (PI-2b) obtained from American type culture collection were used as reference strains. A threshold C_T value is generated when the fluorescence passes through if amplification occurred. We did not investigate isolates for expression of FbsA and BibA.

Serum was collected from the mothers and their infants within 72 h of culture confirmation in cases and at the time of enrolment from controls. We measured IgG antibodies to BibA, FbsA, PI-1, PI-2a, and PI-2b using the Luminex fluorescence micro-bead immunosorbent assay, as well as capsular antibody to serotypes Ia, Ib, III, and V. Antibody concentrations were reported in arbitrary units per milliliter (AU/ml) with a lower limit of detection of 41, 110, 46, 6, and 19 per AU/ml for PI-1, PI-2a, PI-2b, BibA, and FbsA, respectively. The assay methods and specificity standards have been previously described [19]. For PI, antibody was measured to the backbone or AP antigenic targets on the surface of the PI, that is, GBS-80 for PI-1, GBS-67 for PI-2a, and SAN1518 for PI-2b. For BibA, we measured antibodies to the BibA-COH1 antigen.

Statistical analysis

Demographic characteristics and risk factors for invasive disease were compared between cases and controls using the χ^2 or Fisher's exact test, or the Mann–Whitney *U* test to compare medians. Median antibody concentrations were reported but not compared as this was a matched case–control study. For the primary analysis for FbsA and BibA antibody, we compared cases with controls whose mothers were colonized with GBS. For the primary analysis for PI proteins, we compared cases in which the specific PI was identified from the invasive isolate to controls whose mothers were colonized with GBS strains with the homotypic PI. The secondary analysis compared cases with non-colonized controls. We pooled matched sets of cases and controls and reduced the number of strata by combining interchangeable sets [20]. Each stratum contained a case and a colonized control that was matched for all of the following: pilus-type (for pilus protein analysis); EOD or LOD; maternal HIV-status; maternal age as <25, 25 to <35, and ≥35 years; and gestational age as 34 to <37 and ≥37 weeks. Reverse cumulative plots were constructed to show the proportion of mothers with antibody concentrations at various thresholds for each protein. Conditional logistic regression was used to compare the proportion of stratum matched cases with colonized controls, and stratum matched cases with non-colonized controls at different antibody thresholds. The referent was determined by visual analysis of the separation point between the cases and controls from the reverse cumulative plots. We adjusted for variables in which the p value was <0.20 in the univariate analysis. Odds ratios and 95% CI are reported. Two-tailed p value <0.05 was considered statistically significant.

Table 1. Demographic characteristics of matched cases & colonized controls ≥34 weeks of age for FbsA & BibA.

	FbsA/BibA		
	Cases n = 69 (EOD = 34, LOD = 35)	Controls n = 128 (EOD = 75, LOD = 53)	p-value
Maternal			
HIV-infected	29 (42.0)	54 (42.2)	0.983
HIV-uninfected	40 (58.0)	74 (57.8)	
Median age in years (IQR)	25.4 (21.7–30.4)	25.2 (22.7–30.9)	0.430
Median parity (IQR)	1 (0–2)	1 (0–2)	0.567
Black-African race	66 (95.7)	126 (98.4)	0.346
Fever	0/50 (0)	0/118 (0)	0.999
PROM† (>18 h)	11/57 (19.3)	6/123 (4.9)	0.002
IAP	4/69 (5.8)	9/124 (7.3)	0.774
Infant			
Median gestation in weeks (IQR)	40.0 (38.3–40.3)	39.3 (38.0–40.4)	0.255
Median birth weight in grams (IQR)	2995 (2800–3250)	3085 (2800–3410)	0.257
Male gender	39 (56.5)	59 (46.1)	0.163
Day of life at enrollment			
EOD-median (IQR)	4 (3–5)	1 (1–1)	<0.001
LOD-median (IQR)	17 (12–25)	20 (15–24)	0.265

p value: using Chi-squared, Fischer exact or Wilcoxon rank-sum (Mann–Whitney) test;
†Prolonged (>18 h) rupture of membranes.
EOD: Early-onset disease; LOD: Late-onset disease; IQR: Interquartile range; IAP: Intrapartum antibiotic prophylaxis.

Bayesian modeling was used to calculate the probability that a woman with a GBS IgG concentration greater than or equal to *c*, gives birth to a neonate who would develop EOD or LOD, P(D|Ab ≥ *c*). We assumed that the antibody concentrations follow a Weibull distribution.

A β (25, 2500) was used for the prior distribution of the marginal probability of disease P(D).

The most probable marginal risk of disease was equal to 1%, with the central 95% mass falling within 0.64 and 1.41%. The marginal risk was calculated as the proportionate risk of disease and maternal GBS colonization reported in this population [16]. We plotted the posterior mode and the range from 25th to 75th percentile of the posterior distribution. Further details regarding the model have been described [21]. This was undertaken to determine the absolute risk of disease per 1000 live births.

Data were analyzed using STATA version 13.1 (College Station, Texas), R version 2.15 (Vienna, Austria), JAGS [22] and GraphPad Prism version 6.05 for Windows (GraphPad Software; San Diego, CA, USA). The study was approved by the University of Witwatersrand Human Research Ethics Committee (HREC number: M120963) and registered on the South African National Clinical Trial Register (DOH-27-0113-4309). Written informed consent was obtained from women at the time of study enrolment.

