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Parasitic Infections in Pregnancy Decrease Placental Transfer of Anti-Pneumococcal Antibodies

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Running Head: Maternal Parasites and Placental Antibody Transfer

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Abstract

Many factors can influence maternal placental antibody transfer to the fetus, which confers important immune protection to the newborn infant. However, little is known about the effect of maternal parasitic infection on placental antibody transfer. To investigate this, we selected, from a parent study of 576 pregnant Kenyan women, four groups of women with term deliveries (≥37 weeks), including uninfected women (N=30) and women with solo infections of malaria (N=30), hookworm (N=30), or schistosomiasis (N=10). Maternal plasma at delivery and infant cord blood were tested via multiplex fluorescent bead assay for IgG against ten pneumococcal serotypes (PnPs 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F), diphtheria toxoid, and *Haemophilus influenzae* type B. Infants born to mothers with prenatal malaria, hookworm, or *S. haematobium* infections were associated with a significantly reduced ratio of maternal:infant cord blood antibody concentration for *S. pneumoniae* serotypes 1, 4, 5, 6B, 7F, 9V, and 18C compared to infants of uninfected mothers. Anti-diphtheria toxoid and anti-*H. influenzae* type B IgG ratios were not significantly different among infection groups. Prenatal parasitic infections decrease the transfer of maternal IgG antibodies to infants for several serotypes of *S. pneumoniae*.

Introduction

The newborn infant is immunologically disadvantaged. Its naïve adaptive immune system places it at at risk for infection from many pathogens early in life. Morbidity and mortality due to infectious diseases often are greatest during the first few months of life, leading to a high global burden of infectious diseases in young infants. Much of the infant's early protection from infectious diseases results from passive immunity acquired from its mother, both from the placental transfer of immunoglobulin G [IgG] antibodies in utero and in the acquisition of (primarily) mucosal IgA protection via breastfeeding (1). Transplacental antibody transfer, in particular, is the underpinning of many prenatal vaccination strategies (2).

Maternal-fetal antibody transfer is an active process, whereby IgG molecules are transported across the placenta from maternal to fetal circulation (3). This is accomplished via Fcγ receptors [FcRn] on the syncytiotrophoblast. This process favors certain IgG subtypes over others, IgG1 being the most preferentially transferred, followed in order by IgG4, IgG3, and finally IgG2 (4). Transfer of maternal antibodies begins as early as the second trimester, but most activity occurs in the second half of the third trimester (5). Various factors have been shown to affect the magnitude of this transfer, from gestational age and low birth weight to maternal hypergammaglobulinemia (6, 7).

Prenatal infections can dynamically alter this process of antibody transfer. HIV infection has been shown to decrease transplacental antibody transfer for various pathogen-specific

antibodies, including antibodies against *Haemophilus influenzae*, pertussis, pneumococcus, measles, and tetanus (8, 9, 10). In addition, placental malaria has been associated with decreased transfer of antibodies to measles, pneumococcus, tetanus, and RSV (11, 12). The effect of other maternal infections on antibody transfer is not as well understood, however. In the developing world, other parasitic infections such as soil-transmitted helminths and schistosomiasis are common and represent a significant public health challenge (13, 14). Prenatal screening and treatment for these infections have been a part of standard WHO guidelines for many years, but maternal infection with these parasites continue to occur at significant rates. In this study, we investigated the effects of prenatal parasitic infections malaria, hookworm, and schistosomiasis on transplacental antibody transfer of maternal IgG antibody against ten *Streptococcus pneumoniae* serotype polysaccharides (1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F), diphtheria toxoid, and *Haemophilus influenzae* type B [Hib] polysaccharide. As these three bacterial diseases can be particularly fatal to young infants, it is especially important to better understand the maternal-fetal interface for acquisition of immunity and how this may be perturbed by common parasitic infections.

Results

This research was completed as part of an ongoing cohort study in Kenya, with enrollment beginning in July in 2013 and follow up ending in July 2016, investigating the effect of maternal parasitic infections on infant immunity. In that parent study, 576 pregnant women were enrolled in the Msambweni District Hospital Antenatal Clinic (ANC), followed for the course of their pregnancy. Infants born to these mothers were subsequently

followed after birth until up to 3 years of age. The study was performed in a high-risk area for parasitic disease in a predominantly rural location in the southern coastal region of Kenya.

