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MAJOR ARTICLE

Epidemiology of Group B Streptococcus: Maternal Colonization and Infant Disease in Kampala, Uganda

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Background: Child survival rates have improved globally, but neonatal mortality due to infections, such as group B *Streptococcus* (GBS), remains a significant concern. The global burden of GBS-related morbidity and mortality is substantial. However, data from low and middle-income countries is lacking. Vaccination during pregnancy could be a feasible strategy to address GBS-related disease burden.

Methods: We assessed maternal rectovaginal GBS colonization and neonatal disease rates in a prospective cohort of 6062 women-infant pairs. Surveillance for invasive infant disease occurred in parallel at two Kampala hospital sites. In a nested case-control study, we identified infants <90 days of age with invasive GBS disease (iGBS) (n=24) and healthy infants born to mothers colonized with GBS (n=72). We measured serotype-specific anti-capsular immunoglobulin G in cord blood/infant sera using a validated multiplex Luminex assay.

Results: We found a high incidence of iGBS (1.0 per 1,000 live births) within the first 90 days of life across the surveillance sites, associated with a high case fatality rate (18.2%). Maternal GBS colonization prevalence was consistent with other studies in the region (14.7%; 95% confidence interval 13.7-15.6%). IgG geometric mean concentrations were lower in cases than controls for serotypes Ia (0.005 vs 0.12 µg/mL; p=0.05), III (0.011 vs 0.036 µg/mL; p=0.07) and in an aggregate analysis of all serotypes, (0.014 vs 0.05 µg/mL; p=0.02).

Conclusions: We found that GBS is an important cause of neonatal and young infant disease in Uganda and confirmed that maternally derived antibodies were lower in early-onset GBS cases than in healthy exposed controls.

Keywords: Group B Streptococcus; risk reduction; anticapsular antibody; invasive disease; correlate of protection.

BACKGROUND

Although child survival rates have improved, neonatal mortality remains a significant concern, with infections being a leading cause of neonatal deaths [1]. *Streptococcus agalactiae* (group B *Streptococcus*, GBS) is one of the primary causes of sepsis and meningitis in neonates and young infants in most countries [2,3]. It can manifest as early-onset neonatal disease (EOGBS: 0-6 days) or late-onset disease (LOGBS: 7-89 days).

The global burden of GBS-related morbidity and mortality is substantial, resulting in a high number of cases, infant deaths, stillbirths, and long-term neurodevelopmental impairment [2]. However, data from low and middle-income countries (LMICs) are limited. A 2017 systematic

review identified 90 studies on the incidence of infant invasive GBS disease (iGBS), but only 12 were from Africa [3]. Similarly, data on maternal colonization, the main risk factor for EOGBS, are sparse in African countries [4]. Closing these gaps is essential to informing potential preventive interventions.

In high-income countries (HICs), intrapartum antibiotic prophylaxis (IAP) has significantly reduced the incidence of EOGBS [5]. However, implementing such strategies in LMICs is challenging due to financial and practical constraints and limited access to healthcare facilities [6]. Maternal vaccination during pregnancy may offer a feasible strategy to address GBS-related disease burden, especially in settings where microbiologic screening and IAP are not readily available [7]. The licensure of a GBS vaccine faces a major challenge in that over 60,000 pregnant women-infant pairs would need to be enrolled into a prospective clinical trial to demonstrate efficacy [8]. An alternate pathway to licensure could entail demonstrating safety in pregnant women and benchmarking vaccine-induced immunogenicity against correlates of protection (CoP) defined in seroepidemiological studies [9] with subsequent post-licensure evaluation of effectiveness.

We aimed to estimate the incidence of iGBS in infants and maternal colonization rates in Kampala, Uganda. The nested case-control study aimed to compare antibody concentrations in the cord blood of infants with iGBS to healthy controls.

METHODS

Study design

We undertook a longitudinal, observational study at Kawempe National Referral Hospital (KNRH) in Kampala, Uganda. The FY2020/21 GNI per capita for Uganda is $840, classifying the country as low-income according to the World Bank's global income classification issued on 1st July 2022 [10]. Women consented at the time of birth to their and their infant's participation from birth until 90 days postpartum (birth cohort). In parallel, we monitored hospitalized infants ≤ 90 days old who were diagnosed with iGBS at KNRH and Mulago National Referral Hospital (MNRH) in Kampala but were not enrolled in the birth cohort (surveillance cohort) [11]. Details of the study setting are described in **Supplementary Methods S1.**

Birth cohort

Recruitment took place between 24th April 2019 and 1st September 2020. Eligible participants provided verbal consent to collect samples at delivery (cord blood, separate rectal and vaginal swabs) followed by written informed consent after recovery. If the woman had a stillbirth, consent was requested to collect a heart-blood aspirate, which was used for both culture and measuring anti-GBS CPS IgG concentrations in the serum. Participants received phone follow-ups at three time points: within ten days of the infant's life, one month after childbirth, and 90 days post-

delivery. Infants from the cohort diagnosed with iGBS (defined as isolation of GBS from cerebrospinal fluid or blood culture) had a serum sample (acute serum) and a rectal swab collected. Women were eligible if they were over the age of 18 years and delivering at Kawempe hospital or if they were emancipated minors aged between 14 and 17 years of age, and were willing to stay in the area for the first three months of life (or willing to travel to the clinic until their child was 2 years old if their infant had known or presumed GBS infection).

