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Diversity in Naturally Acquired Immunity to Group B Streptococcus: A Comparative Study of Women from Bangladesh, Malawi, and the United Kingdom

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Background. Significant disparities in Group B Streptococcus (GBS) colonisation and neonatal disease rates have been documented across different geographical regions. For example, Bangladesh reports notably lower rates compared to the United Kingdom (UK) and Malawi. This study investigates whether this epidemiological variability correlates with the immune response to GBS in these regions.

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Methods. Qualitative and quantitative analyses of naturally acquired immunoglobulin G (IgG) antibodies against GBS capsular polysaccharides (CPS) and the Alp protein family were conducted in serum samples from women of childbearing age in the UK, Bangladesh, and Malawi. The efficacy of these antibodies in clearing vaginal colonisation or protecting newborns from GBS infection was assessed using humanised mouse models.

Results. Bangladeshi women displayed the highest diversity in serotype distribution, with elevated immunoglobulin G (IgG) levels in the serum against GBS capsular polysaccharides (CPS)- Ia, Ib, II, III, IV, and V, as well as Alp family proteins. In contrast, Malawian sera demonstrated the weakest antibody response. Bangladeshi sera also showed heightened IgG-mediated complement deposition, opsonophagocytic killing and neonatal Fc receptor (FcRn)-binding while tested against CPS Ib. In a humanised FcRn mouse model, Bangladeshi sera led to faster clearance of GBS virulent serotype Ib vaginal colonisation. Additionally, offspring from dams passively immunised with Bangladeshi sera demonstrated notably increased survival rates.

Conclusions. This study demonstrates significant variability in the immune response to GBS across different geographical regions. These findings underscore the importance of understanding GBS-induced immune response in diverse populations, which may significantly impact vaccine efficacy in these regions.

Keywords: Group B Streptococcus, GBS, antibody, immunoglobulin G, Bangladesh, UK, Malawi, capsular polysaccharides, protein, Alp

BACKGROUND

Group B Streptococcus (GBS) is a major cause of neonatal and infant mortality worldwide [1]. Although initiatives are underway to develop effective GBS vaccines, baseline immunity against GBS in the population is largely unknown. Several studies have reported a striking discrepancy in GBS colonisation and disease incidences across geographical regions, with variability observed in both maternal rectovaginal colonisation rates and neonatal disease incidences across Europe, Africa, and Southeast Asia [2-4]. For instance, the maternal rectovaginal colonisation rate was reported as 21.2% in Malawi and 20% in the UK, whereas in Bangladesh, it was 11%, though more recently reported as 17.5% by Kwatra et al. [4-7] . Infant GBS disease incidence is significantly higher in the UK and Malawi than in Bangladesh: 0.94 per 1000 live births in the UK, 1.8 per 1000 live births in Malawi, and only 0.10 per 1000 live births in Bangladesh [2, 8, 9]. These intriguing differences highlight the necessity to explore the underlying factors contributing to variation in GBS epidemiology. Contributing factors include GBS strain virulence, genetic susceptibility, environmental exposure, healthcare practices such as antibiotic use and access, and regional differences in population immunity, whether from natural exposure or passively acquired anti-GBS antibodies, which may significantly shape the epidemiological landscape [10, 11].

Increased levels of naturally occurring antibodies against GBS capsular polysaccharide (CPS) are associated with a lower risk of subsequent acquisition of rectovaginal colonisation in pregnant women [12-14]. This association extends to a reduced risk of both GBS early-onset disease (EOD) and late-onset disease (LOD) in newborns, underscoring the protective role of placentally transferred maternal antibodies that may remain effective up to 90 days after birth. [15-17]. Placentally transferred naturally occurring GBS protein-specific antibodies have also been linked to protection against EOD and LOD [18, 19]]. In addition to antibody concentration, functional properties and subclass specificity of IgG are also important determinants of protection against various pathogens and vaccines, including GBS [20-24]. Furthermore, the binding of IgG to FcRn influences the clearance of GBS vaginal colonisation and transplacental transfer of antibodies[25, 26].

Of the ten GBS serotypes, Ia, Ib, II, III, IV, and V are the most widespread worldwide and account for most colonisation and invasive diseases [27]. Hence, these six CPS antigens have been included in a hexavalent GBS conjugate vaccine currently in phase 2 clinical trials [12, 28]. Alongside GBS polysaccharide-based vaccines, a protein subunit vaccine targeting the N-terminus of the GBS Alpha-like protein (Alp) family of surface proteins, Alp1, Alp2/3, AlphaC (αC), and Rib, has been shown to elicit a functional antibody response in healthy adult non-pregnant women [23].

In the present study, we investigated naturally acquired anti-GBS antibodies in women of childbearing age, targeting CPS Ia, Ib, II, III, IV, and V, as well as the Alp-N, across three geographically distinct populations: the UK, Malawi, and Bangladesh. Through a quantitative and qualitative assessment of IgG using both *in vitro* methods and clinically relevant *in vivo* models, we examined the correlation between the humoral immune response and the reported variations in GBS epidemiology across these regions.

METHODS

Study design and participants. Serum samples used in this study were collected from completed studies or a repository of publicly available samples stored anonymously with generic identification numbers at the University of Liverpool, UK. One hundred samples were randomly selected from each study site: the UK, Bangladesh, and Malawi. Inclusion criteria were women aged 25 to 35, regardless of GBS colonisation or pregnancy status. All samples were collected or stored with informed consent from participants. Details of ethics are included in the supplementary materials.

Quantitation of CPS-specific IgG in sera. A multiplex direct Luminex-based immunoassay was utilised to measure the levels of IgG antibodies in serum samples) as described elsewhere [29]. A brief description is given in the supplementary materials. Twelve Malawian samples were excluded due to insufficient quantity for this assay.

Quantitation of Alp-N-specific IgG in sera. IgG against Alp1-N, Alp2/3-N, αC-N, and Rib-N antigens in the serum samples were quantified using ELISA as described previously [30]. Please see the supplementary file for a detailed method.