Four stratified maternal subgroups were selected for analysis, along with their newborns. These groups were: i) uninfected women (N = 30), ii) women with only malaria infection (N = 30), iii) women with only hookworm infection (N = 30), and iv) women with only *Schistosoma haematobium* infection (N = 10). "Uninfected" was defined as having no evidence of parasitic infections based on testing either in the prenatal clinic or at delivery. The infection groups were limited to women with single infections to prevent confounding by polyparasitism. To account for previously observed effects of gestational age on transplacental antibody transfer, only mothers who delivered at term (≥37 weeks) were included in the study. Since trained ultrasound technicians and equipment were not available, this was estimated by the revised Dubowitz clinical measurement, which includes 34 physical and neurologic assessments to predict the gestational age at birth (15), and has been validated in populations similar to our cohort (16, 17).

Paired sera were available from the time of birth from 100 mother-infant dyads. The selected pairs included 30 uninfected mothers and their infants, 30 malaria-infected mothers and their infants, 30 hookworm-infected mothers and their infants, and 10 *Schistosoma*-infected mothers and their infants. Baseline characteristics of the four groups are shown in Table 1. There were no significant differences among the groups for most individual features, however two baseline characteristics did show significant differences

among the groups. First, mean maternal BMI (measured at first prenatal visit) was significantly higher in the uninfected group (26.8) when compared to the infection groups (23.5 for malaria, 23.7 for hookworm, and 22.4 for schistosomiasis; p=0.023). In addition, on a self-reported survey regarding estimated household expenditures, in thousands of Kenyan shillings per month [Ksh/month], there was a significantly higher amount of mean monthly expenditures in the uninfected group (4.87 thousand Ksh/month) versus the other groups, with 4.53 thousand Ksh/month for malaria, 4.40 thousand Ksh/month for hookworm, and 4.10 thousand Ksh/month for schistosomiasis (p=0.007).

Evaluation of transplacental antibody transfer Serum antibody levels to the ten *S. pneumoniae* serotype polysaccharide antigens, Hib PRP polysaccharide, and diphtheria toxoid were measured using a fluorescent multiplexed bead-based immunoassay. The ratio of the geometric means of infant and maternal plasma antibody concentrations (cord-maternal ratio - CMR) was used to measure placental antibody transfer. The observed CMR values are shown in Figure 1a. There was a significant difference for seven of the pneumococcal serotypes when comparing uninfected mothers to the infected parasite groups (Figure 1b). All three infections showed a reduction in the rate of antibody transfer for PnPs 1: malaria 65%, hookworm 60%, and schistosomiasis 50%. Significant reductions in antibody transfer were observed among the study groups via ANOVA for PnPS 4 (p = 0.006), 5 (p = 0.003), 6B (p = 0.008), 7F (p = 0.003), 9V (p = 0.02), and 18C (p = 0.002). In pairwise comparisons of infected vs. uninfected mothers, for malaria and hookworm the differences in CMR were significant ($p \leq 0.05$, t-test) for all 7 antibodies (PnPs 1, 4, 5, 6B, 7F, 9V, and 18C). For the schistosomiasis group, significance was only reached for

131 antibodies against PnPs 7F and 18C. For PnPs 14, 19F, and 23F, there was no significant
132 difference among the 4 groups in CMR. Placental transfer of diphtheria toxoid (DPT-CRM)-
133 and *Haemophilus influenzae* type B (Hib)-specific antibodies were not significantly different
134 among the study subgroups.
135
136 Given the differences in BMI and monthly expenditures between the uninfected and
137 infected groups, we performed linear regression analyses to test the hypothesis that these
138 baseline variables could be associated with the antibody transfer process by comparing
139 them to the CMR for each antigen. There were no significant associations found between
140 the CMR and BMI or monthly expenditures for any of the tested antigens. Our cohort had a
141 relatively low prevalence of HIV, with a total of 6 mothers (6%) infected, and an equal
142 distribution among the groups (p=0.876). In unpaired t-tests comparing the geometric
143 mean antibody concentration ratios of the 12 antibodies in HIV+ vs. HIV- groups, no
144 significant differences were detected.
145
146 Discussion
147
148 In this study, we observed that prenatal malaria, hookworm, and schistosomiasis infections
149 are associated with a decrease in the transplacental transfer of several anti-*S. pneumoniae*
150 serotype antigen-specific antibodies when compared to that observed for uninfected
151 mothers and their infants. Previous studies have reported the effect of infections such as
152 HIV and malaria on placental antibody transfer. Data on the effect of helminth infections on
153 this process are scarce (18), and this is the first time that prenatal hookworm and

