Reduced placental transfer of antibodies against microbial and vaccine antigens in HIV-infected women in Mozambique

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Abstract

Antibody transplacental transfer is essential for conferring protection in newborns against infectious diseases. This transfer may be affected by gestational age and maternal infections, although the effects are not consistent across studies. We measured total IgG and IgG subclasses by quantitative suspension array technology against fourteen pathogens and vaccine antigens, including target of maternal immunization, in 341 delivering HIV- and HIV+ mother-infant pairs from southern Mozambique. Maternal antibody levels were the main determinant of cord antibody levels. HIV broadly reduced the placental transfer and cord levels of IgG and IgG1, but also IgG2 to half of the antigens. *Plasmodium falciparum* exposure and prematurity were negatively associated with cord antibody levels and placental transfer but this was antigen-subclass dependent. These findings suggest maternal infections may impact the efficacy of maternal immunization and confirm the lower transfer of antibodies as one of the causes underlying increased susceptibility to infections in HIV-exposed infants.

Introduction

Each year, 2.6 million deaths occur during the neonatal period, being infectious diseases the leading cause of mortality, particularly in low-income countries [1, 2]. Newborns are highly vulnerable to pathogens due to their functional immunological differences from adults as a result of living in a semi-allogeneic sterile environment, where exposure to microbial antigens is limited [3–6]. For example, microorganisms such as respiratory syncytial virus (RSV) are generally asymptomatic or cause mild disease in adults but induce acute bronchiolitis, viral pneumoniae and croup in infants, being those between 2 and 6 months of age at the highest risk, especially in low-income countries [7, 8].

Newborns mostly rely on the protection elicited by maternal antibodies transferred across the placenta, which provide passive immunity against common pathogens [9]. Neonatal and child immunization is essential for conferring protection in newborns and infants against vaccine-preventable diseases [10–12]. Vaccination is among the most cost-effective public health measures worldwide [13], and regions with high rates of infant morbidity and mortality like sub-Saharan Africa benefit from the implementation of the Expanded Program of Immunization (EPI) [14]. Nevertheless, acquisition of immunity from vaccination is not immediate and vaccines are not available for all infectious diseases. At present, only three vaccines are being administered at birth in some countries: Bacillus Calmette-Guérin (BCG), hepatitis B virus (HBV) and oral polio vaccine (OPV) [10, 15].

Transplacental transfer of antibodies occurs in utero and it is facilitated by neonatal fragment crystallisable (Fc) region receptor (FcRn), expressed in the human syncytiotrophoblast [16]. Only IgG is transferred across the placenta, being foetal IgG concentrations higher at the third trimester [17], although some studies suggest that maternal IgE is also transferred to the foetus as IgG/IgE complexes [18]. IgG subclasses have different affinities for the FcRn receptor leading to differences in the efficiency of transfer [19]; the greatest transport occurs for IgG1, followed by IgG4, IgG3, and finally IgG2 [20].

To be effective, the transferred IgG must reach protective levels after birth. Maternal immunization is a good strategy to prevent newborn infections, ensuring a sufficient transfer of protective antibodies to the neonate [21]. Maternal vaccination against tetanus, pertussis and influenza has been implemented in many populations and has been effective protecting young infants from these pathogens [22–24], and could be used to protect newborns from RSV [25].

A number of factors have been associated with IgG placental transfer and cord levels, such as maternal antibody concentrations, gestational age, placental integrity, maternal infections and the antigen specificity [26–30], but inconsistently. Placental malaria (PM) has been shown to reduce transplacental transfer of antibodies against tetanus, measles, *Streptococcus pneumoniae* (*S. pneumoniae*), herpes simplex virus type 1 (HSV-1), RSV and varicella-zoster virus (VZV) [26, 31–33]. However, other studies have shown no impact of PM on transplacental transfer of tetanus, *S. pneumoniae*, *Haemophilus influenzae type b* (*Hib*), diphtheria, measles or RSV antibodies [31, 33–36]. The effect of maternal HIV infection is also controversial. Some studies demonstrated that HIV infection leads to a reduction of the transplacental transfer of *Hib*, pertussis, pneumococcus, measles, tetanus and *Plasmodium falciparum* (*P. falciparum*) specific antibodies [26, 27, 37–39], but others have shown no effect [31, 36, 39–41]. Therefore, there are probably confounding variables that should be considered.

In our study, we wanted to assess the impact of different factors, including maternal HIV infection and malaria in pregnancy on the placental transfer and cord levels of IgG and IgG subclasses to a broad range of highly prevalent microbial and vaccine antigens in a sub-Saharan African country, including: *Corynebacterium diphtheriae* (*C. diphtheriae*), *Clostridium tetani* (*C. tetani*), *Bordetella pertussis* (*B. pertussis*), *Hib*, *S. pneumoniae*, *Shigella dysenteriae* (*S. dystenteriae*), *Vibrio cholerae* (*V. cholerae*), hepatitis B virus (HBV), measles, RSV, *Cryptosporidium parvum* (*C. parvum*), *Giardia intestinalis* (*G. intestinalis*) and *P. falciparum*. A better understanding of factors affecting cord IgG levels will help designing better preventive measures and strategies for maternal and child health.