Results

In infants born at ≥34 weeks gestational age, serum was available on 70 mother–infant pairs with invasive GBS disease and 487 controls. After stratum matching, the final FbsA and BibA paired analysis included 69 cases, 128 GBS colonized controls, and 332 non-colonized controls. Risk factors for invasive disease and demographic characteristics were similar between cases and matched controls, except for history of prolonged rupture of membranes during labor being more common in cases (19.3%) than matched colonized controls (4.9%; p = 0.002), and infants with EOD being older (median: 3 days) at the time of enrolment than matched colonized and non-colonized controls (median: 1 day, p < 0.001 for both; Table 1 and Supplementary Table 2).

After strata matching, including specific PI matching, the final paired analysis was conducted on 29 invasive cases with PI-1 containing strains and correspondingly 64 PI-1 colonized and 289 non-GBS colonized controls, 37 invasive cases with PI-2a containing strains and correspondingly 77 PI-2a colonized and 319 non-colonized controls, and 29 invasive cases with PI-2b containing strains and correspondingly 29 PI-2b colonized and 279 non-colonized controls. Maternal and infant demographic characteristics and risk factors for disease were similar between cases and PI-specific controls; apart from

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Table 2. Demographic characteristics of cases & pilus-specific colonized controls ≥34 weeks of age.

	PI-1			PI-2a			PI-2b		
	Cases n = 29 (EOD = 14, LOD = 15)	Controls n = 64 (EOD = 39, LOD = 25)	p-value	Cases n = 37 (EOD = 21, LOD = 16)	Controls n = 77 (EOD = 48, LOD = 29)	p-value	Cases n = 29 (EOD = 11, LOD = 18)	Controls n = 29 (EOD = 14, LOD = 15)	p-value
Maternal									
HIV-infected	12 (41.4)	23 (35.9)	0.616	14 (37.8)	31 (40.3)	0.804	14 (48.3)	14 (48.3)	0.999
HIV-uninfected	17 (58.6)	41 (64.1)		23 (62.2)	46 (59.7)		15 (51.7)	15 (51.7)	
Median age in years (IQR)	25.4 (22.4–31.5)	25.5 (22.1–31.7)	0.772	24.4 (20.9–30.0)	25.2 (22.6–30.5)	0.211	25.4 (22.7–30.3)	28.7 (22.8–31.2)	0.397
Median parity (IQR)	1 (0–2)	1 (0–1)	0.506	0 (0–1)	1 (0–2)	0.121	1 (1–2)	1 (0–2)	0.435
Black-African Race	29 (100.0)	63 (98.5)	0.999	35 (94.6)	76 (98.7)	0.246	29 (100.0)	28 (96.6)	0.999
Fever	0/21 (0)	0/62 (0)	0.999	0/28 (0)	0/73 (0)	0.999	0/19 (0)	0/29 (0)	0.999
PROM[†] (>18 h)	4/24 (16.7)	3/63 (4.8)	0.088	7/32 (21.9)	2/74 (2.7)	0.003	3/22 (13.6)	2/29 (6.9)	0.641
IAP	1/29 (3.5)	6/64 (9.4)	0.428	4/37 (10.8)	5/77 (6.5)	0.469	0/29 (0)	2/29 (6.9)	0.491
Infant									
Median gestation in weeks (IQR)	40.2 (40.0–40.6)	39.4 (38.1–40.4)	0.014	40.0 (38.0–40.2)	39.4 (38.0–40.3)	0.599	40.0 (40.0–40.6)	39.3 (38.2–40.4)	0.058
Median birth weight in grams (IQR)	3100 (2835–3200)	3123 (2805–3405)	0.438	2960 (2770–3270)	3090 (2860–3370)	0.185	3110 (2835–3210)	3150 (2760–3450)	0.367
Male gender	14 (48.3)	33 (51.6)	0.769	24 (64.9)	34 (44.6)	0.038	14 (48.3)	17 (58.6)	0.430
Day of life at enrollment									
EOD-Median (IQR)	3 (3–5)	1 (1–1)	<0.001	4 (3–5)	1 (1–1)	<0.001	3 (3–5)	1 (1–1)	<0.001
LOD-Median (IQR)	17 (11–27)	20 (15–24)	0.334	20 (13–24)	20 (16–23)	0.669	17 (10–27)	23 (13–24)	0.574

p-value: using Chi-squared, Fischer exact or Wilcoxon rank-sum (Mann–Whitney) test;
[†]Prolonged (>18 h) rupture of membranes.
EOD: Early-onset disease; LOD: Late-onset disease; IQR: Interquartile range; IAP: Intrapartum antibiotic prophylaxis.