schistosomiasis have been shown to affect the maternal transfer of anti-pneumococcal antibodies. In addition, we have also observed that there can be differences in anti-pneumococcal antibody transfer among the different tested serotypes.

Although there were no differences in most baseline characteristics among our different study subgroups, we did find significant differences in terms of maternal BMI and average monthly expenditure. With a higher average BMI in the uninfected group, there may have been an association between nutritional status and the efficiency of placental antibody transfer; malnourishment during pregnancy has previously been associated with a 14% reduction in placental antibody transfer (19). The uninfected group also reported a larger monthly expenditure compared to the other groups, a variable that serves as a proxy for socioeconomic status in our study, suggesting a correlation of monthly income and parasite burden.

Unlike previous studies, our results did not find a significant difference in *Haemophilus influenzae* type B placental antibody transfer in malaria-infected women (9). This difference could be related to the fact that, in our study, the malaria cohort was chosen for inclusion if they had any evidence of infection throughout their pregnancy, whereas other studies have only looked at the role of placental malaria at the time of delivery (8, 20).

At present, there is no routine prenatal vaccination for *S. pneumoniae* in Kenya, and participants in our maternal cohort were born before the 2011 introduction of childhood PCV vaccination there. Data on pneumococcal seroprevalence rates in Africa are limited.

177 One group from Burkina Faso surveyed the serotype-specific antibody concentrations of
178 pneumococcal IgG during a meningococcal outbreak in 2006 and showed that antibody
179 concentrations increased with age in the absence of any prior vaccination programs, which
180 suggested that natural immunity develops over time as a result of exposure to circulating
181 pneumococcal serotypes or from other bacteria with cross-reacting polysaccharides (21).
182 By age 10, depending on the serotype, 50-80% of those individuals had a level of antibody
183 presumed to be protective of invasive disease (≥0.35 ug/ml, based on prior studies (22)).
184 The same researchers looked at pneumococcal carriage prevalence in Burkina Faso and
185 found that most serotypes had a 0.5-5% prevalence of asymptomatic carriage in the
186 population (23). In our group, the percentage of mothers with protective antibody levels
187 varied from 5-83% among the different pneumococcal serotypes, and between 5-79% in
188 the infants. Diphtheria protection (defined as ≥0.1 IU/ml (24)) was seen in 73% of mothers
189 and 74% of infants, while Hib protective levels (≥1 ug/ml (25) was only 3% in mothers and
190 11% in the infants.
191
192 It has been observed that the majority of anti-pneumococcal antibody produced is of the
193 IgG2 subclass, similar to other polysaccharide antigens (26), although anti-pneumococcal
194 antibodies af all IgG subclasses can be detected (27). While IgG2 has the least
195 transplacental transfer of all the subclasses, there is on average a 50% transfer of this class
196 (4). One study documented total IgG transfer rates between 77-116% in serotypes 1, 3, 6B,
197 9V, and 14, with no correlation found between the concentrations of serotype-specific IgG
198 subclasses and the transplacental transfer of these antibodies (28). Our study did not look