Results

Description of participants

A total of 341 women (197 HIV-uninfected and 144 HIV-infected) participated in the study (Table 1). HIV-infected women were older than the HIV-uninfected and there were more primigravidae among the HIV-uninfected. HIV-infected women had significantly more anaemia than the HIV-uninfected. There were no significant differences in birth weight or prematurity between infants born to HIV-infected and those born to HIV-uninfected women. Among the 155 infants born from HIV-infected women, 8 tested HIV-positive at 6 weeks of age by polymerase chain reaction (PCR) analysis performed following national guidelines. Placental histology was performed on 307 samples from study participants, of which 3 had acute PM and 8 past PM. In total, 20 women had PM (positive at placental blood, by microscopy or PCR at delivery, or acute or past PM by histology), but there were no differences by HIV infection. Peripheral malaria (positive at peripheral blood by microscopy or PCR at any of the visits during pregnancy) was detected in 51 women, but there were no differences by HIV infection. Finally, *P. falciparum* exposure was lower among HIV-infected women.

Profile of antibody levels in cord blood

We first performed principal component analysis (PCA) of the cord antibody levels and maternal antibody levels separately, including IgG, IgG1, IgG2, IgG3 and IgG4 to all antigens tested, to reduce the dimensionality of the data and get insights into the overall antibody patterns. Cord and maternal PCA looked very similar (data not shown). Cord antibody responses clearly clustered by IgG subclasses (Fig. 1a) and antigens (Fig. 1b) suggesting different antibody profiles depending on the antigen specificity. IgG and IgG1 clusters were closer, showing similar responses, whereas IgG4 and IgG3 were the most distant. *Hib* cluster was clearly separated from the rest indicating a different antibody profile. Consistently, median IgG and IgG1 levels were higher than the rest of IgG subclasses and were both similar between them for most of the antigens with the exception of *Hib* (Fig. 1c). IgG2 had lower median levels than IgG1, followed by IgG3 and the lowest levels were shown for IgG4.

We also determined the placental transfer of antibodies, measured as the ratio of cord blood levels to the maternal levels. The transfer efficiency was greatest for IgG1, IgG3 or IgG4 depending on the antigen: IgG1 for *C. diphtheriae, P. falciparum*, HBV and rotavirus, IgG3 for *B. pertussis, C. tetani, Hib* and *V. cholerae* and IgG4 for *C. parvum, S. dysenteriae,* measles and RSV. The less efficiently transferred subclass was IgG2 for most of the antigens, with the exception of *G. intestinalis* and *S. pneumoniae* for which IgG2 was the greatest (Fig. 1d).

Altered maternal and cord blood antibody levels in HIV-infected women

HIV-infected and HIV-uninfected women did not show significant differences between antibody levels except for *C. tetani* (IgG and IgG1), *S. pneunomiae* and RSV (IgG2), and *C. diphtheriae* and *P. falciparum* (IgG4), with lower antibody levels in HIV-infected compared to HIV-uninfected women (Fig. 2a-2d and Figure 2-figure supplement 1). In contrast, higher *G. intestinalis* and HBV IgG levels were found in HIV-infected women (Fig. 2a).

IgG cord blood levels were lower in HIV-infected than HIV-uninfected women for *C. tetani, B. pertussis, S. pneumoniae*, measles, rotavirus and *C. parvum*. Similarly, HIV-infected women had lower IgG1 cord levels for *C. diphtheriae, C. tetani, B. pertussis, Hib, S. dysenteriae, V. cholerae* and measles for IgG1 (Fig. 2b). Lower *C. diphtheriae and P. falciparum* IgG4 levels were also found in cord blood of HIV-infected than HIV-uninfected women, whereas no differences were observed between groups for IgG2 and IgG3 (Fig 2c-2d and Figure 2-figure supplement 1).

Altered placental transfer of antibodies in HIV-infected women

Placental transfer of IgG and IgG1 was significantly lower in HIV-infected women for all antigens except for *Hib* and *V. cholerae* (IgG) and *C. tetani, S. pneumoniae, V. cholerae* and RSV (IgG1) (Fig. 3a-3b, Figure 3-figure supplement 1 and Figure 3-figure supplement 2). For IgG2, only *G. intestinalis, B. pertussis* and HBV had significantly lower transfer in HIV-infected women, while *S. pneumoniae* and RSV had higher transfer in HIV-infected women (Fig. 3c and Figure 2-figure supplement 3). For IgG3, only *C. tetani* antibodies had a significantly lower transfer in HIV-infected compared to HIV-uninfected women (Fig. 3d and Figure 3-figure supplement 4). No

142 significant differences in placental transfer between the two groups were found for IgG4 (Figure
143 3-figure supplement 5).

144 Factors associated with cord blood levels of IgG and IgG subclasses

145 Maternal antibodies, HIV infection and *P. falciparum* exposure were the only variables with a
146 clear general impact on univariable models and were selected for multivariable models, in which
147 maternal antibody levels had the strongest positive correlation with cord antibody levels for all
148 the antigens and subclasses (Fig. 4a). However, the effect of maternal antibody levels was more
149 variable for IgG3-4 than for IgG and IgG1 subclasses. On average, a 10% increase in maternal
150 IgG levels was associated with 8.1% to 9.7% increases in IgG cord blood levels, depending on
151 the antigen. For IgG subclasses, a 10% increase in maternal antibody levels was associated with
152 increases of cord blood levels from 7.6% to 10.9% for IgG1, 5.4% to 9.6% for IgG2, 5% to 9.9%
153 for IgG3 and 5.3% to 9.3% for IgG4.