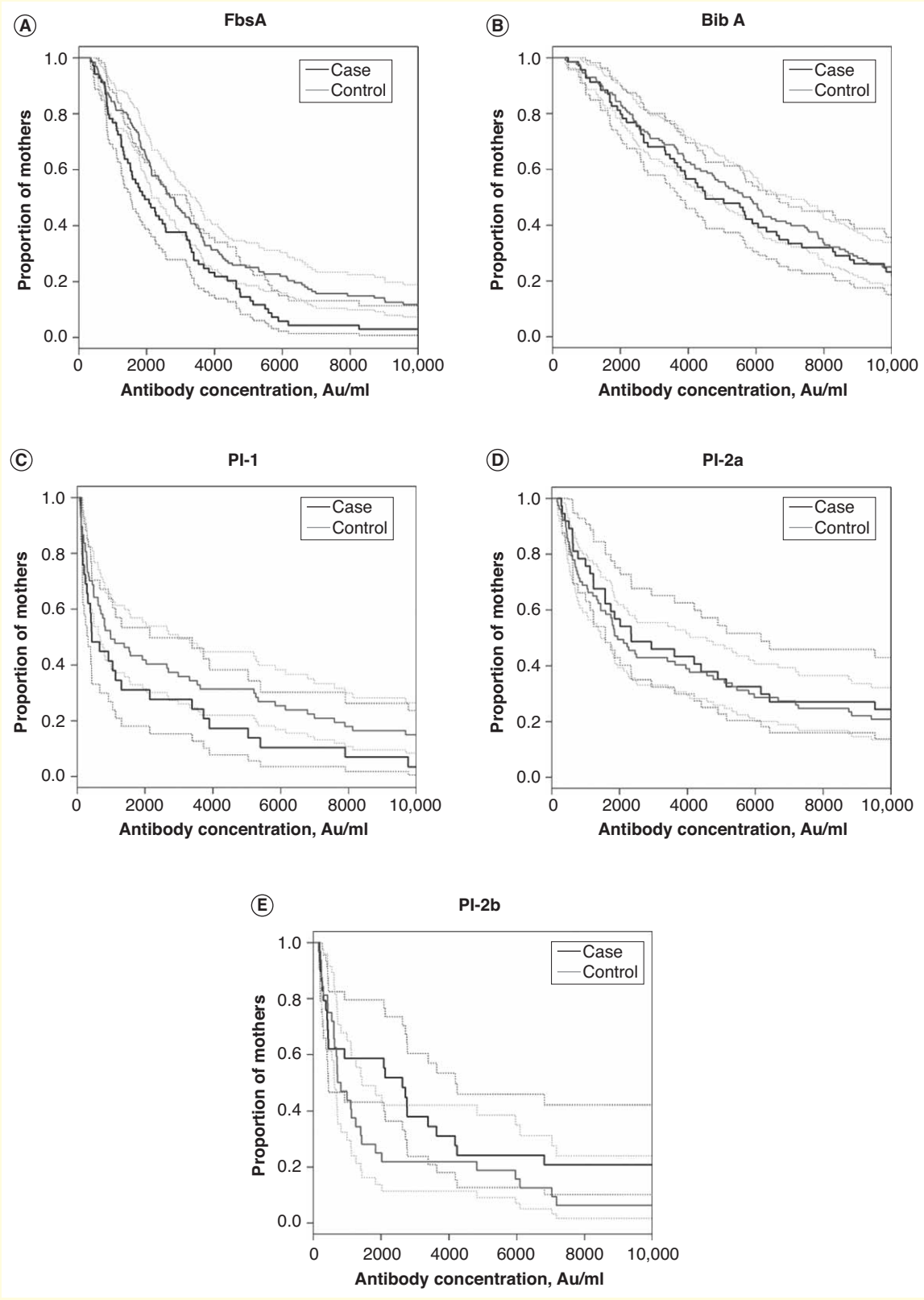

Figure 1. Reverse cumulative plots demonstrating the proportion of mothers of cases and colonized controls to antibody concentrations for (A) FbsA, (B) BibA, (C) PI-1, (D) PI-2a, & (E) PI-2b.
The *solid line* represents the proportion of mothers at various antibody thresholds. The *upper and lower dotted lines* are the 95% confidence intervals.

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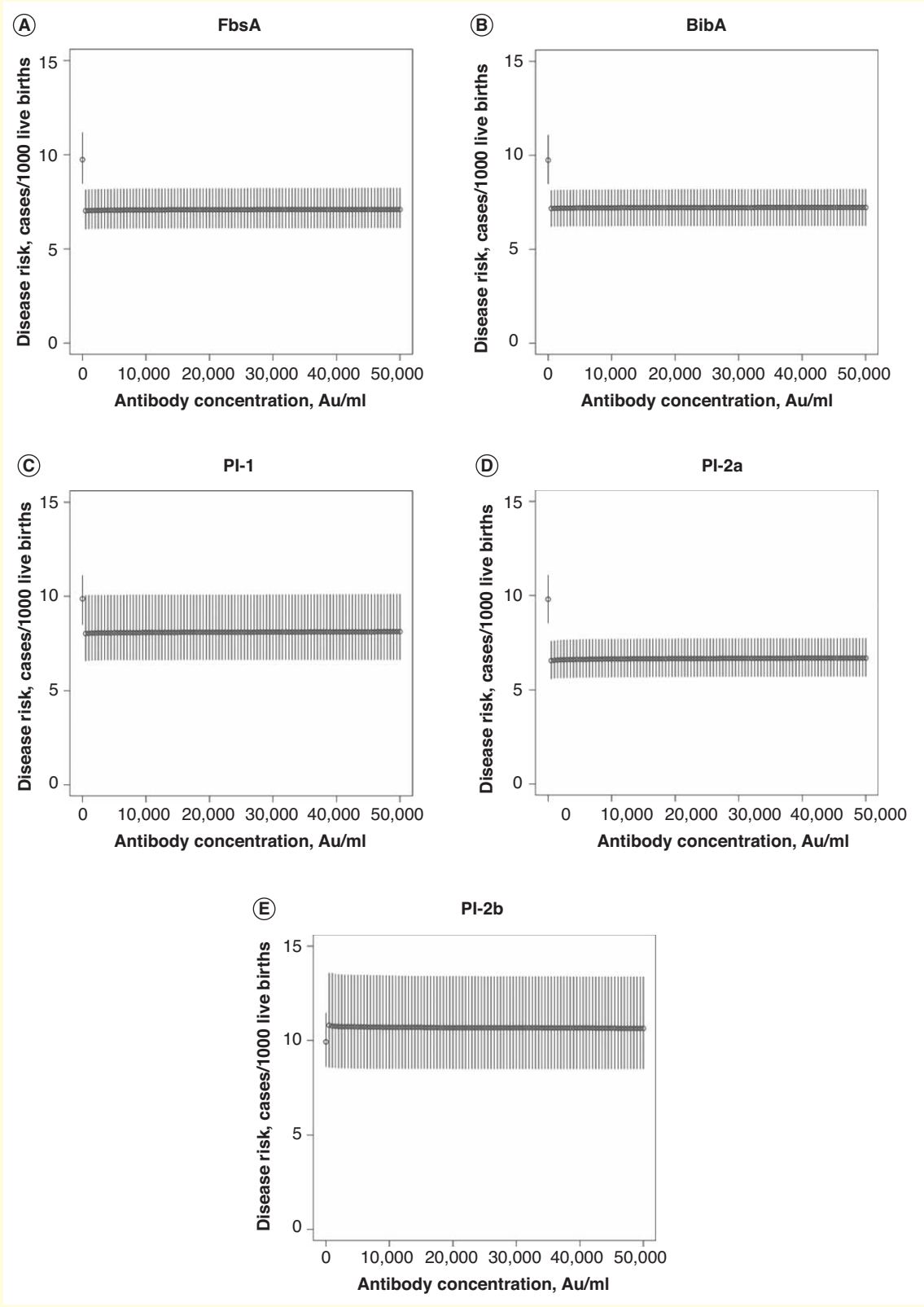