199 at IgG subclass-specific data, as it was not possible to selectively measure these data with
200 the multiplex platform we utilized.
201
202 Our reports of differences between antibody transfer between pneumococcal serotypes
203 and the lack of an effect on *H. influenzae* and diphtheria antibody transfer may be explained
204 by several possible mechanisms. It has been observed that parasitic infections can raise
205 levels of total IgG in the host, and since Fcγ receptors can be saturated (5), there could be a
206 decrease in serotype-specific transfer in mothers having higher levels of IgG, as a
207 consequence of subclass competition for limited Fc binding sites. There may also be a role
208 for antigen specificity in transport, where higher levels of maternal IgG against herpes
209 simplex virus, tetanus toxoid, streptolysin O, and *S. pneumoniae* have been shown to have
210 an inverse relationship with transfer of these antibodies to babies (8). It may be possible
211 that IgG against certain serotypes of pneumococcus are more easily transferred than others
212 (supported by the variable transfer rates of serotypes (28)), and that this could be further
213 altered by the inflammatory changes induced by different parasitic infections. Ultimately,
214 more research is warranted to better understand the specifics of this process.
215
216 This study was observational in nature, with the potential for experimental bias and
217 confounding. A more robust randomized clinical trial, however, would have been difficult
218 given the current standard of care for anti-parasitic treatment in pregnancy. We could not
219 control for the timing of the infection with this study, as infection status was determined
220 not by proximity to birth but instead by the presence of parasites at any point throughout
221 the pregnancy. Because the majority of antibody transfer occurs later in the third trimester,

it remains unclear how differences in the timing of exposure might ultimately affect placental antibody transfer by the time of birth. All of the women in the study received anti-parasitic therapy for malaria and intestinal helminths during their pregnancies, which involved IPTp and mebendazole, so it is not possible to analyze the effect of treatment on the antibody transfer process in this group. Despite this consistent anti-parasitic antenatal treatment, many mothers in the parent study from which this group was selected were found to be infected at delivery with either malaria (8.5%), hookworm (5.7%), and/or *S. haematobium* (29%). These diseases are continuously circulating in the community and none of the mothers were on continuous prophylaxis – only intermittent treatment. Furthermore, mebendazole does not effectively all intestinal helminths, particularly *Trichuris*, while *S. haematobium* infections, which require praziquantel therapy, went untreated for the duration of the pregnancy based on Kenya MoH guidelines.

HIV infections have been shown to have a significant effect on placental antibody transfer in previous studies (8, 9,10), a potential source for confounding in these results. Our cohort had an HIV prevalence of 6%, comparable to recent prevalence data for this region (5.9% in Kwale county as of 2016 (29)), and infected participants were evenly distributed within the study groups. When comparing the CMR of the 12 antibodies in HIV+ vs. HIV- groups, there was no association found between infection status and antibody transfer.

This study was limited by a smaller sample size in the schistosomiasis group. Despite there being over 40% of women in the parent study infected with this parasite, the majority of participants with *Schistosoma* exposure had other parasitic infections. Because the

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presence of two or more concurrent infections may have led to confounding, the infected groups in this study were selected from mothers with single-parasite infections only. With that exclusion, there remained only a limited number of women infected with *Schistosoma* only.

Vaccine-preventable infectious diseases remain a serious health issue for very young infants in developing countries. Where there is a potential for prenatal parasitic infections to decrease the transfer of protective maternal antibodies to infants via transplacental transport, it will be important to develop prenatal care and vaccination strategies to mitigate this effect. The present study adds to the evidence in favor of anti-parasite control among expectant mothers, so that their infants may have the best possible protection before they receive their standard vaccinations. Because this observational study was limited in its ability to provide a detailed analysis of the effects of treatment on the transfer of antibodies from mother to fetus, future prospective studies are needed to further define this important aspect of early immunity.

Materials and Methods

Ethical oversight Ethical approval of this study was obtained from the Kenyatta National Hospital Ethical Review Committee (protocol # P85/03/2013), from the Institutional Review Board for Human Studies at Case Western Reserve University (IRB # 01-13-13) and from the Stanford University School of Medicine IRB (protocol # IRB-31468). Mothers provided written informed consent for their own participation and that of their infants.

268

269 *Inclusion and exclusion criteria* To be included as a study participant, mothers must have a)
270 been permanent residents of Msambweni, b) been willing to participate in pre-natal and
271 post-natal care at ANC, c) exclusively used study health facilities for primary health care, d)
272 delivered at Msambweni District Hospital (now Msambweni County Referral Hospital), e)
273 been willing to provide blood, urine and stool samples, and f) been willing to allow
274 examination, blood, urine, and stool testing of their infants. Pregnant mothers were
275 excluded if they: a) presented with a complicated delivery resulting in significant infant
276 morbidity at birth, b) delivered an infant ≤27 weeks gestation, and/or had: c) known
277 chronic illness, e.g. TB, diabetes, renal failure, d) severe anemia requiring hospitalization
278 (Hgb < 6 g/dl accompanied by symptoms requiring urgent treatment), e) permanent
279 disability that impeded study participation and/or comprehension, f) known multiple
280 pregnancy and/or g) plans to relocate after delivery.