Surveillance cohort

Recruitment took place between 1st April 2019 and 11th January 2022. Infants aged 0-90 days seeking care at KNRH or MNRH and diagnosed with iGBS were eligible for recruitment into the surveillance cohort. If parents agreed, we collected a serum sample (acute serum) and a rectal swab.

For both the birth cohort and the active surveillance cohort, recruitment was suspended between March 2020 and May 2020 due to lockdown restrictions associated with the COVID-19 pandemic.

Nested case-control study

In a nested case-control study, each eligible patient case was matched to three healthy controls (infants born to mothers colonized with the same serotype, not exposed to intrapartum antibiotics as evidenced by clinical history and in-vitro testing, and who survived to 90 days of life and were not admitted with an illness between birth and 90 days).

Laboratory methods

Rectal and vaginal swabs were collected in Amies transport medium and transported for processing to the MRC/UVRI and LSHTM Research Unit, Entebbe, Uganda. Samples were sent to MRC with a cold chain maintained at the end of each working day and plated on the same day. They were cultured in Todd Hewitt Broth and incubated for 24 hours before being sub-cultured on Chromagar Strep B ®. Blood and cerebrospinal fluid cultures were processed at Makerere Microbiology Laboratories, Kampala. Detailed laboratory methods have been previously published [11]. Antimicrobial susceptibility testing was performed at Cardiff University (**Supplementary Methods S1**). Minimum inhibitory concentrations (MICs) were determined for tetracycline, benzylpenicillin, gentamicin, erythromycin, clindamycin, levofloxacin and chloramphenicol. High-level gentamicin resistance (HLGR) defined as gentamicin MIC >128. Extracted genomic DNA using the QiaSymphony method was sent for whole genome sequencing at the Wellcome Sanger Institute, Hinxton, UK (**Supplementary Methods S1)**. Whole genome sequencing was performed on the Illumina NovaSeq 6000 platform (Illumina, USA) with 150 bp paired-end reads as previously described (**Supplementary Methods S1**) [12]. Serotype-specific anti-GBS CPS IgG concentrations for serotypes Ia, Ib, II III, IV and V were determined using the GASTON-adopted multiplex immunoassay (MIA) in cord and infant sera (**Supplementary Methods S1**) [13].

Statistical analysis

Demographic and clinical characteristics were presented using proportions for categorical variables and medians for quantitative variables. The prevalence of GBS colonization was expressed as a proportion of the total number of participants in the birth cohort swabbed. The incidence of iGBS in the birth cohort was estimated using denominators of facility births in KNRH. Multivariable logistic regression models were used to assess the association between GBS colonization and pregnancy outcomes as described in **Supplementary Methods S1.** Geometric mean concentrations (GMCs) were reported, and the Mann-Whitney U test was used to compare concentrations between cases and controls. For patients with both cord and acute serum collected, cord serum was used. Serotypes with fewer than five cases were excluded from individual analysis due to insufficient sample size, but were included in the aggregate analysis. Statistical analyses were carried out in R version 4.3.0.

Ethical considerations

Ethical approval for this study was granted by the Makerere University College of Health Sciences School of Medicine Research Ethics Committee (Ref 2018-130) and registered with the Uganda National Council for Science & Technology (UNCST) (Ref. HS 2496). Ethical approval for this study was also granted by St George's, University of London Research Ethics Committee (Ref. 2020.0024).

RESULTS

Recruitment and Patient Characteristics

There were a total of 19,653 deliveries at KNRH during the birth cohort recruitment study period (18,807 (95.7%) live births and 846 (4.3%) stillbirths). Overall, 6,479/19,653 (32.9%) women were screened prospectively for eligibility, of whom 6,062 (93.6%) were enrolled into the study, resulting in 6,170 live births in the birth cohort (**Figure 1**). The demographic and clinical characteristics of pregnant women and infants are summarised in **Tables 1** and **2**.

Maternal gbs colonisation

Overall, 5,746/6,062 (94.8%) swabs were collected. Of these, 842 women (14.7%; 95% confidence interval 13.7-15.6%) were colonized with GBS at delivery. GBS colonization was not associated with any of the maternal baseline characteristics modelled (**Table S1**). There was no evidence of an association between maternal GBS colonization and adverse pregnancy outcomes in multivariable models (**Tables S2-S6**).

CPS serotyping and alpha-like protein (alp) gene analysis from whole-genome sequencing (WGS) was done on positive GBS swabs from 766/842 (91.0%) colonized pregnant women. Serotypes Ia (244/766, 31.9%) and III (222/766, 29.0%) were most common among colonizing isolates,

followed by V (142/766, 18.5%), Ib (76/766, 9.9%), II (69/766, 9.0%), IV (10/766, 1.3%) and VI (1/766, 0.1%) (**Figure 2A**). The large majority of the isolates (778/766, 93%) clustered into five major GBS clonal complexes (CCs): CC23 (242/766, 31.6%), CC17 (207/766, 27.0%), CC10 (103/766, 13.4%), CC1 (83/766, 10.8%), CC19 (73/766, 9.5%) (**Figure 2B**). A total of 764/766 (99.7%) isolates were positive for the presence of an alp gene (alp1, alp2/3, alphaC, and rib). The most prevalent was rib (276/766, 36.0%), followed by alp1 (267/766, 34.9%), alphaC (127/766, 16.6%), and alp2/3 (94/766, 12.3%) (**Figure 2C**). The CPS serotype and alp gene distribution among the main GBS CCs are shown in **Figure S1**. The alp gene distribution among the CPS serotypes is shown in **Figure S2**.