Figure 2. Probability of invasive GBS disease risk to (A) FbsA, (B) BibA, (C) PI-1, (D) PI-2a, & (E) PI-2b at varying maternal antibody concentrations using a Bayesian model.
The *circles* represent the posterior mode (i.e., the most likely value) and *vertical lines* represent the 50% credible interval.

Table 3. Maternal antibody (AU/ml) thresholds to FbsA and BibA surface-protein epitopes in mothers of cases & colonized controls.

	Cases	Controls	OR (95% CI)[†]	p-value	aOR (95% CI)[‡]	p-value
FbsA	n = 69 (%)	n = 128 (%)				
<1000	16 (23.2)	20 (15.6)	Ref			
≥1000	53 (76.8)	108 (84.4)	0.55 (0.26–1.18)	0.124	0.56 (0.24–1.32)	0.182
≥2000	34 (49.3)	82 (64.1)	0.41 (0.18–0.94)	0.035	0.40 (0.16–1.04)	0.061
≥5000	10 (14.5)	32 (25.0)	0.37 (0.12–1.33)	0.082	0.22 (0.05–1.02)	0.053
≥10,000	2 (2.9)	15 (11.7)	0.20 (0.03–1.26)	0.086	0.04 (0.01–0.69)	0.027
BibA	n = 69 (%)	n = 128 (%)				
<2000	13 (18.8)	20 (15.6)	Ref			
≥2000	56 (81.2)	108 (84.4)	0.62 (0.28–1.38)	0.237	0.54 (0.22–1.36)	0.191
≥5000	34 (49.3)	71 (55.5)	0.66 (0.26–1.50)	0.293	0.53 (0.19–1.48)	0.214
≥10,000	15 (21.7)	32 (25.0)	0.39 (0.13–1.16)	0.092	0.30 (0.08–1.17)	0.083
≥15,000	11 (15.9)	19 (14.8)	0.47 (0.14–1.56)	0.218	0.43 (0.11–1.71)	0.231

[†]Calculated odds ratio with 95% confidence using conditional logistic regression.
[‡]Adjusted odds ratio with 95% confidence using conditional logistic regression (BibA and FbsA: adjusted for prolonged rupture of membranes, infant gender, day of life at enrollment).

gestational age (40.2 vs 39.4 weeks, respectively; p = 0.014) in PI-1, infant gender (64.9 vs 44.6% males; p = 0.038) and the occurrence of prolonged rupture of membranes (21.9 vs 2.7%; p = 0.003) in cases for PI-2a (Table 2). When comparing cases with non-colonized controls, gestational age differed for PI-1 and PI-2b (Supplementary Table 3). The timing of enrolment for EOD cases differed (median: 3 or 4 days) compared with PI-specific (median: 1 day) and non-colonized controls (median: 1 day; Table 2 and Supplementary Table 3).

Antibody levels to FbsA

There was a higher proportion of colonized controls than cases at higher antibody thresholds; the adjusted odds ratio for disease decreased from 0.40 (95% CI: 0.16–1.04), 0.22 (95% CI: 0.05–0.02), and 0.04 (95% CI: 0.01–0.69) with antibody threshold ≥2000, ≥5000, and ≥10,000 AU/ml, respectively (Figure 1A and Table 3). The odds ratio for disease also decreased with increasing antibody concentrations when comparing cases with non-colonized controls (Supplementary Table 4). The median maternal FbsA antibody concentrations (in AU/ml) was 1942 (interquartile range (IQR): 1120–3688) compared with colonized controls (2752; IQR: 1620–5108) and non-colonized controls (2296; IQR: 1408–4627; Supplementary Table 5). The median infant FbsA antibody concentrations was 1131 (IQR: 679–2104) compared with infants of colonized controls (1744; IQR: 775–3303) and non-colonized controls (1696; IQR: 859–3486, Supplementary Table 6).

Antibody levels to BibA

The proportion of cases and controls (colonized and non-colonized) with antibody concentrations at various thresholds were similar and the adjusted odds ratios were not significant (Table 3, Figure 1B and Supplementary Table 4). The median BibA maternal antibody concentrations (in AU/ml) was 4512 (IQR: 2587–9774) in cases compared with 5727 (IQR: 2560–9913) in colonized controls and 5243 (IQR: 2420–9871) in non-colonized controls (Supplementary Table 5). The median infant BibA antibody concentrations was 1866 (IQR: 787–3919) in cases compared with infants of colonized 2901 (IQR: 1554–6593) and non-colonized controls 3063 (IQR: 1397–6447) (Supplementary Table 6).