154 Maternal HIV infection (Fig. 4b) had a negative effect on IgG cord blood levels to all antigens,
155 except for *C. diphtheriae*, *Hib* and *V. cholerae*. HIV infection was associated with a 2.1% to 4.1%
156 reduction in the IgG cord blood levels. For IgG1, HIV infection negatively impacted cord blood
157 levels against *C. diphtheriae*, *B. pertussis*, *S. dysenteriae*, HBV, measles, *C. parvum* and *G.*
158 *intestinalis* (2.9% to 7.1% reduction). For IgG2, an HIV negative effect was observed against *B.*
159 *pertussis*, *S. dysenteriae*, HBV and *G. intestinalis* (2.9% to 7.1% reduction), whereas HIV was
160 associated with an increase of 3.3% of IgG2 to RSV. Finally, we only detected a negative effect
161 of HIV infection on IgG3 levels to *C. tetani* (1.1% reduction) and IgG4 to *P. falciparum* (1.8%
162 reduction).

163 *P. falciparum* exposure was negatively associated with cord blood IgG levels against *S.*
164 *dysenteriae* and HBV, IgG1 against *S. pneumoniae* and rotavirus, IgG2 against HBV and IgG3
165 against *C. diphtheriae* and rotavirus (Fig. 4c). Depending on the IgG sublcass and antigen, 10%
166 increseas in *P. falciparum* exposure reduced the cord blood levels from 0.3% to 0.8%.

Previous studies suggest that PM rather than peripheral malaria affect transplacental transfer of antibodies and lead to adverse outcomes due to the damaged placental tissue [35, 42, 43]. Therefore, we explored the effect of PM on cord blood levels and placental transfer instead of *P. falciparum* exposure despite the low number of women with any evidence of PM. When analysing HIV-infected women only, PM was associated with lower *B. pertussis* IgG1, *C. diphtheriae* IgG2 and HBV IgG3 levels in cord blood (Figure 4-figure supplement 1).

Prematurity (Fig. 5a), previously shown to have a detrimental effect on placental transfer of antibodies [44], increased the quality (AIC) of some of the above multivariable models. Prematurity was associated with lower cord blood IgG levels against *Hib* (4.2% reduction compared with on-term cord blood levels), *V. cholerae* (2.3% reduction), measles (5.8% reduction) and *C. parvum* (3,8% reduction without statistical significance after adjusting for multiple testing)

The rest of the variables (age, maternal anaemia, gravidity, low birth weight, IPTp treatment, seasonality; and $CD4^+$ T cell counts, ART and viral load for HIV-infected women) did not provide an added value to the multivariable models. Univariable models did not show a consistent effect of any variable across antigens or IgG subclasses, but some significant associations were found for age and gravidity (Supplementary Material 1).

Factors associated with placental transfer of IgG and IgG subclasses

In multivariable models including HIV infection and *P. falciparum* exposure, HIV infection (Fig. 6a) was associated with a generalized reduced placental transfer of IgG and IgG1 (from 2.1% to 6.7% reduction depending on the antigen). HIV infection was also associated with a reduced transfer of IgG2 against *B. pertussis*, HBV and *G. intestinalis*, but was associated with an increase in IgG2 RSV transfer (5.4% increase). Although adjusted p-values were not significant, a similar trend of positive correlation was found for *S. pneumoniae* IgG2 and *Hib* and *V. cholerae* IgG3 and IgG4.

192 *P. falciparum* exposure (Fig. 6b) had a negative effect on the placental transfer of antibodies for
193 some antigens. An increase of 10% in *P. falciparum* exposure reduced the placental transfer of
194 IgG against *S. dysenteriae* and HBV by 0.3% and 0.5%, respectively, IgG2 against HBV by 0.9%
195 and IgG3 against *C. diphtheriae* by 0.6%.

196 PM, in contrast to *P. falciparum* exposure, did not have any impact on transplacental transfer of
197 antibodies in exploratory analyses and did not improve any of the models, although it had a similar
198 trend of correlation on IgG. Nevertheless, PM was associated with a diminished placental transfer
199 on IgG1 *B. pertussis* among the HIV-positive subset of women (Figure 6-figure supplement 1).

200 When prematurity was added to the multivariable models, this additional covariable had a
201 negative effect on placental transfer of *Hib* and *V. cholerae* IgG antibodies (4.5% and 2.3%
202 reduction in premature vs on-term newborns, respectively) (Fig. 5b).

203 The rest of variables were not added to the placental transfer multivariable models, because almost
204 none of the models improved when included. Similar to cord blood levels ones, the placental
205 transfer univariable models did not show a consistent effect of any variable not included in the
206 multivariable models across antigens or IgG subclasses (Supplementary Material 1).

207 **Discussion**

208 Our comprehensive analysis of maternal and cord plasma IgG and IgG subclasses against a wide
209 range of microbial and vaccine antigens allowed a depth immunoprofiling, that is essential to
210 decipher the mechanisms affecting antibody placental transfer and maternal and newborn
211 immunity in women chronically exposed to pathogens. We confirmed that the main determinant
212 of cord IgG and IgG subclass levels are the maternal corresponding antibody levels, and that
213 maternal HIV infection is associated with a generalized diminished IgG levels in the cord due to
214 low maternal levels but also to a broadly reduction of IgG and IgG1 placental transfer.