Quantitation of CPS- and Alp-N-specific IgG subclasses. Fluorescent magnetic beads (Luminex Corp) were coupled with CPS-Ib and Alp-N antigens following the manufacturer's instruction. Antigen-coated beads ($5 \times 10^{2-3}$ beads/well, 80 µl) were mixed with 20 µl serum samples pre-diluted in assay wash buffer (PBS-1X, 0.1% BSA, 0.05% Tween 20, pH 7.4) in a 96-well plate and incubated for 2 hours at room temperature on an orbital shaker (450 rpm). Sample dilutions are of 1:250 for IgG1, IgG2, and IgG3, and 1:50 for IgG4. RPE-coupled detection antibodies for each subclass (Southern Biotech) were added at a concentration of 0.65 µg/ml and incubated for 1 hour at room temperature on an orbital shaker. Beads were then washed and thoroughly mixed in the buffer by briefly vortexing and sonicating for 30-60 seconds. The binding between antigens and antibodies was quantified using the BioPlex-200 system (Bio-Rad), with results reported as mean fluorescence intensity (MFI).

Antibody Dependant Complement Deposition (ADCD) assay. Total IgG in serum samples was purified using the Melon™ Gel IgG Purification kit (ThermoFisher Scientific, UK) following the manufacturer's instructions. CPS Ib and Alp protein antigen-coated beads ($5 \times 10^{2-3}$ beads/well, 5 µl) were incubated with 80 µl purified IgG at a final dilution of 1:250, followed by a 2-hour incubation at 37°C. After washing four times with wash buffer (PBS+1%BSA), a 1:150 dilution of human complement (Sigma, S1764) was added and further incubated for 30 minutes at 37°C. Subsequently, Biotinylated monoclonal anti-human C3d (Quidel, A702, 1 µg/ml in PBS, 80 µl) was added and incubated for 30 minutes at room temperature in the dark. After washing, Streptavidin-RPE (1 µg/ml in PBS) was added, followed by another 30 minutes of incubation at room temperature in the dark. Finally, the beads were washed and resuspended in wash buffer, and C3d binding was measured using the BioPlex-200 system, with results expressed as MFIs.

FcRn-binding assay. Recombinant Human FCRN Protein (R&D, 8639-FC) was biotinylated using the EZ-LinkTM Sulfo-sNHS-LC-Biotin labelling kit (ThermoFisher). Streptavidin-PE (Prozyme, PJ31S) was added to FcRn-biotin (0.65µg/ml) at 4:1 to create a PE-conjugated FcRn detection reagent. After adding 1% v:v of free biotin, the reagent was stored at 4°C in the dark until use.

CPS-Ib or Alp-N antigen-coated beads (5 × 102-3 beads/well, 80 µl) were mixed with 20 µl serum in a black 96-well microplate and incubated for 2 hours at room temperature on an orbital shaker (450 rpm) in the dark. Following incubation, beads were washed with the assay buffer (PBS, 0.1% BSA and 0.05% Tween-20, pH 7.4), and the previously prepared detection reagent was added and further incubated for one hour. After the final wash in the wash buffer adjusted to pH 5.8, beads were resuspended in the same buffer to measure the FcRn binding with IgG, which was measured using the BioPlex-200 system, and the results were expressed in MFIs.

Opsonophagocytosis killing assay (OPKA). The OPK assay was performed using the HL-60 cell line as described by Leung *et al.*, 2023 [31]. Please see the supplementary file for a detailed method.

Mouse model of passive immunisation, vaginal colonisation, and neonatal protection. Serum samples from the UK, Malawi, and Bangladesh were pooled within each country group and normalised with PBS to achieve a consistent IgG concentration of 6 µg/ml. Details on the selected serum samples are provided in Supplementary Table 1. All animal experiments were performed per the Home Office Scientific Procedures Act (1986) regulations, project licence PP2832279 and the University of Liverpool Ethical and Animal Welfare Committee.

Female Balb/C mice, aged six to eight weeks, were intraperitoneally injected with 0.5 mg of β-oestradiol suspended in 100 µl of sesame oil. The following day, mice received intraperitoneal injections of 100 µl serum samples or PBS. Twelve hours later, mice were vaginally inoculated with $1x10^5$ colony-forming units (CFU) of GBS serotype Ib, a clinical strain isolated from a neonatal meningitis case and tested for virulence in a murine model. Vaginal tissue was collected from mice euthanised on days 1, 3, and 7 post-inoculations, homogenised, serially diluted in PBS, and plated on blood agar. Colony-forming units (CFU) per millilitre were enumerated after overnight incubation at 37°C to determine bacterial load.

Humanised FcRn mice (014565B6.Cg-FcgrttmlDcr Tg(FCGRT)32Dcr/DcrJ), purchased from the Jackson Laboratory, were bred in-house for the neonatal protection model. Pregnant dams (n=5/ country) were injected (i.p.) with serum samples or PBS on day 16 of gestation. Pups were challenged intraperitoneally with GBS within 24 hours of birth and monitored for disease signs and mortality over 72 hours. To calculate the IgG transfer ratio, a blood sample was collected from another batch of hFcRn pregnant mice after 24 hours of passive immunisation via tail bleeding and from the pups within 12-18 hours of birth from the chest cavity after dissecting the heart. IgG concentration to CPS Ib was measured using the immunoassay described above.

Statistical analysis. Statistical analyses were conducted using GraphPad Prism version 9.0.1 software (www.graphpad.com). Differences across three countries were determined by the non-parametric Kruskal-Wallis one-way ANOVA test with Dunn's multiple comparisons test. Two-way ANOVA with Dunnett's post hoc test was used to compare CFU between the three countries and PBS control. Survival curves were compared by the log-rank (Mantel-Cox) test. For all analyses, an adjusted p-value < 0.05 was considered significant to account for multiple comparisons and control for type I errors. Reverse Cumulative Distribution (RCD) curves were created using Microsoft Excel version 1808 to present the IgG concentrations against CPS and Alp antigens across countries.