Antibody levels to pilus-island proteins

A larger proportion of PI-1 colonized controls had antibody concentrations at higher thresholds than cases resulting in a decreased odds ratio for disease; however, the adjusted odds ratio for disease did not significantly differ between cases and colonized controls (Table 4 & Figure 1C). The median PI-1 antibody concentrations (in AU/ml) was 432 (IQR: 203–3391) in mothers of invasive cases compared with controls with PI-1 colonization (1052; IQR: 301–6463) and those not colonized by GBS (789; IQR: 317–2419; Supplementary Table 5). The median infant PI-1 antibody concentrations was 408 (IQR: 76–1452) in cases, 901 (IQR: 215–5534) in infants of women colonized with PI-1 strains and 595 (IQR: 196–1852) in control infants whose mothers were not colonized by GBS (Supplementary Table 6).

The proportion of mothers with PI-2a and PI-2b antibodies at increasing thresholds were higher in cases than controls (Table 4 & Figure 1D, E). Similarly, median maternal antibody concentrations trended to being higher in cases than controls (Supplementary Table 5). Comparing maternal PI-2a and PI-2b

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Table 4. Maternal antibody (AU/ml) thresholds to pilus island surface-protein epitopes in mothers of cases & colonized controls.

	Cases	Controls	OR (95% CI)[†]	p value	aOR (95%CI)[‡]	p-value
PI-1	n = 29 (%)	n = 64 (%)				
<500	15 (51.7)	23 (35.9)	Ref			
≥500	14 (48.3)	41 (64.1)	0.57 (0.23–1.42)	0.226	0.64 (0.20–2.03)	0.446
≥1000	12 (41.4)	32 (50.0)	0.55 (0.21–1.47)	0.236	0.59 (0.17–2.06)	0.408
≥2000	9 (31.0)	27 (42.2)	0.47 (0.17–1.33)	0.156	0.39 (0.10–1.58)	0.189
≥5 000	5 (17.2)	20 (31.3)	0.28 (0.07–1.11)	0.070	0.29 (0.06–1.43)	0.130
≥10,000	1 (3.5)	10 (15.6)	0.15 (0.02–1.31)	0.086	0.10 (0.01–1.31)	0.079
PI-2a	n = 37 (%)	n = 77 (%)				
<1000	9 (24.3)	24 (31.2)	Ref			
≥1000	28 (75.7)	53 (68.8)	1.48 (0.57–3.81)	0.417	1.44 (0.45–4.59)	0.533
≥2000	21 (56.8)	38 (49.4)	1.54 (0.57–4.16)	0.392	1.14 (0.33–3.97)	0.834
≥5000	13 (35.1)	27 (35.1)	1.12 (0.37–3.35)	0.844	0.58 (0.14–2.50)	0.466
≥10,000	9 (24.3)	16 (20.8)	1.04 (0.31–3.46)	0.945	0.83 (0.18–3.88)	0.812
PI-2b	n = 29 (%)	n = 29 (%)				
<1000	12 (41.4)	17 (58.6)	Ref			
≥1000	17 (58.6)	12 (41.4)	1.99 (0.69–5.72)	0.202	1.72 (0.56–5.26)	0.342
≥2000	17 (58.6)	6 (20.7)	4.15 (1.15–14.96)	0.030	3.32 (0.88–12.45)	0.076
≥5000	7 (24.1)	6 (20.7)	1.87 (0.46–7.63)	0.383	1.65 (0.40–6.87)	0.490
≥10,000	6 (20.7)	2 (6.9)	3.21 (0.52–19.95)	0.210	3.03 (0.46–19.76)	0.248

[†]Calculated odds ratio with 95% confidence using conditional logistic regression.
[‡]Adjusted odds ratio with 95% confidence using conditional logistic regression (PI-1: adjusted for prolonged rupture of membranes, gestational age and day of life at enrollment; PI-2a: adjusted for parity, prolonged rupture of membranes, birth weight, infant gender and day of life at enrollment; PI-2b: adjusted for gestational age and day of life at enrollment).

antibody concentrations between cases and controls at varying thresholds did not demonstrate differences in the adjusted odds ratio for disease for PI-2a and PI-2b antibodies (Table 4, Supplementary Table 7).

Absolute risk of GBS disease & surface-protein antibodies

Using Bayesian modeling, we were able to estimate the absolute risk of invasive disease. None of the studied surface-protein antibodies demonstrated a protective threshold against invasive disease, nor were there any significant reductions in the risk of disease with increasing antibody concentrations (Figure 2A–E). Although the adjusted odds ratio showed a significant difference between cases and colonized controls for FbsA antibodies at thresholds above 10,000 Au/ml and a similar trend for PI-1, no decrease in the absolute risk of disease was observed.

Furthermore, in an exploratory analysis, we measured whether there were any correlations between the select surface-protein antibody concentrations in serotypes I and III cases and homotypic controls, of which there was none (Supplementary Figure 1).