215 Maternal and cord blood antibody levels are usually correlated in many studies, suggesting that
216 maternal levels are the main determinant for transfer efficiency [9, 45, 46]. However, the effect
217 of HIV infection on placental transfer has not been consistently assessed and the few studies

looking at its effect on maternal and cord blood levels mainly focussed on total IgG. Our results showed that HIV infection reduced the IgG maternal levels for some antigens, the cord blood levels overall, and also had a negative effect on transplacental transfer of IgG antibodies. It is interesting that although we found higher maternal HBV and *G. intestinalis* IgG levels among HIV-infected women, cord blood levels and transplacental transfer were lower than in HIV-uninfected women. Higher maternal antibody levels against these pathogens in HIV-infected women may be due to an increased susceptibility to co-infections with these pathogens, as described before [47–49], but it seems that they are not being transferred as efficiently as in HIV-uninfected women. This could be due to hypergammaglobulinemia, demonstrated to be common among HIV-infected individuals [50] and previously shown to impair transplacental transfer of antibodies [9, 33].

Our results are consistent with previous studies reporting that HIV infection led to a reduction of the cord blood levels and transplacental transfer of total IgG against *B. pertussis* [40, 41], *C. tetani* [26, 38, 40, 41], *S. pneumoniae* [31, 38, 41, 51], RSV [52, 53] and measles [37, 38]. Some studies also found a negative effect on *Hib* [27, 40, 51] that is not appreciated in our study (although we found reduced IgG1 levels in cord in univariable analyses). However, our results differ from other studies that did not find any effect of HIV status on IgG levels against *C. diphtheriae* [36], *C. tetani* [31, 36], *S. pneumoniae* [53], HBV [36] and measles [31, 36].

IgG subclasses may be differently elicited depending on the pathogen, the antigen or the epitope [54] and the efficiency of the antibody placental transfer is different for each subclass due to differential affinity of the receptors FcRn. Furthermore, the Fc region of IgG, that mediates effector functions, vary between IgG subclasses, conferring them different roles during infection and pathogen clearance [55]. We found that HIV infection reduced mainly IgG1 cord levels due to an HIV impairment of the transplacental transfer, similarly to IgG. Interestingly, maternal HIV infection increased the placental transfer of IgG2 to *S. pneumoniae* and RSV, although in multivariable models it was only significant for RSV. We also found that HIV infection had a positive effect on RSV IgG2 cord blood levels, although IgG2 maternal levels were lower among

HIV-infected women. To our knowledge an increased placental transfer by HIV infection has not been described before. This may have implications for maternal immunization with RSV vaccines under development.

The efficacy of IgG placental transfer also depended on the antigen. IgG1, IgG3 or IgG4 transferred better than IgG2, except for *S. pneumoniae* and *G. intestinalis*, for which IgG2 transfer was higher. This was unexpected because it has been previously described that the greatest transport occurs for IgG1, followed by IgG4, IgG3, and finally IgG2 [9, 20]. However, IgG1 levels were the highest for almost all antigens in cord blood, probably because the overall higher levels of this IgG subclass in maternal blood. One exception was *Hib* that presented higher IgG2 cord levels than IgG1, although IgG1 transplacental transfer was higher than IgG2 consistently with previous studies [56]. The mothers had a IgG2-predominant response to *Hib*, and consequently higher IgG2 than IgG1 levels were found in cord blood as previously described [57, 58].

Regarding other variables, our results did not show any significant association between $CD4^+$ T cell counts or HIV viral load on cord blood levels and transplacental transfer of antibodies. Even though these results agree with previous studies that did not find any associations [40, 51, 59], other studies described that lower $CD4^+$ T cell counts and higher HIV viral load led to a reduction on the transfer of some pathogen-specific antibodies and vaccines such as measles and *S. pneumoniae* [35, 60, 61]. Some studies described that HIV-infected women receiving ART transferred higher pathogen-specific antibodies than those who were not under ART [59] or who initiated it during pregnancy [62]. However, in our cohort we did not find any significant associations in regards to ART.

At the time of the study, malaria transmission intensity was very low in the area and only a few women had active malaria during pregnancy. Nonetheless, we found a negative correlation between *P. falciparum* exposure and both placental transfer and cord blood antibody levels for some antigens and IgG subclasses. Previous studies are contradictory, as some found that PM led to a reduction of the transplacental transfer of some pathogen-specific IgG to *C. tetani* [32],

measles [33, 37], RSV [35] and *S. pneumoniae* [31], but others did not find any effect for IgG against *C. diphtheriae* [35, 36], *C. tetani* [26, 33, 36], *Hib* [35], HBV [36], measles [36] RSV [34] and *S. pneumoniae* [35]. Discrepancies between studies could be due to the different study areas, with different prevalence of malaria and study sample sizes, different type of antigens used in the studies, the different sensibilities among the serological methods used, different exposure to the pathogens tested, and other co-infections.

We found prematurity to be associated with lower cord blood IgG levels and placental transfer for some antigens, as previous studies have shown [44, 63, 64], although the effect was not consistent among subclasses. It has already been reported that the greatest transport occur in the third trimester of gestation [17], and due to this fact, preterm infants may have lower amounts of transplacental IgG than term infants.