RESULTS

Serotype diversity and quantity of GBS CPS-specific antibody

We conducted a comprehensive analysis of naturally developed antibodies against GBS in women aged 25-35 residing in three geographically diverse regions: the UK (n=100), Bangladesh (n=100), and Malawi (n=88). Serum samples were evaluated for IgG concentration against GBS CPS Ia, Ib, II, III, IV, and V. Bangladeshi women exhibited the highest seroprevalence against all six serotypes, with serotype II-specific antibodies being the most abundant across all three countries. (Figure 1A). Multiple GBS serotype-specific antibodies were detected in women from all regions, with a notably higher number in Bangladeshi women (Figure 1B). Approximately 31% of Bangladeshi women had IgG against all six serotypes, compared to 23% in Malawi and only 4% in the UK. Reverse cumulative distribution curves revealed higher antibody concentrations in Bangladeshi serum for all six serotypes. (Figure 1C). The geometric mean concentration (GMC) of IgG against the six serotypes was highest in Bangladeshi sera (Supplementary Table 2). Overall, Bangladeshi women demonstrated the highest seroprevalence, presence of IgG against multiple CPS types, and IgG concentrations against all six serotypes assessed in this study.

The distribution of the CPS Ib-specific igg subclasses and effector functions across three countries

While analysing IgG subclass prevalence in the serum samples, the MFI of IgG1 was found to be lower in Malawi sera compared to the UK (not statistically significant) and Bangladesh ($p<0.05$). The most significant difference was found in IgG2 levels, with the Malawian population exhibiting substantially lower IgG2 values than the UK and Bangladesh (Figure 2A). Bangladeshi sera displayed significantly higher IgG4 levels, while IgG3 levels remained similar across all three countries.

Bangladeshi women exhibited notably higher levels of FcRn-binding and ADCD, whereas Malawi sera demonstrated the lowest ADCD and FcRn-binding capabilities (Figures 2B and 2C). Additionally, Bangladeshi sera displayed the highest OPK response against the CPS Ib-expressing GBS strain (NCTC, 14092), while comparable titres were observed in UK and Malawi sera (Figure 2D). These results indicate enhanced IgG-mediated effector functions and FcRn-binding in Bangladeshi women in response to GBS.

Seroprevalence, subclass distribution and effector functions of Alp-N-specific igg across three countries

Subsequently, we profiled the naturally acquired humoral response against the GBS surface proteins of the Alp family (n=100/ country). No significant variations were noted in the prevalence of anti-Alp-N antibodies among the three countries, with high Rib-N seropositivity observed across all countries. However, a notably lower seropositivity against Alp2/3-N was

evident in the Malawian population (Figure 3A). The reverse cumulative distribution curves for antibody concentrations in Figure 3B depict a significantly lower Alp-N-specific IgG concentration in Malawi compared to the UK and Bangladesh. Significant differences were observed in geometric mean concentrations across the three countries, with the Malawian population displaying the lowest antibody levels (Supplementary Table 3).

Although the IgG subclasses were comparable for some Alp proteins amongst the three countries, a general trend of higher subclass levels was observed in Bangladeshi sera, particularly in IgG1 and IgG3 (Figure 3C, Figure 3E). In contrast, Malawi exhibited the lowest levels for all subclasses against Alp-N proteins, with a significantly low IgG2 level compared to the UK and Bangladesh (Figure 3D).

Among the four Alp-N proteins, the greatest ADCD was exhibited by IgG specific for Alp1-N and Rib-N (Figure 4A). Interestingly, ADCD to Alp1-N and Rib-N antigens was significantly higher in Malawi than in other countries. In contrast to ADCD, FcRn-binding by anti-Alp protein IgG was similar among all four protein antigens, although there were marked inter-country variations (Figure 4B). Across all antigens, the lowest FcRn-binding was observed in Malawi, most notably with Alp2/3-N and αC-N-specific IgG, with no significant difference between the UK and Bangladesh.

Overall, Malawian sera had the lowest levels of Alp-N-specific IgG, while Bangladeshi sera showed a stronger IgG1 and IgG3 response compared to other countries. Variable antibody effector functions were observed across the three countries for different Alp proteins.

Effect of passive immunisation with sera in mouse vaginal GBS colonisation and neonatal protection

Following significant variation observed in CPS and Alp-specific functional antibodies across three countries, we evaluated the serum samples in protection against GBS colonisation in a mouse model of vaginal colonisation. Bangladeshi sera initiated bacterial clearance within 24 hours post-inoculation, contrasting with no clearance observed in mice treated with UK or Malawian sera or in the PBS control group (Figure 5A). By day 7, only two mice in the Bangladeshi sera group had a low GBS count, while PBS-treated mice retained a high bacterial load. Although colonisation density was reduced in mice treated with UK and Malawi sera, 80% remained colonised with GBS at day 7.

Subsequently, we assessed protective efficacy in a humanised neonatal Fc receptor (hFcRn) mouse model. Pregnant mice were passively immunised with sera from the three countries, and pups were challenged with a lethal dose of serotype Ib GBS post-birth. Bangladeshi sera provided significantly higher protection than UK ($p=0.03$) and Malawian sera ($p=0.01$), with over 70% pup survival, compared to approximately 40% with UK and Malawian sera (Figure 5B). Although not statistically significant, Bangladeshi sera demonstrated a higher transfer ratio of IgG against CPS Ib to the pups (Figure 5C).

In summary, these results underscore the superior efficacy of Bangladeshi sera in reducing vaginal GBS colonisation and protecting mouse pups from lethal GBS infection.

DISCUSSION

In this study, we identified significant diversity in the seroprevalence of antibodies specific for GBS capsular polysaccharides and surface Alp proteins among three geographically distinct areas: the UK, Bangladesh, and Malawi. We found notable variations in CPS and Alp-specific antibody concentrations and their functional properties, with Bangladeshi women showing the highest response and Malawian women the lowest, particularly in CPS-specific antibodies. Moreover, Bangladeshi sera exhibited a more rapid reduction in GBS vaginal colonisation and provided the most significant protection against GBS infection in neonates in a humanised mouse model. Our *in vitro* and *in vivo* findings may not apply to all GBS serotypes, as these assays were conducted using only serotype Ib, one of the six most prevalent serotypes in maternal colonisation and disease in infants [4, 7, 32-34].