Discussion

To the best of our knowledge, this is the first study to report FbsA, BibA and PI GBS surface-protein antibody concentrations and the risk of invasive GBS disease in infants. We found no association between maternal BibA and the PI surface-protein antibodies and the risk of invasive GBS disease in infants; however, we observed a relative association between maternal FbsA antibody concentrations and a similar trend for PI-1 antibody concentrations and invasive GBS disease in their infants. Although the odds ratios for disease declined with increasing antibody concentrations to FbsA and PI-1, only a small proportion of cases (2.9 and 3.5%, respectively) and controls (11.7 and 15.6%, respectively) had antibody levels >10,000 AU/ml. Importantly, determining the odds ratio for disease at various antibody levels requires a given arbitrary threshold that may not be clinically relevant. An alternative method, using Bayesian modeling, is to estimate the probability of disease at various antibody thresholds, and choose a protective threshold based on a sharp decrease in probability of disease. We were unable to identify any changes in the probability of disease at any antibody threshold and therefore

unable to define correlates of protection for the studied surface-proteins. Our findings of lack of associations between maternal antibodies to these proteins and protection against invasive disease in their infants are contradictory to the promise shown for these antigens as potential vaccine epitopes in animal-model studies.

Animal-model studies have identified FbsA and BibA as highly immunogenic, and antibodies to these proteins protected mice from GBS inoculums [8,23,24]. Furthermore, using neonatal pup challenge models, maternal mice immunized with fragments of the FbsA and BibA proteins were more likely to survive GBS challenges than those not vaccinated [23]. Similarly, maternal mice vaccinated with antigen components of PI-1, PI-2a, and PI-2b antigens compared with unvaccinated controls had improved survival in their litters after GBS inoculation [25,26]. In addition, *in vitro* opsonophagocytic assays demonstrated enhanced killing by polymorphonuclear cells using the vaccinated BibA and PI sera of adult mice [24–26].

Other studies addressing the role of antibodies to various GBS surface-proteins in mothers of infants or newborns with invasive disease include: surface immunogenic protein (Sip), resistance to proteases immunity group B (Rib), αC protein and βC protein [9–13]. Overall, most studies that addressed the association between natural surface-protein antibodies and the risk of GBS disease have reported similar geometric mean concentrations in the mother of infected neonates compared with GBS colonized mothers with well newborns. An association between Rib antibody levels and invasive GBS disease has, however, been demonstrated [11]. Median antibody concentrations were lower in Rib expressed cases (n = 14) compared with controls (n = 60), including an association for disease.

Studies have reported an inverse association between capsular antibody levels and the risk of invasive GBS disease [4]. We found no correlation between invasive GBS disease and any of the studied surface-protein antibody in serotype Ia or III disease cases and homotypic controls, further corroborating that these surface-protein antibodies are unlikely to be a marker of protection against invasive GBS disease.

A limitation of our study was that we did not measure whether GBS-cultured isolates expressed FbsA and BibA in cases and controls so as to compare protein antibody concentration by type specificity. It is, however, thought that BibA may be expressed more universally (>90%) in GBS strains, and approximately half of strains express FbsA [8,24]. A further limitation is that we measured antibody concentrations using our own in-house references as no references are currently reported. In addition, we have not measured the association between IgA antibodies to these surface-proteins and the risk of invasive GBS disease, as suggested to be protective by others [8]. Furthermore, most participants were of black African descent and the results thereof cannot be extrapolated to other racial groups.

In conclusion, our study failed to identify a definitive association for higher maternal antibodies to the five studied GBS surface-proteins and risk for invasive GBS disease in young infants, suggesting a low likelihood that these proteins have potential for being developed into successful vaccine candidates.

Financial & competing interests disclosure

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Key issues

- Group B Streptococcus (GBS) is a common cause of sepsis and meningitis in young infants.
- An alternative strategy to GBS prevention is maternal vaccination, to which a trivalent capsular polysaccharide conjugate vaccine has completed Phase II trials.
- This vaccine, although covers the most prevalent GBS serotypes globally, may be less effective in certain regions and the risk for replacement disease is a possibility.
- Immunogenic GBS surface-proteins have been identified and favor survival in mice challenge studies.
- This is the first study conducted in infants with invasive disease, in which no association was demonstrated for antibodies to fibrinogen-binding protein A, GBS-immunogenic bacterial adhesin, and pilus island proteins.
- These surface-proteins are unlikely to be suitable vaccine candidates.

doi: 10.1586/14760584.2015.1085307

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• of interest
•• of considerable interest