Infants with igbs

A total of 35 cases of iGBS were identified from KNRH and MNRH. Of these, 18 cases involved infants born at KNRH, including five from the birth cohort, while 17 involved infants transferred to Mulago from other health centres (**Figure 1**). The incidence of iGBS within the birth cohort was 0.8 cases per 1,000 live births (5/6,170). For babies born at KNRH, the incidence rate, based on birth data between 24th April 2019 and 1st September 2020, was 1.0 cases per 1,000 live births (18/18,807). Most cases (31/35, 88.6%) were EOGBS, and almost one-third (11/35, 31.4%) were born preterm. Among the four LOGBS cases (symptom onset on days 8, 14, 21, and 23), three were admitted directly from home, while one was referred from another health center after initially presenting from home. Follow-up calls did not identify any additional cases. The case fatality rate was 18.2%. (**Table S7)**.

Among the case patients, 18/35 (51.4%) had serotype III, 8/35 (22.9%) had serotype Ia, 5/35 (14.3%) had serotype II, 3/35 (8.6%) had serotype Ib, and 1/35 (2.9%) had serotype V disease (**Figure 2A).** WGS was completed for 32/35 cases. All invasive isolates grouped into five major GBS clonal complexes (CCs): CC17 (16/32, 50.0%), CC23 (8/32, 25.0%), CC1 (4/32, 12.5%), CC10 (3/32, 9.4%), and CC19 (1/32, 3.1%) **(Figure 2B)**. All isolates tested positive for alp genes, with the most common being rib (17/32, 53.1%), followed by alp1 (8/32, 25.0%), alp2/3 (4/32, 12.5%), and alphaC (3/32, 9.4%) **(Figure 2C)**.

Antimicrobial susceptibility

All colonizing isolates were analyzed for the presence of genes associated with antimicrobial resistance (**Table S8**). A total of 739/766 (96.5%) carried at least one tetracycline resistance gene (tetM or tetO). A total of 207/766 (27.0%) carried at least one macrolide-lincosamide-streptogramin B (MLSB) resistance gene (ermA, ermB, ermT, lnuB, lsaC, lsaE, mefA, mphC, msrA, or msrD). A total of 12/766 (1.6%) harboured the aac(6') – aph(2'') gene associated with HLGR and 7/766 (0.9%) carried PBP2x transpeptidase sequence variants associated with reduced beta-lactam susceptibility. Phenotypic antimicrobial susceptibility testing (AST) results were available for 748/766 (97.7%) isolates (**Table S8**). All isolates, including those with PBP2x variants, were susceptible to penicillin. A total of 5/748 isolates (0.7%) had a benzylpenicillin MIC

of 0.125 mg/L, but none exceeded 0.25 mg/L. HLGR was observed in 17/748 (2.3%) isolates. There was some discordance between genotype and phenotype for erythromycin resistance, with 207/766 (27.0%) isolates showing genotypic resistance compared to 288/748 (38.5%) displaying phenotypic resistance. Phenotypic resistance was found in 732/748 (97.9%) isolates for tetracycline, and 39/748 (5.2%) for levofloxacin.

WGS was completed for 32/35 cases (**Table S8**). All 32 invasive isolates carried at least one tetracycline resistance gene and 13/32 (40.6%) carried at least one MLSB resistance gene. None of the invasive isolates carried PBP2x variants. Phenotypic AST results were available for 32/35 isolates (**Table S8**). All 32 isolates were susceptible to penicillin and clindamycin, and none exhibited HLGR. Phenotypic resistance was observed in 31/32 isolates (96.9%) for tetracycline, 15/32 (46.9%) for erythromycin, and 1/32 (3.1%) for levofloxacin.

The prevalence of MLSB resistance genes among invasive and colonizing isolates was higher in CC1 (79/83, 95.2%) and CC19 (51/73, 69.9%) and lower in CC23 (7/242, 2.9%) and CC17 (29/207, 14.0%). MLSB resistance genes were most prevalent among isolates with serotype V (114/143, 79.7%) and less common among isolates with serotype Ia (16/252, 6.3%) and III (39/240, 16.2%) (**Figure S3**).