Our study demonstrates distinctive serotype-specific antibody responses across the UK, Bangladesh, and Malawi, shedding light on variations in GBS immune profiles in these regions. Our data suggest that a multivalent GBS vaccine might elicit strong responses to serotype II while generating a relatively lower response against serotype V across all three regions (Figure 1A). While we found high seroprevalence of the two predominant serotypes for maternal colonisation, Ia and II, only serotype Ia is consistently reported as a significant cause of neonatal disease in these countries, and serotype II is less frequently associated with disease [5-9, 32, 35]. Additionally, Bangladeshi women exhibited significantly higher antibody titres against serotype III despite its low detection in Bangladesh. These variations between serotype prevalence in carriage or disease and immune responses may reflect the influence of factors beyond capsular types, such as specific sequence types (STs) and clonal complexes (CCs), and the dynamic relationship between GBS exposure and resulting antibody profiles [14, 36-38].

Several studies have demonstrated a correlation between functional antibody concentration and GBS colonisation in women. Fabbrini et al. and Haeusler et al. reported higher anti-GBS antibody levels in colonised women compared to non-colonised women [37, 38]. Conversely, Kwatra et al. showed that women with higher serotype-specific antibodies had a significantly lower likelihood of acquiring a new homotypic serotype, with high OPK titres associated with protection from colonisation throughout the study [14]. A longitudinal study by Le Doare et al. in Gambia found that mothers with increased C3b/iC3b deposition were less susceptible to GBS colonisation [20]. The relationship between pathogen exposure and the antibody response is challenging to discern in our cross-sectional study. Consequently, whether the high antibody levels observed in the Bangladeshi population result from recent GBS colonisation or prior repeated exposures remains unclear. The high prevalence of multiple CPS-specific antibodies in

Bangladesh may suggest increased exposure, potentially leading to a durable antibody response, contributing to the lower colonisation rates observed in this population (Figure 1B).

Significant intercountry variations in IgG levels were observed against GBS Alp proteins, but the differences were less pronounced than those of the anti-CPS antibodies (Figure 3B). This outcome was anticipated since over 99% of GBS isolates possess at least one of the Alp proteins, leading to the production of Alp-reactive antibodies regardless of CPS types [39, 40]. Notably, the heightened antibody response observed in Bangladesh and the diminished response in Malawi appears to be specific to GBS, as significantly elevated IgG levels were observed in Malawian women to other antigens, such as Tetanus Toxoid and Cytomegalovirus Glycoprotein B (Supplementary Figure 1).

Malawian women exhibited significantly reduced antibody-mediated effector functions against CPS Ib and most Alp proteins (Figure 2B-2D, Figure 4), which may correlate with lower levels of IgG1 and IgG2 against the capsular polysaccharides and IgG1 and IgG3 against Alp proteins (Figure 2A, Figure 3C, 3E). These IgG subclasses are vital for complement deposition and phagocytosis during infections with encapsulated bacteria like pneumococci and GBS [24, 41, 42]. Notably, serum IgG levels may have influenced the variability in subclass composition and effector functions across countries. However, subclasses against CPS Ib and protein antigens did not consistently correlate with corresponding IgG concentrations (Supplementary Figures 2, 4-6). While CPS-specific ADCD and FcRn-binding showed weak correlations with IgG concentrations, OPK did not, likely due to the presence of IgM, antibody avidity, or other serum inhibitory factors (Supplementary Figure 3) [21, 43-46]. The correlations between protein-specific IgG ADCD and FcRn-binding were also variable (Supplementary Figures 7 and 8), potentially reflecting differences in Alp protein expression among prevalent GBS serotypes or other IgG structural properties.

The diversity observed in IgG quantity and functionality across the three countries was strongly reflected in GBS colonisation and passive immunisation outcomes in humanised FcRn mice. Bangladeshi sera demonstrated a substantial reduction in vaginal carriage density of GBS and provided enhanced protection against GBS invasive disease in mice (Figure 5). In addition to complement-mediated opsonophagocytic killing, the high FcRn-binding ability of IgG in Bangladeshi sera may have contributed to improved protection against both vaginal colonisation and invasive disease in mice. FcRn-mediated recycling and transcytosis of IgG not only prolong IgG half-life but have also been linked to GBS clearance from the mouse vaginal tract and efficient transplacental transfer of IgG in humans [25, 26]. However, since a significant amount of maternally derived IgG is transferred via breast milk, the protective effects observed in the pups may not be solely due to placental IgG transfer [47].

A significant limitation of our study is the lack of essential demographic information, such as details on women's GBS colonisation or pregnancy status, recent antibiotic usage, and other comorbidities that could affect antibody responses to GBS [14, 48]. Additionally, the ethnic representation of the women in our UK cohort could not be determined retrospectively. However, we anticipate that the cohort mainly consists of white British individuals, as the majority of the population (90%) in Exeter, where the samples originated, identified as white, according to the 2021 Census [49]. Future research incorporating detailed demographic and health data, including GBS colonisation status of the women, could help refine these findings further.

In conclusion, our study underscores a potential correlation between GBS epidemiology and the humoral immune response across various populations. The substantial variations in IgG concentration and functionality in different regions call for further exploration through a comprehensive system serology approach, including assessing antibody avidity, glycosylation profiling, and considering epigenetic factors. Finally, our study emphasises the importance of expanding seroprevalence studies to include more diverse demographics for a comprehensive understanding of GBS immunity and for informing effective vaccine implementation strategies globally.

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Author contribution: S.K., A.M., K.L.D., A.K., and N.F. conceptualised the study and designed the experiments. Z.B.A. and S.K.S collected patient data and samples in Bangladesh. S.K., S.S., S. Lim, T.H., S. Leung, and J.L. conducted the experiments. S.K., S. Lim, T.H., S. Leung, J.L., A.M., K.L.D., A.K., and N.F. analysed the data. S.K. drafted the manuscript, and S.K., S.S., T.H., A.G., A.M., K.L.D., A.K., and N.F. edited and revised it. All authors reviewed and approved the final version of the manuscript.

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Conflict of interests JL is an employee at MinervaX and receives salary and has been allocated warrants in MinervaX in accordance with the Company's articles of association. The

employment presents both a financial and non-financial interest, and the warrant allocation presents a potential financial interest. Other authors declare no competing interests.

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FIGURES

Figure 1. Diverse seroprevalence to Group B Streptococcus capsular polysaccharide observed in the UK, Bangladesh, and Malawi.