Our results are important for maternal immunization implementation in settings with a high prevalence of HIV infection. In our study cohort, the only vaccine given during pregnancy was tetanus. Although HIV infection was associated with lower maternal and cord blood tetanus toxoid IgG and IgG1 levels in univariable models, HIV did not affect cord blood IgG1 levels in multivariable models adjusted by maternal levels. Systemic tetanus vaccination during pregnancy has been implemented in Africa and has demonstrated a high efficacy [65]. Pertussis vaccination in pregnancy has also been implemented in some countries, but not in Africa. Acellular pertussis vaccine induces mainly IgG and IgG1 responses that are thought to confer protection [66, 67]. We found lower cord blood levels and a reduced placental transfer of IgG and IgG1 against *B. pertussis* among HIV-infected women and those exposed to *P. falciparum*. These results highlight the need for further studies assessing the impact of these infections on pertussis vaccine efficacy and antibody placental transfer when implemented in pregnant women from African countries. A current vaccine in development for maternal immunization is RSV [68]. Natural RSV infection seems to elicit an IgG1 and IgG2 response against the F protein, the major target of the host's immune response [69] and of some vaccines in development [70]. Antibodies binding to the F protein were protective [71] and Palivizumab, an IgG1 monoclonal antibody against RSV F

299 protein with neutralizing function, has shown to be effective [72]. Here, IgG and IgG1 against
300 RSV F protein had the highest levels in cord blood compared to other subclasses, but HIV
301 infection reduced IgG cord blood levels and placental transfer in multivariable models. Instead,
302 IgG2 cord blood levels were increased by maternal HIV infection. Therefore, HIV infection could
303 compromise the levels of RSV neutralizing antibodies transferred to the newborn and,
304 consequently, diminish the effectivity of a RSV vaccine.

305 Unfortunately, we do not know what are the thresholds of antibody levels that confer protection
306 in our study, therefore it is difficult to infer the clinical relevance of the reductions in antibody
307 levels detected in cord blood from the-HIV infected women. A study in South Africa reported
308 that the frequency of HIV-infected and HIV-uninfected pregnant women with protective antibody
309 levels against pertussis, tetanus or HBV was similar, although the overall frequencies were low
310 (32%, 41% and 30%, respectively) [40]. This same study demonstrated that the proportion of
311 HIV-infected pregnant women reaching anti-*Hib* protective antibody levels was lower than HIV-
312 uninfected women (35% vs 59%). Thus, for the implementation of maternal immunization
313 programs, the effect of HIV infection and *P. falciparum* exposure must be taken into account,
314 especially after demonstrating that both infections reduce the levels of antibodies in the cord blood
315 and therefore may compromise vaccines protective effect in the newborn.

316 In conclusion, our results demonstrate that maternal HIV infection was associated with reduced
317 levels of antibodies against a broad range of pathogens and vaccine antigens in cord blood. Part
318 of this reduction in antibody levels was due to altered antibody levels in the mother, which are
319 the main determinants of cord blood levels, but HIV-infection also diminished transplacental
320 transfer of antibodies. Importantly, IgG1 was the most affected by maternal HIV infection but,
321 depending on the pathogen, other subclasses were also affected. *P. falciparum* exposure also
322 reduced the levels and transfer of some antibodies, although overall the effect was lower than
323 HIV infection. Our findings are important for effective maternal immunization strategies and for
324 newborn and infant's health.

325 **Materials and methods**

Study design and sample collection

A total of 197 HIV-uninfected and 144 HIV-infected women were recruited among those participating in two clinical trials of antimalarial intermittent preventive treatment in pregnancy (IPTp, ClinicalTrialGov NCT00811421) (Fig. 7) in the Manhiça District, Southern Mozambique [73, 74], between May 2011 and September 2012, to perform an immunology ancillary study. The first clinical trial evaluated mefloquine (MQ) as an alternative IPTp drug to sulfadoxine-pyrimethamine (SP) in HIV-uninfected pregnant women. The study arms were (1) SP, (2) single dose MQ (MQ full), and (3) split dose over two days MQ (MQ split). The second trial evaluated MQ as IPTp drug in HIV-infected pregnant women in whom SP is contraindicated and who received daily cotrimoxazole (CTX), and women received either three monthly doses of MQ or placebo. All women received bed nets treated with long-lasting insecticide and supplements of folic acid and ferrous sulphate. Women also received tetanus vaccination. At the time of the study, the intensity of malaria transmission was low/moderate [75]. Antiretroviral therapy (ART) with daily monotherapy with zidovudine (AZT) was recommended when $CD4^+$ T cell count was below <350 cells/μL and/or when women were in III or IV HIV WHO clinical stage [76].

At delivery, blood samples from women (peripheral, placental and cord blood) were collected into sodium heparin and EDTA vacutainers. Plasma samples from peripheral blood and cord blood were available for this study from 332 (195 HIV-uninfected and 137 HIV-infected) and 303 women (178 HIV-uninfected and 125 HIV-infected), respectively. There were 294 mother-cord paired samples.

For the detection of *P. falciparum* species, thick and thin blood smears were assessed according to standard procedures [73, 74]. Fifty μl of maternal peripheral, placental, and cord blood samples were collected on filter papers for the detection of *P. falciparum* by means of a real-time quantitative polymerase-chain-reaction (qPCR) assay targeting the 18S ribosomal RNA [77]. Tissue samples from the maternal side of the placenta were also collected for the assessment of placental malaria. Microscopy data of peripheral and placental blood smears at delivery were

352 available for 308 and 340 women, respectively. Peripheral and placental blood qPCR data were
353 available for 242 and 236 women, respectively.