Anti-CPS igg concentration in infants with igbs compared to healthy controls

The demographic and clinical characteristics of the case and control groups are summarised in **Table 3**. Case patients were more likely to be born preterm and with low birth weight. Among the 24 infants with enough sera to be included in the case group, 19/24 (79.2%) were diagnosed with EOGBS within 0-2 days of birth, 3/24 (12.5%) developed LOGBS with symptom onset on days 8, 14, and 23, respectively, and 2/24 (8.3%) were antepartum stillbirths born at term. Cord blood samples were available for 7/24 (29.2%) cases (four with EOGBS, one with LOGBS and two stillbirths). Acute disease sera were collected from 19/24 (79.2%) cases, with a median of six days (range: 3-8 days) between symptom onset and serum collection. For serotype Ia, controls had higher geometric mean concentrations (GMCs) of anti-CPS IgG compared to the case patients (0.12 vs 0.005 µg/mL; p=0.05) (**Figure 3A**). For serotype III, controls had higher GMCs than case patients (0.036 vs 0.011 µg/mL; p=0.07) (**Figure 3B**). In an aggregate analysis of all serotypes, controls had higher GMCs of anti-CPS IgG than case patients (0.05 vs 0.014 µg/mL; p=0.02) (**Figure 3C**). When considering only EOGBS cases, controls had marginally higher GMCs than case patients for serotype Ia (0.12 vs 0.005 µg/mL; p = 0.05) and serotype III (0.036 vs 0.010 µg/mL; p = 0.05). The difference was statistically significant when all serotypes were combined (0.05 vs 0.011 µg/mL; p = 0.02) (**Figure S4**).

DISCUSSION

This study provides the first estimates of iGBS incidence in newborns and young infants in Kampala, Uganda. For the first time, it also compares serum anti-GBS CPS IgG levels between Ugandan infants with iGBS and healthy controls born to women colonised with GBS at delivery. Our data confirmed that GBS is an important cause of neonatal and young infant disease in Uganda [14]. In HICs, where there is good capture of cases and routine laboratory surveillance, GBS has been well-recognized as one of the leading causes of early and late-onset disease in neonates and young infants since the 1990s [15]. However, there is still uncertainty about the incidence of iGBS in LMICs. Well-conducted studies in healthcare facilities in South Africa, Kenya, and The Gambia have indicated that GBS is an important neonatal pathogen in those settings [16–18]. Our study confirms these findings, reporting a disease incidence similar to the previous estimate of iGBS in Africa (1.0 vs 1.12 per 1000 live births) [19]. The case fatality ratio in our cohort was high, in keeping with previous regional estimates [2].

The maternal colonization rate was 14.7% (95% CI 13.7-15.6%), which is lower than the 28.8% reported in a 2016 study at Mbarara Regional Referral Hospital in rural Southwestern Uganda [20]. However, it is consistent with the most recent region-specific GBS colonization prevalence for sub-Saharan Africa, estimated at 16.1% (13.7-19.0%) [2]. These are high-quality data, given that the study used the gold-standard sampling method: rectal/vaginal swabs collected at delivery with selective enrichment in the lab, addressing some of the biases noted in earlier studies [4]. It is also important to highlight that data from Africa outside of South Africa have been more sparse, making this estimate from East Africa particularly valuable.

Overall, a hexavalent vaccine (serotypes Ia, Ib, II, III, IV and V) and an alp-based vaccine would provide comprehensive cover against invasive (100% and 100%, respectively) and colonizing GBS strains (99.7% and 99.7%, respectively) in our cohort. This aligns with a previous systematic review indicating that 93-99% of iGBS in Africa could potentially be prevented by a hexavalent or alp-based vaccine [21]. Additionally, a large multi-country colonization study found that a hexavalent vaccine would cover 97.3% of all maternal colonizing isolates [22]. While six main serotypes are most common, some rarer serotypes have also been identified in Ghana and Egypt [23].

Reassuringly, all invasive and colonizing isolates were susceptible to penicillin. This is of clinical importance, given that penicillin is the first-line treatment and prevention of GBS infections, which is now jeopardized by the emergence of GBS with reduced penicillin susceptibility in HICs and LMICs [24,25]. In contrast, high rates of phenotypical resistance to erythromycin were observed in both colonizing and invasive isolates. Resistance to erythromycin in GBS strains has risen dramatically in recent years, with rates varying according to geographical regions between 22.5% and 74.1% [26]. The two clonal complexes with the highest prevalence of MLSB resistance genes in this and previous studies, CC1 and CC19, could be potential reservoirs of high-risk lineages and require further genomic surveillance [12].

Serotype Ia and III anti-GBS CPS IgG levels were higher in the cord blood of healthy babies born to colonized women compared to those with EOGBS due to the same serotype. Although the difference was small, likely due to the small sample size in our study, these findings are in line with previous research in this area [27–31]. In our aggregate analysis of all serotypes, only one case had IgG concentrations above the threshold associated with a 90% risk reduction, as proposed in a seroepidemiological study from South Africa that used a similar design and laboratory methods [32]. However, a key difference between the studies is the proportion of LOGBS cases—12.5% in this study compared to 48% in the South African study. This may be due to case ascertainment in our highly mobile population.