(A) Presence of IgG against GBS serotypes Ia, Ib, II, III, IV and V in serum samples collected from the UK (n=100), Bangladesh (n=100), and Malawi (n=88). The bar plot represents the percentage of women with IgG concentration equal to or above the Lower Limit of Quantification (LLOQ) for the assay. (B) Simultaneous presence of IgG antibodies against multiple serotypes of GBS in a single woman in three countries. The analysis included serum samples with a concentration equal to or above the LLOQ. Box and Whisker plots indicate median, IQR and minimum/maximum values. UK denotes the United Kingdom, and BD denotes Bangladesh (*p<0.05, ****p<0.0001). (C) Reverse Cumulative Distribution curves show IgG levels in the serum samples. Analysis included all serum samples in the study. Sample concentrations below the LLOQ were assigned half the LLOQ value for the corresponding serotypes. Green, blue, and yellow lines represent the sera from the UK, Bangladesh, and Malawi, respectively.

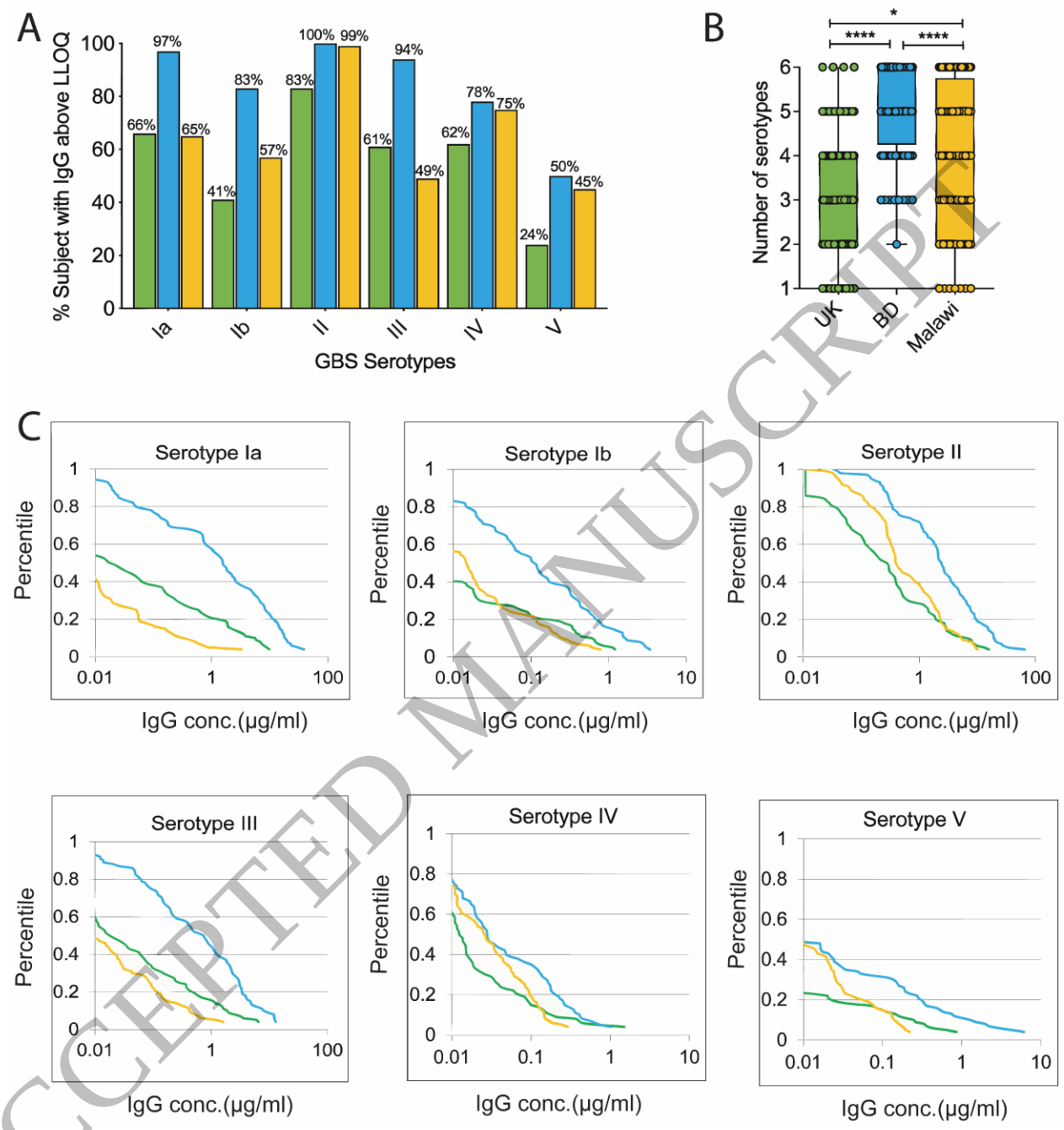


Figure 2. The distribution of the CPS Ib-specific IgG subclasses and IgG functionality across three countries shows a dominance of Bangladeshi sera.

(A) GBS CPS Ib-specific IgG subclasses (IgG1, IgG2, IgG3, and IgG4) in serum samples collected from the UK, Bangladesh and Malawi (n=55/ country). Data is depicted as mean fluorescence intensity (MFI). (B-D) Effector functions of CPS Ib-specific IgG were determined as FcRn receptor binding, antibody-dependent complement deposition (ADCD), and opsonophagocytic killing (OPK) in the serum samples (n=55/ country). The Luminex-based assay was used to measure FcRn-binding and ADCD, and the results were expressed as MFI. OPK was determined by *in vitro* OPK assay, and the results are expressed as OPK titre determined as 50% killing by HL60 neutrophils compared to no serum control. Samples with titres below the lowest value were assigned half of the minimum detectable titre.. Statistical analysis to evaluate intercountry variation was performed by a non-parametric Kruskal-Wallis test with Dunn's multiple comparisons tests (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, 'ns' used for non-significant differences). Box and Whisker plots indicate median, IQR and minimum/maximum values. UK denotes the United Kingdom, and BD denotes Bangladesh.