354 Antibody assays

355 Quantitative suspension array technology (qSAT) assays applying the xMAP™ technology
356 (Luminex Corp., TX) were used to measure antigen-specific IgG, IgG1, IgG2, IgG3 and IgG4
357 responses to vaccine and pathogen antigens. A total of 16 recombinant proteins were selected for
358 the analysis: diphtheria toxoid (*Corynebacterium diphtheriae,* Alpha Diagnostic DTOX15-N-
359 500), tetanus toxin (*Clostridium tetani,* Santa Cruz SC222347), pertussis toxin (*Bordetella*
360 *pertussis,* Santa Cruz SC200837), *Hib* Oligosaccharide (BEI Resources NR12268),
361 pneumococcal surface protein A (PspA, *Streptococcus pneumoniae,* BEI Resources NR33179),
362 shiga toxin (*Shigella dysenteriae,* BEI Resources NR4676), anti-O-specific polysaccharide (OSP,
363 *Vibrio cholerae,* Massachusetts General Hospital, MA, USA) [78], hepatitis B surface antigen
364 (HBsAg, Abcam ab91276), hemagglutinin (measles, Alpha Diagnostic RP655), viral protein 6
365 (VP6, rotavirus, Friedzgerald 80-1389), F protein (respiratory syncytial virus, BEI Resources
366 NR31097), 17-kDA surface antigen (Cp17, *Cryptosporidium parvum,* Centres for Disease
367 Control and Prevention, GA, USA) [79], variant-specific surface protein 5 (VSP5, *Giardia*
368 *intestinalis*) [79], 42 kDA fragment of merozoite surface protein 1 ($MSP1_{42}$, *P. falciparum,*
369 WRAIR) [80], merozoite surface protein 2 (MSP2, *P. falciparum,* University of Edinburgh) [81]
370 and exported protein 1 (EXP1, *P. falciparum,* Sanaria) [82]. $MSP1_{42}$ antigen was selected for
371 representing *P. falciparum* infection. Eight recombinant proteins represent the most prevalent
372 pathogens circulating in the study area [83–85] and 6 were from the vaccines administrated to the
373 infants through the EPI in Mozambique [86].

374 qSAT assays were previously standardized and optimized to control for sources of variability
375 [87–89]. Briefly, antigens covalently coupled to MagPlex beads were added to a 96-well µClear®
376 flat bottom plate (Greiner Bio-One) in multiplex resuspended in 50µL of PBS, 1% BSA, 0.05%
377 Azide pH 7.4 (PBS-BN). Fifty µL of test samples, negative or positive controls [90] were added
378 to multiplex wells and incubated overnight at 4ºC protected from light. After incubation, plates

were washed three times with PBS-Tween 20 0.05%, and 100µL of anti-human IgG (Sigma B1140), anti-human IgG1 (Abcam ab99775), anti-human IgG2 (Invitrogen MA1-34755), anti-human IgG3 (Sigma B3523) or anti-human IgG4 (Invitrogen MA5-16716), each at their corresponding dilution, were added and incubated for 45 min. Then, plates were washed three times more and 100µL of streptavidin-R-phycoerythrin (Sigma 42250) at the appropriate dilution were added to all wells and incubated 30 min for IgG, IgG1 and IgG3. For IgG2 and IgG4, 100 µL of anti-mouse IgG (Fc-specific)−biotin (Merck B7401) were added and incubated for 45 min, followed by another washing cycle and the incubation with streptavidin-R-phycoerythrin for 30 min. Finally, plates were washed and beads resuspended in 100 µL/well of PBS-BN. Plates were read using the Luminex 100/200 analyser, and at least 20 microspheres per analyte were acquired per sample. Antibody levels were measured as median fluorescence intensity (MFI). Data were captured using xPonent software.

Test samples were assayed at 2 dilutions for IgG (1/250 and 1/10000), and IgG1 and IgG3 (1/100 and 1/2500) to ensure that at least one dilution fell in the linear range of the respective standard curve. For IgG2 and IgG4 only 1 dilution was tested (1/50) because their usual low levels. Twelve serial dilutions (1:3, starting at 1/25) of a positive control (WHO Reference Reagent for anti-malaria *P. falciparum* human serum, NIBSC code: 10/198) were used for QA/QC and to select optimal sample dilution for data analysis. Two blanks were added to each plate also for quality control purposes. Sample distribution across plates was designed to ensure a balanced distribution of groups and time-points. Single replicates of the assay were performed.

Statistical Analysis

To stabilize the variance, the analysis was done on $\log_{10}$-transformed values of the MFI measurements. To select the sample dilution for each antigen-isotype/subclass-plate, the dilution nearest to the midpoint between the two standard curve serial dilutions ranging the maximum slope was chosen. If the maximum MFI value of a standard curve did not reach 15000, the reference value was automatically set up at 15000, since below this point, standard curve data does not seem trustworthy. If the MFI of the first sample dilution was lower than the MFI of the

second dilution (hook effect), the second one was chosen. Plates were normalized using the standard curve in each plate and the average standard curve from all plates -in both cases using the dilution of the latter with the value closest to 15000 MFI. The MFI values of samples were multiplied by the corresponding normalization factor (MFI value of the chosen dilution from the average standard curve divided by the MFI value of same dilution in the plate curve).