281

282 *Clinical testing* Maternal participants were screened at each prenatal visit and at delivery
283 for malaria (blood smear by light microscopy and DNA PCR from RBC pellet (30)),
284 schistosomiasis (urine filtration egg counts (31) and plasma anti-soluble worm adult
285 protein [SWAP] IgG4 (32)), and intestinal helminths (stool microscopy following the
286 Ritchie method (33)). Mothers found positive for malaria and/or intestinal helminths
287 received treatment within 72 hours per Kenya Ministry of Health [MOH] guidelines.
288 Current standard-of-care includes a single dose of mebendazole for helminths and 4
289 monthly doses of intermittent preventive treatment (IPTp) for malaria with
290 sulfadoxine/pyrimethamine. This prophylaxis and treatment for malaria followed MOH

291 guidelines for antenatal care. Any pregnant woman developing symptoms of infection
292 between study visits was asked to return for further evaluation and care. For the mother-
293 infant pairs included in this study, both maternal venous blood and umbilical cord blood
294 were collected at delivery, as previously described (34). Plasma was stored at -80º C until
295 antibody assays were performed.
296
297 *Determination of antibody levels* Serum antibody levels to the ten *S. pneumoniae*
298 polysaccharide serotypes, Hib PRP polysaccharide, and diphtheria toxoid were measured
299 using a fluorescent multiplexed bead-based immunoassay employing Luminex multiple
300 analyte profiling technology (Luminex, Austin, TX) (35, 36). Briefly, the ten pneumococcal
301 polysaccharide (PnPs) antigens (serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F) were
302 obtained from the American Type Culture Collection (ATCC; Virginia, USA) and conjugated
303 to carboxylated microspheres (Luminex, Austin, TX) using 4-(4,6-dimethoxy[1,3,5]triazin-
304 2-yl)-4-methyl-morpholinium [DMTMM] (37). Hib PRP capsular polysaccharide conjugated
305 to human albumin and diphtheria toxoid were obtained from the National Institute for
306 Biological Standards and Control (NIBSC, Potters Bar, UK). These were coupled to
307 carboxylated microspheres (Luminex, Austin, TX) using a two-step carbodiimide reaction,
308 as previously described (38).
309
310 For the assay standard, a 1:1 mixture of 007SP (anti-PnPs human serum – NIBSC) and
311 09/222 (anti-Hib human serum – NIBSC) reference sera was used. Dilutions of the mixture
312 were tested against each reference serum, as well as the reference serum for diphtheria
313 toxoid (10/26, NIBSC) to define the concentrations of all 12 antibodies in the 007SP-

09/222 mixture. A series of seven 3-fold dilutions of the standard serum starting at 1:50 was prepared using a standard diluent buffer of PBS 1% BSA 0.05% Tween 20 with 5 µg/ml of pneumococcal cell wall polysaccharide (CWPS, Statens Serum Institute, Copenhagen, Denmark) added to quench non-specific binding of *S. pneumoniae* antibodies (39). Mother and infant test serum samples were diluted 1:50 in PBS 1% BSA, 0.05% Tween 20 containing 5 µg/ml CWPS at 50 µl/well and tested in duplicate. Antigen-coupled beads were added to the samples in a mixture containing 1000 beads/antigen target in the same diluent serum at 50 µl/well of a 96-well plate and incubated on a rotator plate at 4º C overnight. After incubation, the beads were washed with PBS 0.05% Tween 20 and stained with goat anti-human IgG Fcγ-specific R-phycoerythrin (R-PE)-conjugate for an additional 30 minutes at room temperature. After a final wash, the beads were resuspended in 100 µl PBS 0.05% Tween 20 and data was acquired using a BioPlex MAGPIX multiplex reader (BioRad, Hercules, CA).