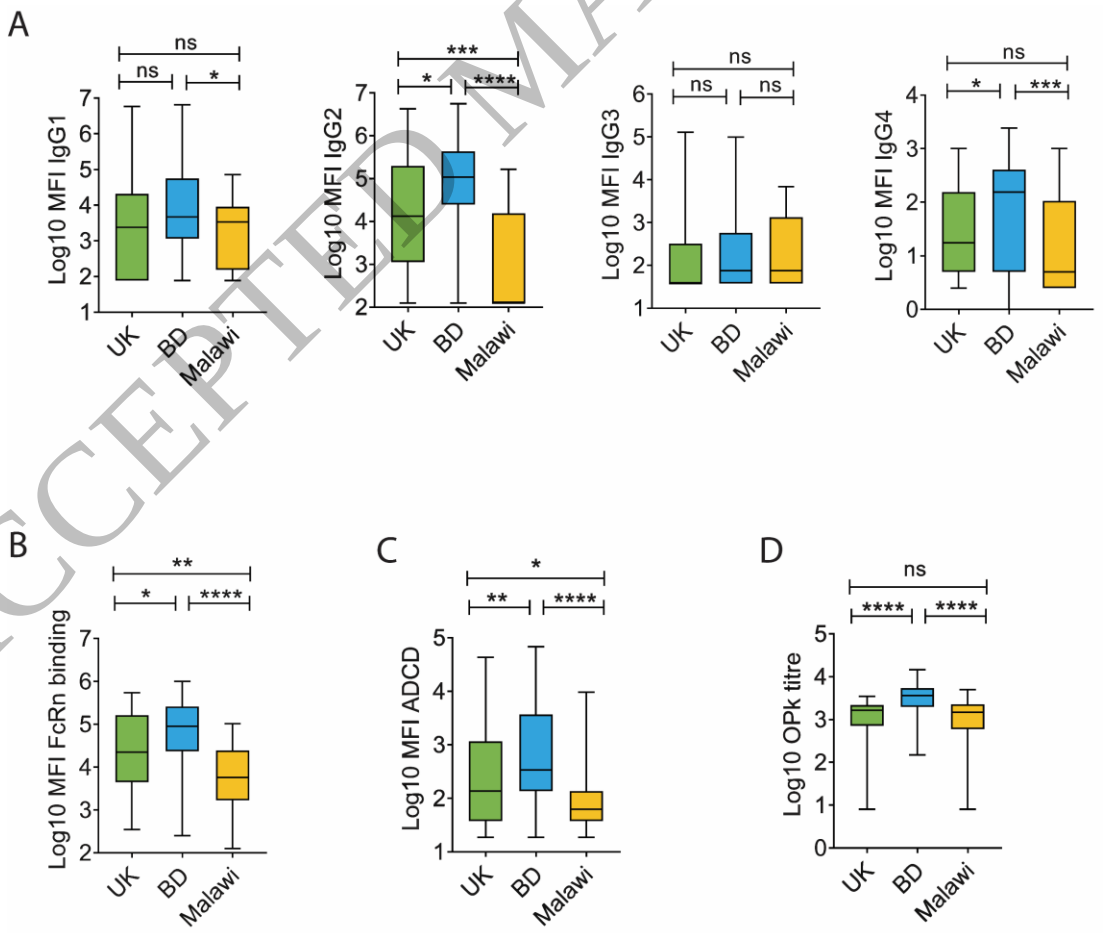


Figure 3. Differential seroprevalence and IgG subclass distribution observed against Alp-N-specific IgG across the countries.

(A) IgG against GBS Alp proteins in the serum samples from the UK, Bangladesh, and Malawi (n=100/ country) where the bar plot represents the percentage of women possessing IgG above or equal to the LLOQ against GBS surface protein antigens Alp1-N, Alp2/3-N, αC-N and Rib-N. (B) Reverse Cumulative Distribution curves show IgG levels (μg/ml) against GBS Alp proteins in the serum samples of all study participants (n=100/ country). Sample concentrations below the LLOQ were assigned half the LLOQ value of the corresponding serotypes. Maroon, red, and pink lines represent the sera from the UK, Bangladesh, and Malawi, respectively. (C-F) Subclass distribution of Alp-specific IgG in the serum samples (n=55/ country) where data are presented as mean fluorescence intensity (MFI). Statistical significance was determined using the Kruskal-Wallis test with Dann's multiple comparison tests (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ****, and 'ns' used for non-significant differences). Box and Whisker plots indicate median, IQR and minimum/maximum values. UK denotes the United Kingdom, and BD denotes Bangladesh.