The Shapiro-Wilk test of normality confirmed that most of the antibody data were not normally distributed. The Chi-square and the non–parametric Wilcoxon-Mann-Whitney tests were used to compare categorical and continuous variables, respectively, between HIV-infected and HIV-uninfected women. Comparisons of crude Ig levels across antigens and subclasses between HIV exposure groups were assessed by Wilcoxon-Mann-Whitney tests. Univariable linear regression models were fit to determine the effect of variables on the cord blood antibody levels (log_{10}) or the cord blood/mother ratio (log_{10}). The variables considered in this analysis were log_{10} maternal antibody levels, maternal HIV infection, *P. falciparum* exposure, PM (acute, defined by the presence of parasites on sections without malaria pigment; chronic, by presence of parasites and pigment; or past, by the presence of pigment alone), age, gravidity (defined as *primigravidae* and *multigravidae*), maternal anaemia (defined as haemoglobin level <11g/dL), low birth weight (defined as <2500g at birth), prematurity (defined as delivery before 37 weeks of gestational age), gestational age (measured by Ballard score [91]), treatment (defined as MQ or placebo in the HIV-infected women ancillary study and MQ full, MQ split or SP in HIV-uninfected women ancillary study), antiretroviral therapy (ART) received before the initiation of the study, $CD4^+$ T cell counts (<350 cells/μL or ≥350 cells/μL), HIV viral load (<400, 400-999, 1000-9999 and >9999 copies/mL), and seasonality (dry or rainy). Exposure to *P. falciparum* was computed as the sum of the maternal IgG antibody levels (MFI) for the following immunogenic *P. falciparum* antigens: $MSP1_{42}$, MSP2 and EXP1, as antibody levels to these antigens have been shown to reflect exposure to malaria [92, 93]. Seasonality was computed for each woman based on the pregnancy period - if at least 4 of the pregnancy months felt under the category of rainy period (November through April), the season was defined as such. In any other case, the season was

433 defined as dry. A base multivariable model including maternal antibody levels, maternal HIV
434 infection and *P. falciparum* exposure was established for each antigen and IgG or IgG subclass.
435 Base model for MSP_{42} did not include *P. falciparum* exposure as this variable includes antibodies
436 to this antigen. We performed additional regression models testing exhaustively all possible
437 combinations of predictor variables (added to our base model) and selected the models based on
438 the Akaike information criterion (AIC), Bayesian information criterion (BIC) and Adjusted r-
439 square parameters. All p-values were considered statistically significant when <0.05 after
440 adjusting for multiple testing through Benjamini-Hochberg. All data collected were pre-
441 processed, managed and analysed using the R software version 3.6.3 and its package *devtools*
442 [94]. The *ggplot2* package was used to perform boxplot graphs [95]. The *FactoMineR* and
443 *factoextra* packages were used to perform Principal Component Analysis (PCA) [96, 97].

444 **Additional files**

445 Supplementary material 1: Cord blood levels and placental transfer of antibodies univariable
446 models.

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455 **Competing interests**

456 The authors declare that they have no competing interests.

457 **References**

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Figures legends and tables

Fig. 1: Overview of levels of IgG and IgG subclasses to all pathogen antigens. a) Principal component analysis (PCA) plots of IgG and IgG subclass levels against all antigens clustered by subclass type. b) PCA plots of IgG and IgG subclass levels clustered by antigen type. The two principal components (Dim 1, Dim2) that explained the highest percentage of the variance of the data (percentage in parenthesis) were chosen for representation. Representation of the a) 10 b) 5 first variables that contributed to the principal components c) Medians of IgG and IgG subclass levels (log_{10} MFI) in cord blood for each antigen d) Median IgG and IgG subclass placental transfer for each antigen, represented as the cord/mother ratios. Source files of the medians of each antigen/subclass are available in the Figure 1-source data 1.

Fig. 2: Mother and cord blood antibody levels (log_{10} MFI) in HIV-infected and HIV-uninfected women. Boxplots illustrate the medians and the interquartile range for IgG (a) and IgG1 (b), IgG2 (c) and IgG4 (d) subclasses. Levels between HIV-infected and HIV-uninfected women were compared by parametric Wilcoxon-Mann–Whitney test and p-values were adjusted for multiple testing by the Benjamini-Hochberg approach. Statistically significant differences between HIV infected and uninfected women levels are highlighted with an asterisk. Red represents HIV-infected women and blue HIV-uninfected women. Source files of the mother and cord levels of each antigen/subclass are available in the Figure 2-source data 1.

Fig. 3: Antibody placental transfer in HIV-infected and HIV-uninfected women. Radar charts representing the medians of each analyte antibody cord/mother ratio in HIV-infected and HIV-uninfected women for IgG (a) and IgG subclasses (b-d). Ratios between HIV-infected and uninfected women were compared by parametric Wilcoxon-Mann-Whitney test and p-values were adjusted for multiple testing by the Benjamini-Hochberg approach. Statistically significant differences between HIV-infected and uninfected women ratios are highlighted with an asterisk. *** = p-val < 0.0001, ** = p-val < 0.001, * = p-val < 0.01. Red represents HIV-infected women and blue HIV-uninfected women. Source files of the medians and p-values of each antigen/subclass are available in the Figure 3-source data 1.

Fig. 4: Factors associated with IgG and IgG subclass levels in cord blood. Forest plots show the effect of a) maternal antibody levels, b) HIV infection and c) *P. falciparum* exposure (Pf exposure) on cord blood levels of IgG and IgG subclasses, for all the antigens tested. Beta values, representing the increase or decrease of cord blood levels (log_{10}MFI) were obtained from multivariable regression models using cord blood (log_{10}MFI) levels as the outcome. Beta values are shown when raw p-vals are significant. Asterisks are shown when adjusted p-vals by Benjamini-Hochbert are significant **** = p-val < 0.0001, *** = p-val < 0.001, ** = p-val < 0.01, * = p-val < 0.05. Source files of the multivariable model are available in the Figure 4-source data 1.

Fig. 5: Association of prematurity with cord blood levels and placental transfer of IgG and IgG subclasses. Forest plots show the effect of a) prematurity and cord blood levels and b) prematurity and transplacental transfer of IgG and IgG subclasses, for all the antigens tested. Cord antibody levels are represented in log_{10}MFI. Placental transfer is represented as cord/mother ratio (log_{10}). Beta values are shown when raw p-vals are significant. Asterisks are shown when adjusted p-vals by Benjamini-Hochbert are significant. **** = p-val < 0.0001, *** = p-val < 0.001, ** = p-val < 0.01, * = p-val < 0.05. Source files of the multivariable model are available in the Figure 5-source data 1.