Luminex data analysis was performed using Bio-plex Manager 6.1 software (BioRad). Antibody concentrations were derived by interpolating the measured median fluorescence intensity (MFI) values of samples against a 5-parameter logistic curve fit from MFI values of the standard curve. We used the geometric means of the infant (cord blood) antibody concentrations and maternal (delivery blood) antibody concentrations due to the variability and skewed distribution of IgG concentrations between individuals.

Statistical analysis Results were analyzed in Excel (Microsoft, 2011) and on STATA (Statacorp, 2015). The study groups for this research were chosen as a pilot study with cost

and sample availability limiting the group selection. Assuming a mean antibody transfer ratio in healthy pregnancies of 120% (+/- 40%) (40), a sample size of 28 per group would give 80% power to detect a 25% decrease in placental antibody transfer. Significance between groups was first confirmed by analysis of variance (ANOVA), with Bonferroni correction for multiple comparisons setting $p \leq 0.017$ as significant. Subsequent two-sided t-tests were performed between each infection group and the uninfected group.

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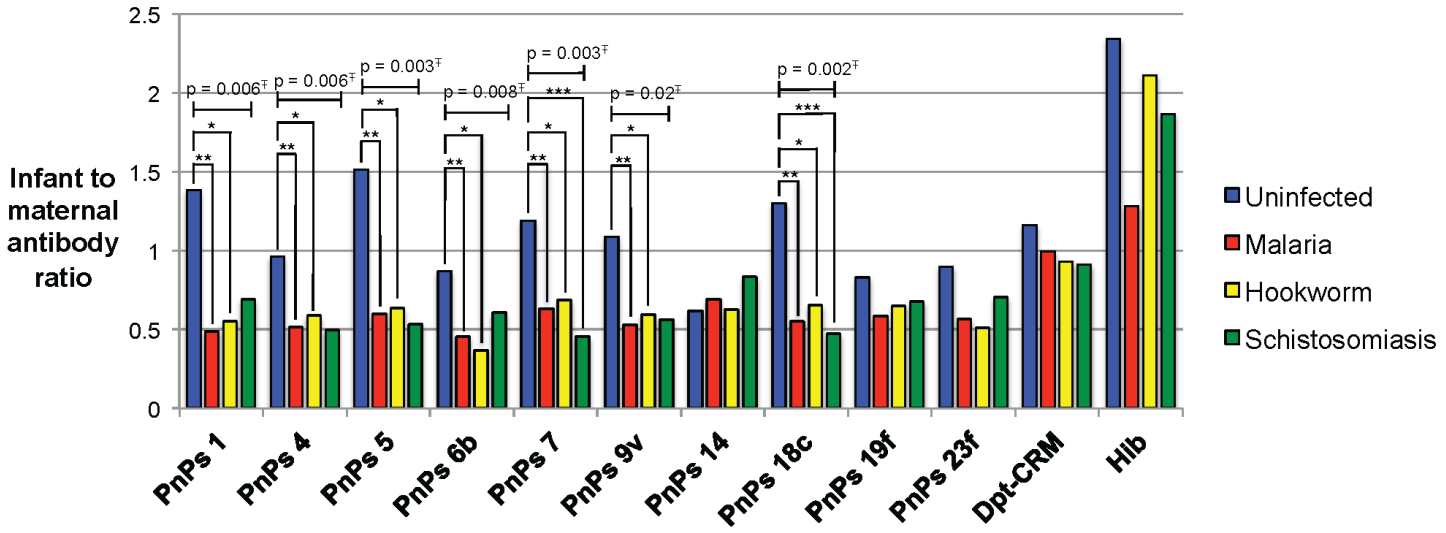
Table 1. Baseline Characteristics