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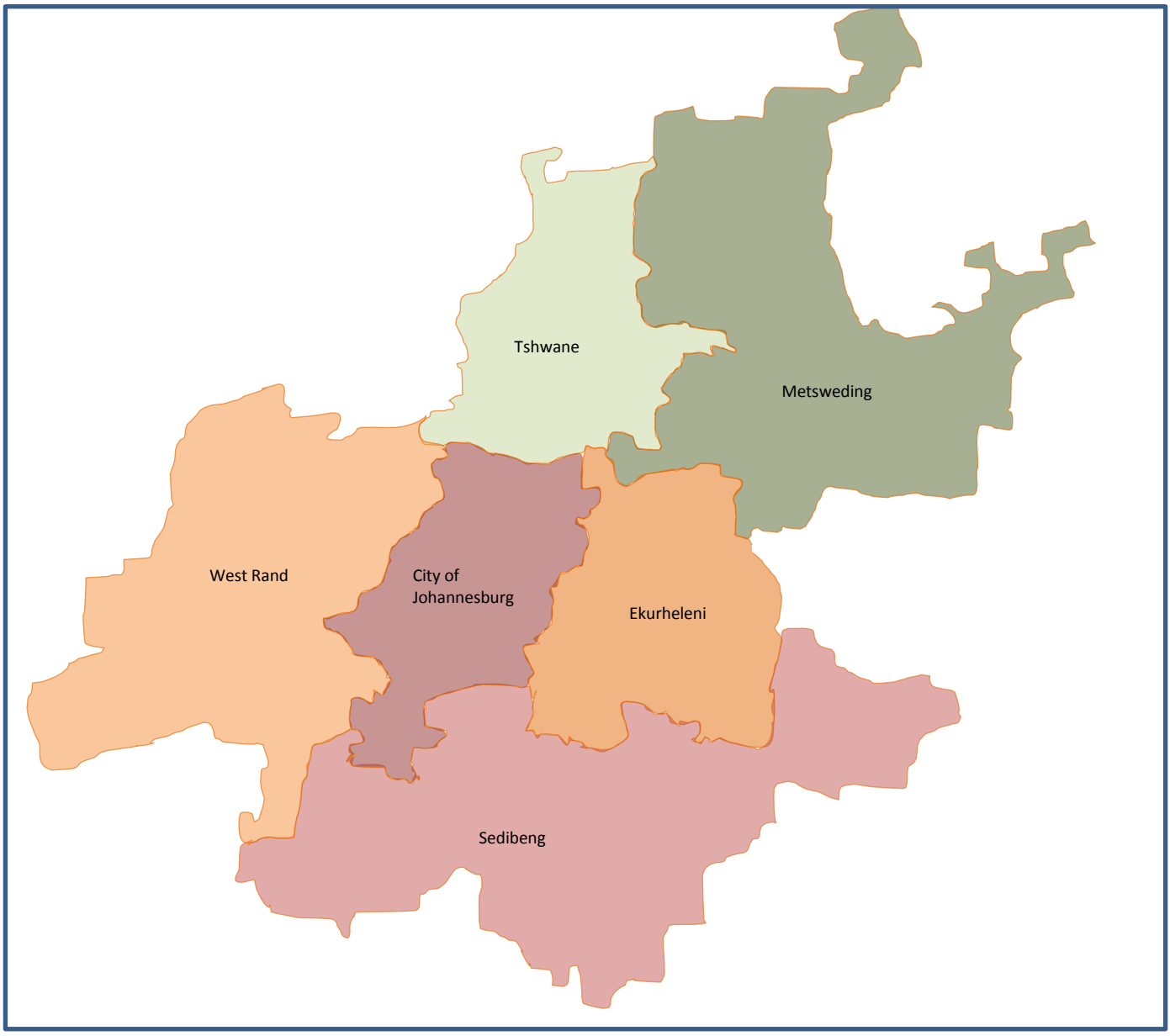
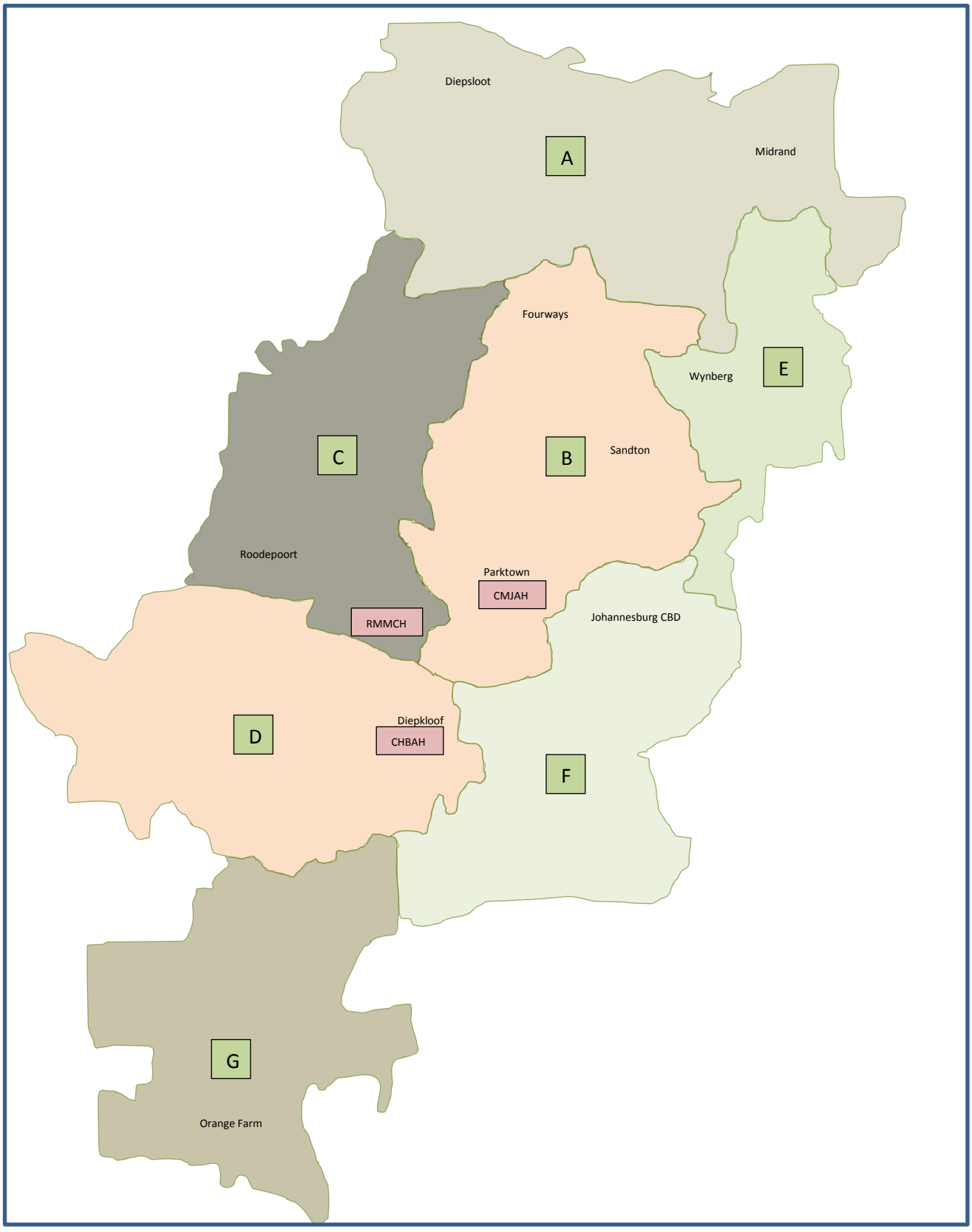
Appendix 5