Our study has several limitations. First, the birth cohort included only a third of the total pregnancies in Kawempe, which limits the generalisability of our results to the entire catchment population. However, our careful review of hospital logs enables us to have relative certainty that the women recruited are typical of those presenting to the hospital and those presenting to public hospitals across Uganda. Second, the very low proportion of LOGBS cases suggests a recruitment bias, possibly indicating under-ascertainment of cases in the community or outpatient settings due to limited access to care for infants not included in the birth cohort during the study. Also, because lumbar punctures are infrequently undertaken in this population, it is possible that GBS meningitis, the main clinical presentation of LOGBS, may have been significantly underestimated. Third, the lower IgG concentrations observed in cases compared to controls may have been confounded by the higher proportion of preterm births. Due to the small sample size, a sub-analysis of term births could not be performed. Fourth, since we did not have cord blood samples from all cases, we measured IgG concentrations in acute disease sera. This method is likely a reliable surrogate for EOGBS cases, which formed the vast majority of cases in our study, but it may not be as accurate for LOGBS due to the decline in antibodies after birth. Fifth, due to the small number of case patients, we did not determine the serotype-specific or serotype-aggregate disease risk-IgG concentration relationships. To address the issue of small sample size, we propose to combine the antibody data with that derived from cases and controls from several African and European countries (Malawi, UK, Netherlands, Italy, and France) and process using the same assay, in order to determine anti-CPS IgG concentration thresholds associated with reduced risk of disease [33]. Finally, we cannot exclude the possibility that we did not capture all infants with infections in the first three months of life who were born at one of our study sites. To minimise this, in addition to regular telephone follow up, we reviewed all admission records and compared these to birth records to ensure that case capture was as accurate as possible.

In conclusion, GBS is an important, potentially preventable cause of neonatal disease and death in urban Uganda. Maternal GBS vaccination provides a key opportunity to reduce morbidity and mortality in this high-burden region.

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TABLES

Table 1: Demographic and clinical characteristics of enrolled pregnant women.

Characteristic	**Overall, N = 6,062[1]**
Age	25 (21, 29)
<18 years	157 (2.6%)
18- 24 years	2,773 (45.7%)
25-34 years	2,584 (42.6%)
35-41 years	513 (8.5%)
>41 years	35 (0.6%)
Education Level	
No formal education	309 (5.1%)
Primary	1,697 (28.0%)
Secondary	3,303 (54.5%)
Tertiary/University	753 (12.4%)

Gravidity	
1	2,007 (33.1%)
2-4	3,165 (52.2%)
>4	890 (14.7%)
Previous Stillbirth among those with previous pregnancy (n=4,055)	
Previous Stillbirth	183 (4.5%)
No previous stillbirth	3,872 (95.5%)
Previous abortion among those with previous pregnancy (n=4,055)	
Previous abortion	1,099 (27.1%)
No previous abortion	2,956 (72.9%)
Previous child death (<5years) among those with previous livebirth (n=3,697)	
Yes	277(7.5%)
No	3,420 (92.5%)
Total number of pregnancy tetanus immunizations	
0	117 (1.9%)
1	682 (11.3%)
2-4	3,265 (53.9%)
>4	1,946 (32.1%)
Unknown	52 (0.8%)
Delivery	
Vaginal	5,522 (91.1%)
C-Section	461 (7.6%)

Assisted vaginal	78 (1.3%)
Unknown	1 (<0.1%)
Duration of ROM	
< 18 hours	4,787 (79.0%)
>=18 hours	785 (12.9%)
Unknown	490 (8.1%)
Antibiotics during labour	
Yes	116 (1.9%)
No	5,945 (98.1%)
Unknown	1 (<0.1%)
Multiple Pregnancy	
Singleton	5,897 (97.3%)
Twins	162 (2.7%)
Triplets	3 (<0.1%)
Maternal Death	3 (<0.1%)
Fever during labour	32 (0.5%)
HIV status	
Negative	5,452 (89.9%)
Positive	599 (9.9%)
Unknown	11 (0.2%)
Syphilis	
Positive	100 (1.6%)
Negative	5928 (97.8%)
Unknown	34 (0.6%)

Hepatitis B (n=6,037)	
Positive	131 (2.2%)
Negative	5906 (97.4%)
Unknown	25 (0.4%)
Maternal nutrition, Mid-upper arm circumference	
Malnourished (MUAC <21cm)	61 (1.0%)
Underweight (21≤ MUAC <23cm)	147 (2.4%)
Normal weight (23≤ MUAC < 27cm)	2,787 (46.0%)
Overweight (27≤ MUAC < 31cm)	2,630 (43.4%)
Obese (MUAC ≥ 31cm)	435 (7.2%)
Unknown	2 (<0.1%)
Tobacco/cigarettes	
Currently smokes	8 (0.1%)
Has quit smoking during pregnancy	5 (<0.1%)
Never smoked	6,049 (99.8%)
Pipe	
Currently smokes from a pipe	15 (0.2%)
Has quit pipe smoking during pregnancy	5 (<0.1%)
Never smoked a pipe	6,042 (99.7%)
Alcohol	
Currently drinks alcohol	336 (5.5%)
Has quit drinking alcohol during pregnancy	153 (2.5%)
Never drinks alcohol	5,573 (92.0%)
[1] *n (%)*	

Table 2: Demographic and clinical characteristics of all live newborns and stillbirths in the birth cohort.