	Uninfected	Malaria	Hookworm	Schistosomiasis	*p*
	N = 30	N = 30	N = 30	N = 10	(ANOVA)
Maternal Age, mean	26.1	26.5	27.2	22.5	0.248
Previous pregnancies, mean	1.67	2.63	2.6	1.7	0.168
Household expenditures (thousand Ksh/month), mean	4.87	4.53	4.4	4.1	0.007
Maternal BMI, mean	26.8	23.5	23.7	22.4	0.023
Maternal Hgb at delivery (g/dL), mean	9.62	9.97	10.15	10.3	0.067
Maternal HIV infection, total (%)	2 (6.7%)	1 (3.3%)	2 (6.7%)	1 (10%)	0.876
Newborn weight (g), mean	3061.3	3035.7	3044.7	3087	0.986
Newborn head circumference (cm), mean	33.3	33.5	33.7	33.4	0.504
Gestational age (Dubowitz score in weeks), mean	40	41.3	41.3	38.5	0.132

Figure 1a. Infant to maternal antibody concentration ratios (geometric means) by infection status



Ŧ ANOVA between all groups

* p < 0.05 by two-sided t test (comparing malaria to uninfected group)

** p < 0.05 by two-sided t test (comparing hookworm to uninfected group)

*** p < 0.05 by two-sided t test (comparing schistosomiasis to uninfected group)

PnPs – *S. pneumoniae* polysaccharide; **Dpt-CRM** – diphtheria-CRM; **Hib** – *Haemophilus influenzae* type b

Figure 1b. Placental transfer (CMR)[a] of antigen-specific antibodies by infection status

	Uninfected	Malaria	Hookworm	Schistosomiasis	p^b
	CMR (95% CI)	CMR (95% CI)	CMR (95% CI)	CMR (95% CI)	
PnPs 1	1.39 (0.67 - 2.86)	0.49 (0.37 - 0.64)	0.55 (0.45 - 0.67)	0.69 (0.33 - 1.43)	0.006[c]
PnPs 4	0.96 (0.63 - 1.47)	0.52 (0.43 - 0.62)	0.59 (0.48 - 0.72)	0.49 (0.38 - 0.65)	0.006[c]
PnPs 5	1.51 (0.80 - 2.85)	0.60 (0.52 - 0.69)	0.63 (0.45 - 0.89)	0.53 (0.32 - 0.88)	0.003[c]
PnPs 6b	0.87 (0.60 - 1.27)	0.46 (0.35 - 0.59)	0.37 (0.23 - 0.58)	0.61 (0.31 - 1.18)	0.008[c]
PnPs 7	1.19 (0.89 - 1.60)	0.63 (0.48 - 0.82)	0.69 (0.51 - 0.93)	0.45 (0.20 - 1.04)	0.003[d]
PnPs 9v	1.09 (0.67 - 1.77)	0.53 (0.41 - 0.68)	0.59 (0.43 - 0.83)	0.56 (0.37 - 0.85)	0.02[c]
PnPs 14	0.62 (0.39 - 0.98)	0.69 (0.54 - 0.89)	0.62 (0.43 - 0.90)	0.83 (0.55 - 1.25)	0.827
PnPs 18c	1.30 (0.74 - 2.30)	0.55 (0.47 - 0.64)	0.65 (0.50 - 0.86)	0.47 (0.37 - 0.61)	0.002[d]
PnPs 19f	0.83 (0.50 - 1.39)	0.58 (0.48 - 0.71)	0.65 (0.47 - 0.89)	0.68 (0.43 - 1.06)	0.547
PnPs 23f	0.90 (0.54 - 1.49)	0.57 (0.49 - 0.66)	0.51 (0.32 - 0.81)	0.70 (0.44 - 1.12)	0.185
Dpt-CRM	1.16 (0.82 - 1.65)	1.00 (0.87 - 1.15)	0.93 (0.71 - 1.23)	0.91 (0.66 - 1.26)	0.612
Hib	2.34 (1.17 - 4.69)	1.28 (0.71 - 2.32)	2.11 (1.16 - 3.85)	1.87 (0.62 - 5.65)	0.53

a CMR - ratio of infant cord blood plasma to maternal delivery plasma antibody concentration (geometric means)

b ANOVA

c $p \leq 0.05$ by non-paired t-test compared to uninfected for malaria and hookworm groups only

d $p \leq 0.05$ by non-paired t-test compared to uninfected for all infection groups

PnPs, *Streptococcus pneumoniae* polysaccharide; **Dpt-CRM**, Diptheria toxoid; **Hib**, *Haemophilus influenzae* type b