Map outlining the six districts in Gauteng Province. (Modified from Department of Health and Social Development)

Appendix 6



Map outlining the sub-districts/Regions of the Johannesburg metropolitan and location of the three site hospitals. (Modified from Department of Health and Social Development)

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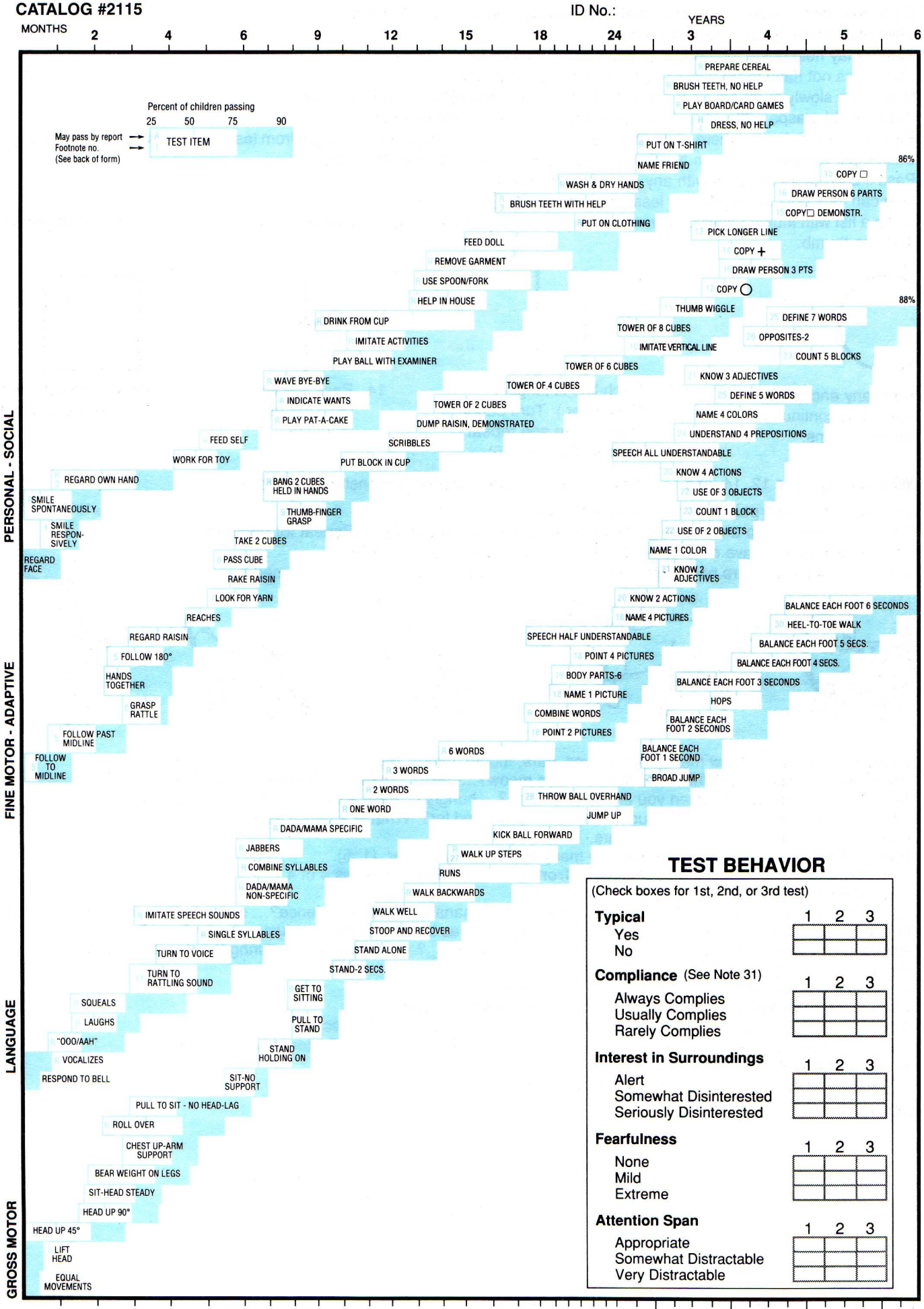

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UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Ziyaad Dangor

CLEARANCE CERTIFICATE	**M120963**
PROJECT	Clinical and Immunological Epidemiology of Invasive Group B Streptococcus (GBS) in South Africa: A Case Control Study
INVESTIGATORS	Dr Ziyaad Dangor.
DEPARTMENT	Department of Paediatrics
DATE CONSIDERED	28/09/2012
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 28/09/2012 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Prof Shabir Madhi

DECLARATION OF INVESTIGATOR(S)
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I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Ziyaad Dangor

CLEARANCE CERTIFICATE	**M120905**
PROJECT	Effect of Maternal HIV-Infection on Trans-Placental Transfer of Group B Streptococcus (GBS) Antibodies
INVESTIGATORS	Dr Ziyaad Dangor.
DEPARTMENT	Department of Clinical Medicine/Paediatrics
DATE CONSIDERED	28/09/2012
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 07/11/2012 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Prof Shabir Madhi

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