Characteristic	**Overall, N = 6,231[1]**
Sex (n=6,229)	
Male	3,133 (50.3%)
Female	3,096 (49.7%)
Unknown	2 (<0.1%)
HIV exposed (n=6,220)	
Negative	5,601 (89.9%)
Positive	619 (9.9%)
Unknown	11 (0.2%)
Birth outcome	
Liveborn	6,170 (99.0%)
Stillborn	61 (1.0%)
Infant death between birth and 90 days	161 (2.6%)
Deaths in Labour ward	12 (7.5%)
Deaths at Postnatal ward/NICU	78 (48.4%)
Deaths after hospital discharge	71 (44.1%)
Term/preterm (Ballard)	
≥ 37 weeks	5,756 (92.4%)
≥ 32 to < 37 weeks	365 (5.9%)
< 32 weeks	44 (0.7%)
Unknown	66 (1.0%)
Birth weight	
< 2500 grams	724 (11.6%)

>= 2500 grams	5,502 (88.3%)
Unknown	5 (0.1%)
Birth weight < 2000 grams	222 (3.6%)
Resuscitation at birth	
Required resuscitation	721 (11.6%)
Did not require resuscitation	5,448 (87.4%)
Unknown	62 (1.0%)
Congenital Abnormalities (n=5,846)	
Yes	72 (1.2%)
No	5,774 (92.6%)
Unknown	385 (6.2%)
[1] n (%)	

Table 3. Demographic and clinical characteristics of patient cases and controls.

Characteristic	Cases (n=24) [1]	Controls (n=72) [1]	P Value [2]
Serotype Distribution			
Ia	5 (20.8%)	15 (20.8%)	**
Ib	3 (12.5%)	9 (12.5%)	
II	4 (16.7%)	12 (16.7%)	
III	11 (45.8%)	33 (45.8%)	
V	1 (4.2%)	3 (4.2%)	
Onset of Disease (days age)		NA	
Stillbirth	2 (8.3%)		
0	10 (41.6%)		
1	8 (33.3%)		
2	1 (4.2%)		
3-6	0 (0.0%)		
7-13	1 (4.2%)		
14-20	1 (4.2%)		
>20	1 (4.2%)		
Gestational age at birth	37 (32.5-38.5)	39 (38-40)	0.002
Term (≥ 37 weeks)	17 (70.8%)	69 (95.8%)	
Preterm (< 37 weeks)	7 (29.2%)	3 (4.2%)	
34 - <37 weeks	2 (8.3%)	2 (2.8%)	
32 - <34 weeks	3 (12.5%)	1 (1.4%)	

28 - <32 weeks	2 (8.3%)	0 (0.0%)	
Sex			0.6
Male	12 (50.0%)	40 (55.6%)	
Female	12 (50.0%)	32 (44.4%)	
Birthweight (grams)	2,585 (2,120-3,350)	3,240 (2,925-3,580)	<0.001
LBW (< 2,500 grams)	11 (45.8%)	1 (1.4%)	
Normal BW (≥ 2,500 grams)	13 (54.2%)	71 (98.6%)	
Maternal HIV Status			1.0
Living with HIV	2 (8.3%)	5 (6.9%)	
Not living with HIV	22 (91.7%)	67 (93.1%)	
Antibiotics during labour			1.0
No	24 (100.0)	72 (100.0)	
Yes	0 (0.0)	0 (0.0)	

*[1]Median (IQR); n (%) [2]Fisher's exact test ** p value for serotype distribution is missing as the study is matched for 1 case to 3 controls*