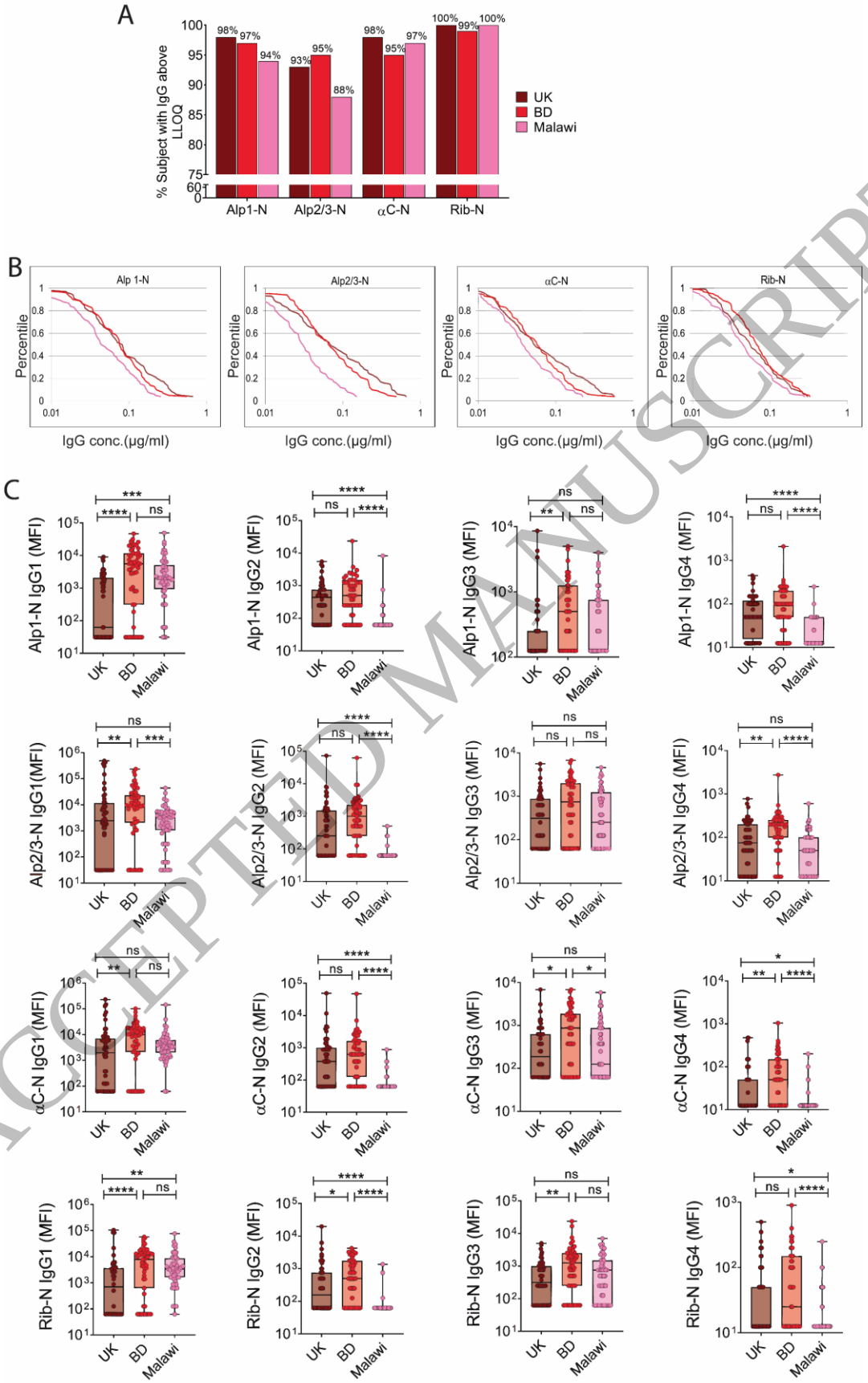


Figure 4. Variable IgG effector functions observed against different Alp-N proteins in different country settings.

(A) Antibody-dependent complement deposition (ADCD) exerted by IgG against Alp1-N, Alp2/3-N, αC-N, and Rib-N proteins in the serum of women from the UK, Bangladesh and Malawi.

(B) FcRn receptor binding of IgG against four Alp proteins across the three countries.

Statistical significance was determined using the Kruskal-Wallis test with Dann's multiple comparison tests (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ****, 'ns' denotes non-significant). n=55/country for both assays. Box and Whisker plots indicate median, IQR and minimum/maximum values. UK denotes the United Kingdom, and BD denotes Bangladesh.