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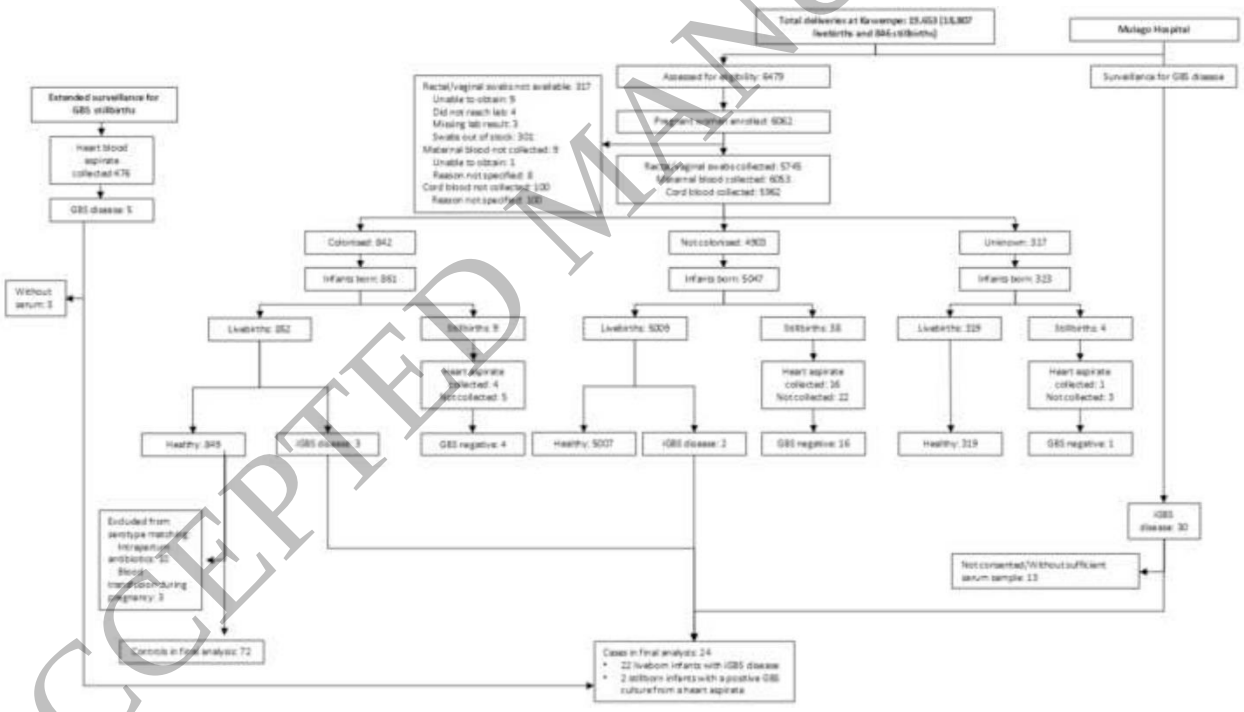
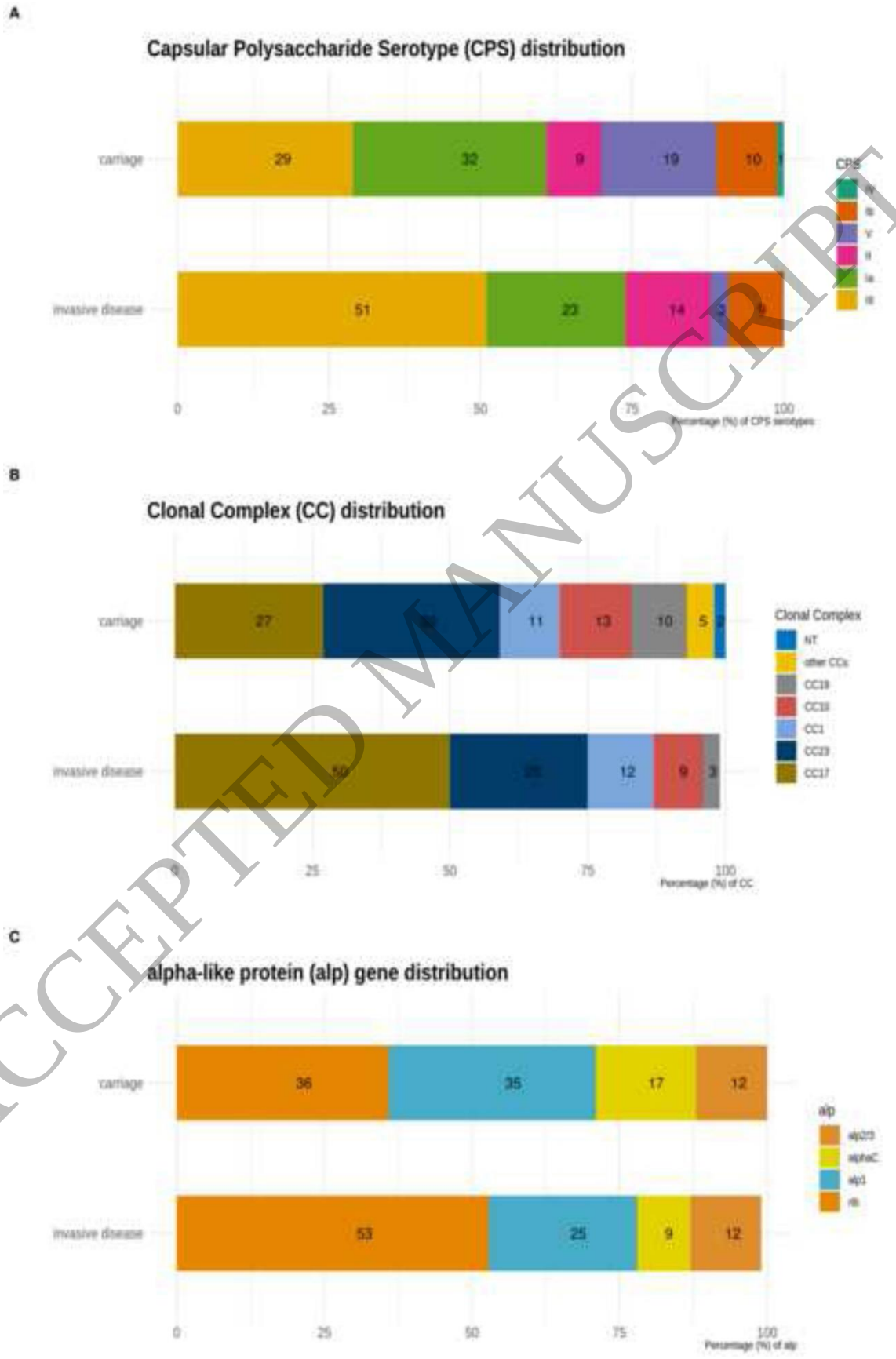
FIGURES LEGENDS AND ALT TEXT

Figure 1: Study cohort diagram. GBS: Group B Streptococcus; iGBS: invasive GBS disease



Alt text: Flow diagram of the study cohort, showing the selection process, inclusion/exclusion criteria, and grouping of participants. The visual outlines the progression of the cohort through different stages of the study.

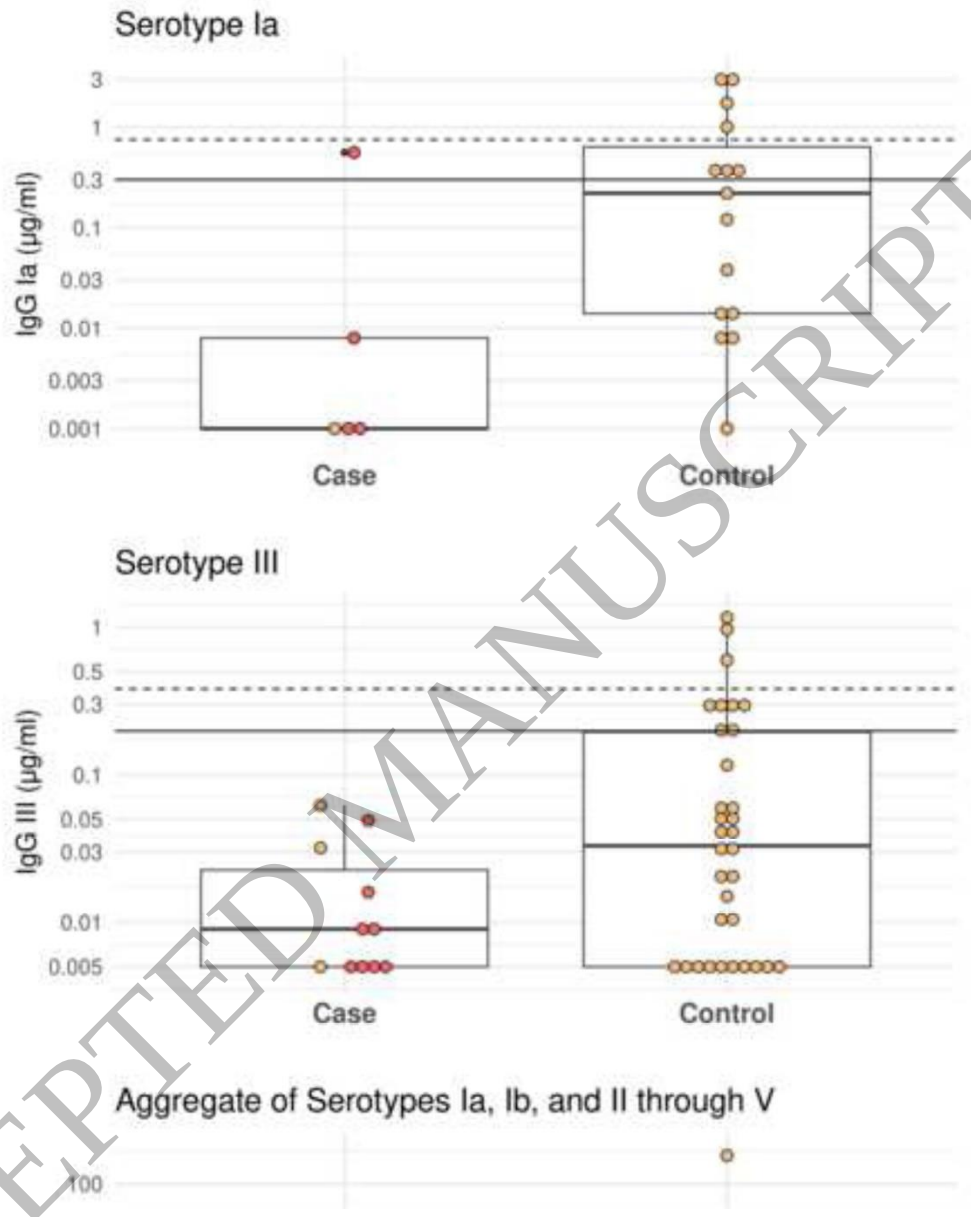
Figure 2: A. The percentage of different serotypes in GBS isolates from maternal colonization, and invasive GBS disease; B. The percentage of different clonal complexes in GBS isolates from maternal colonization, and invasive GBS disease; C. The percentage of different alpha-like protein genes in GBS isolates from maternal colonization, and invasive GBS disease. GBS: Group B Streptococcus; CPS: Capsular Polysaccharide; alp: alpha-like protein; NT: Not typed.



DOI: 10.1093/ofid/ofaf167

Alt text: Bar charts displaying the distribution of characteristics among isolates from maternal colonization and invasive GBS disease. (A) Percentage of different serotypes. (B) Percentage of different clonal complexes. (C) Percentage of different alpha-like protein genes.

Figure 3: A. Anti-CPS IgG concentrations among infant case patients and controls for serotype Ia; B. Anti-CPS IgG concentrations among infant case patients and controls for serotype III; C. Anti-CPS IgG concentrations among infant case patients and controls for aggregated serotypes. The horizontal continuous line indicates the recently proposed threshold associated with 80% risk reduction. The horizontal dashed line indicates the recently proposed threshold associated with 90% risk reduction. Each point represents an individual sample. Yellow points indicate cord serum. Red points indicate infant serum collected during the acute phase of the disease. CPS: Capsular Polysaccharide; IgG: Immunoglobulin G.



Alt text: Dot and box plots showing anti-CPS IgG concentrations in infant case patients and controls for (A) Serotype Ia, (B) Serotype III, and (C) Aggregated serotypes. Yellow dots represent cord serum, and red dots represent infant serum from the acute phase. Horizontal lines indicate proposed risk reduction thresholds.