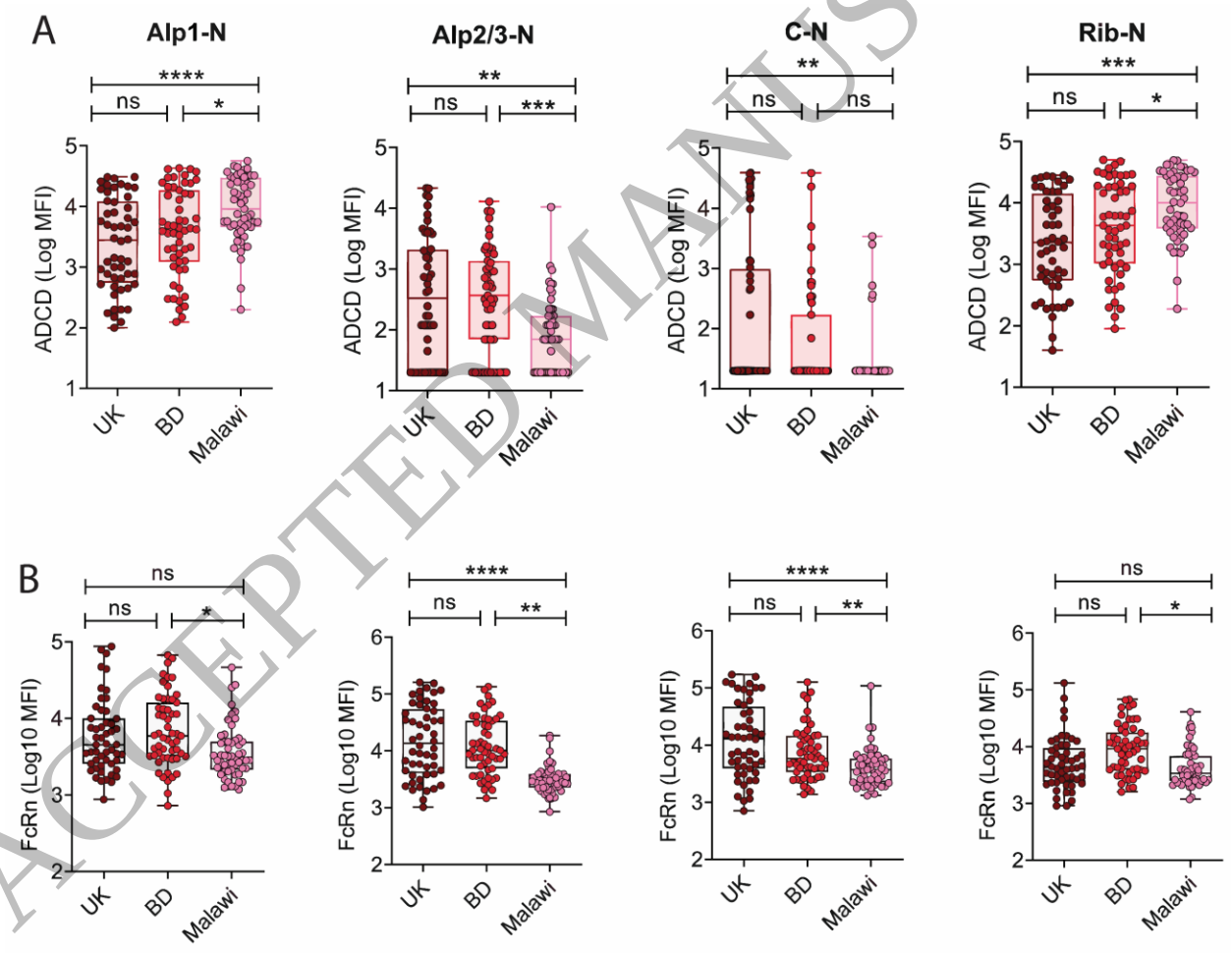
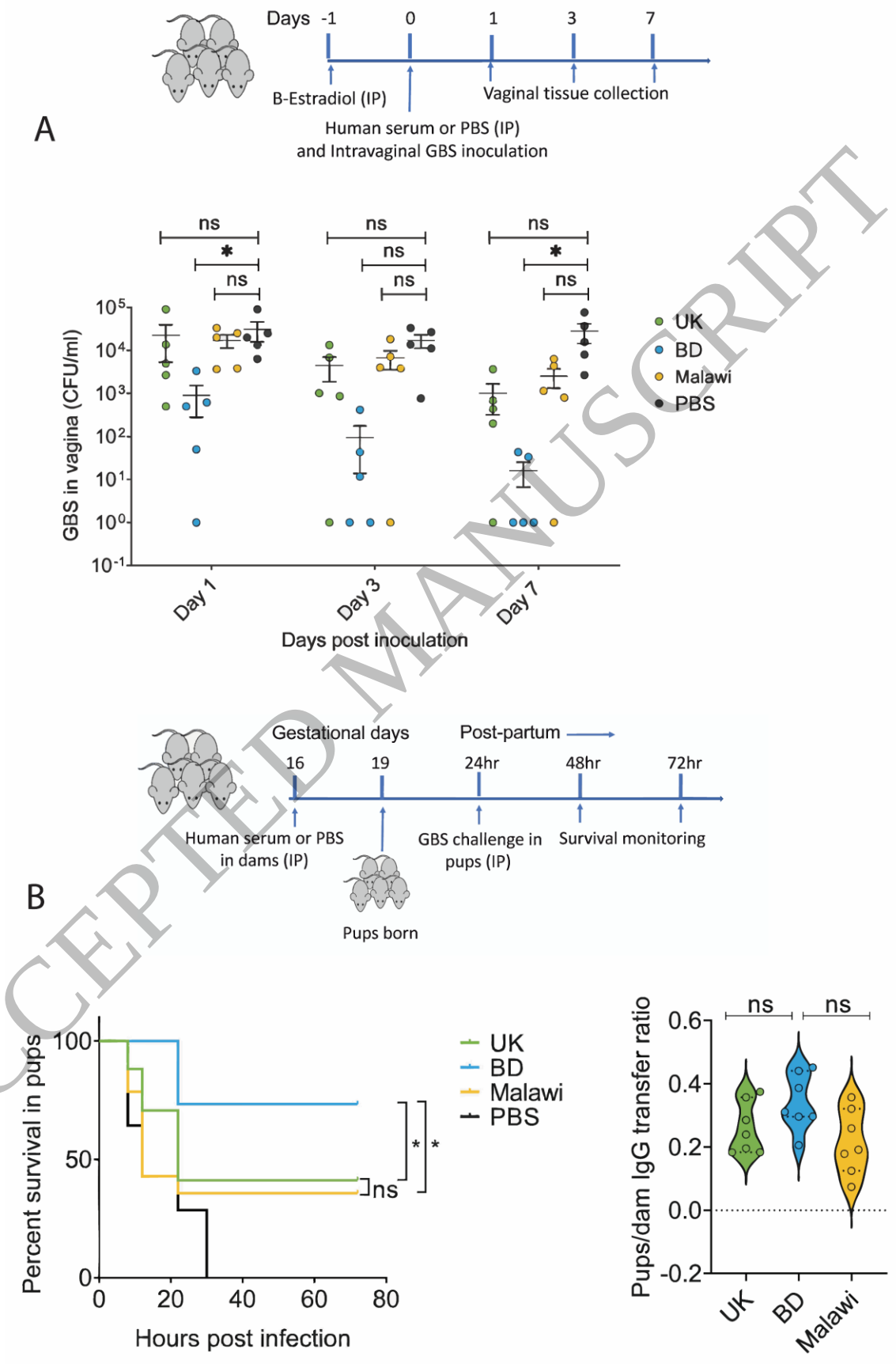


Figure 5. Bangladeshi serum conferred superior protection against GBS vaginal colonisation and neonatal sepsis.

(A) Schematic representation of GBS vaginal colonisation in female Balb/c mice treated with serum samples (n = 5 per group). (B) Bacterial load in the mouse vaginal tissues is plotted, where each dot represents a single mouse. Data expressed as mean ± SEM. CFU in the serum-treated groups were compared with the PBS-treated group at each time point by Two-way ANOVA with Dunnett's test for multiple comparisons. P value <0.05 was considered significant, and 'ns' refers to the non-significant results. (C) Schematic representation of neonatal protection from GBS infection following passive immunisation of pregnant mice with serum samples mothers (n=5 per group). (D) Kaplan Meier survival curve showing the survival of pups born from dam received the UK sera (n=17), Bangladeshi sera (n=15), and Malawi sera (n=14) or PBS (n=14). The survival curves were compared using the log-rank Mantel-Cox test. Data were pooled from three independent experiments. (E) The violin plot shows the IgG transfer ratios of pups to dam for the UK, Bangladesh, and Malawi serum measured in hFcRn pregnant mice (n=3 per country) and their pups (n=7 per country). Statistical analysis was performed by Kruskal-Wallis one-way ANOVA with Dunn's test for multiple comparisons. 'ns' indicates non-significant differences.



FOOTNOTES:

Conflict of interests

Shadia Khandaker- No conflict

Shilpee Sharma- No conflict

Tom Hall- No conflict

Suzanna Lim- No conflict

Janne Lehtonen- JL is an employee at MinervaX and receives salary and has been allocated warrants in MinervaX in accordance with the Company's articles of association. The employment presents both a financial and non-financial interest, and the warrant allocation presents a potential financial interest.

Stephanie Leung- No conflict

Zabed Bin Ahmed- No conflict

Andrew Gorringe- No conflict

Samir K Saha- No conflict

Arnaud Marchant- No conflict

Kirsty Le Doare- No conflict

Aras Kadioglu- No conflict

Neil French- No conflict

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Centre for Vaccine Research annual meeting, October 2019, Liverpool, United Kingdom

IMPRINT Network annual meeting, October 2021 (Online)

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