Fig. 6: Factors associated with IgG and IgG subclass placental transfer. Forest plots show the effect of a) HIV infection and b) *P. falciparum* exposure (Pf exposure) on placental transfer of IgG and IgG subclasses, for all the antigens tested. Beta values are shown when raw p-vals are significant. Asterisks are shown when adjusted p-vals by Benjamini-Hochbert are significant. **** = p-val < 0.0001, *** = p-val < 0.001, ** = p-val < 0.01, * = p-val < 0.05. Source files of the multivariable model are available in the Figure 6-source data 1.

Fig. 7: IPTp trial profile.

Fig. 2-figure supplement 1: Mother and cord blood antibody levels (log_{10} MFI) in HIV-infected and HIV-uninfected women. Boxplots illustrate the medians and the interquartile range for IgG3. Levels between HIV-infected and HIV-uninfected women were compared by parametric Wilcoxon-Mann–Whitney test and p-values were adjusted for multiple testing by the Benjamini-Hochberg approach. Statistically significant differences between HIV infected and uninfected women levels are highlighted with an asterisk. Red represents HIV-infected women and blue HIV-uninfected women.

Fig. 3-figure supplement 1: Cord/mother log_{10} antibody ratios in HIV-infected and HIV-uninfected women. Boxplots illustrate the medians, the interquartile range (IQR) and the outlier points that are further 1.5*IQR and black dots show the arithmetic means for IgG. Levels between HIV-infected and uninfected women were compared by Wilcoxon test and p-values were adjusted for multiple testing by the Benjamini-Hochberg approach. ns = not significant. Red represents HIV-infected women and blue HIV-uninfected women.

Fig. 3-figure supplement 2: Cord/mother log_{10} antibody ratios in HIV-infected and HIV-uninfected women. Boxplots illustrate the medians, the interquartile range (IQR) and the outlier points that are further 1.5*IQR and black dots show the arithmetic means for IgG1. Levels between HIV-infected and uninfected women were compared by Wilcoxon test and p-values were adjusted for multiple testing by the Benjamini-Hochberg approach. ns = not significant. Red represents HIV-infected women and blue HIV-uninfected women.

Fig. 3-figure supplement 3: Cord/mother log_{10} antibody ratios in HIV-infected and HIV-uninfected women. Boxplots illustrate the medians, the interquartile range (IQR) and the outlier points that are further 1.5*IQR and black dots show the arithmetic means for IgG2. Levels between HIV-infected and uninfected women were compared by Wilcoxon test and p-values were adjusted for multiple testing by the Benjamini-Hochberg approach. ns = not significant. Red represents HIV-infected women and blue HIV-uninfected women.

Fig. 3-figure supplement 4: Cord/mother log_{10} antibody ratios in HIV-infected and HIV-uninfected women. Boxplots illustrate the medians, the interquartile range (IQR) and the outlier points that are further 1.5*IQR and black dots show the arithmetic means for IgG3. Levels between HIV-infected and uninfected women were compared by Wilcoxon test and p-values were adjusted for multiple testing by the Benjamini-Hochberg approach. ns = not significant. Red represents HIV-infected women and blue HIV-uninfected women.

Fig.3-figure supplement 5: Cord/mother log_{10} antibody ratios in HIV-infected and HIV-uninfected women. Boxplots illustrate the medians, the interquartile range (IQR) and the outlier points that are further 1.5*IQR and black dots show the arithmetic means for IgG4. Levels between HIV-infected and uninfected women were compared by Wilcoxon test and p-values were adjusted for multiple testing by the Benjamini-Hochberg approach. ns = not significant. Red represents HIV-infected women and blue HIV-uninfected women.

Fig.4-figure supplement 1: Forest plots show the effect of placental malaria on cord blood levels of IgG and IgG subclasses, for all the antigens tested, in HIV-infected women. Cord antibody levels are represented in log_{10}MFI. Beta values are shown when raw p-vals are significant. Asterisks are shown when adjusted p-vals by Benjamini-Hochbert are significant. **** = p-val < 0.0001, *** = p-val < 0.001, ** = p-val < 0.01, * = p-val < 0.05.

Fig. 6-figure supplement 1: Forest plots show the effect of placental malaria on placental transfer of IgG and IgG subclasses, for all the antigens tested, in HIV-infected women. Placental transfer is represented as cord/mother ratio (log_{10}). Beta values are shown when raw p-vals are significant. Asterisks are shown when adjusted p-vals by Benjamini-Hochbert are significant. **** = p-val < 0.0001, *** = p-val < 0.001, ** = p-val < 0.01, * = p-val < 0.05.

Table 1: Characteristics of study participants.

	All **N=341**	**HIV-uninfected** **N=197**	**HIV-infected** **N=144**	**p–value** [a]
Age [a] (years median [IQR])	25.0 [19.0; 29.0]	21.0 [18.0; 28.0]	27.0 [22.0; 31.0]	< 0.001
Gravidity (n, %)				< 0.001
Multigravidae	259 (76.0)	128 (65.0)	131 (91.0)	
Primigravidae	82 (24.0)	69 (35.0)	13 (9.0)	
Maternal haemoglobin (n, %)				0.025
Anaemia (< 11 g/dL)	208 (61.5)	109 (56.2)	99 (68.8)	
Normal (≥ 11 g/dL)	130 (38.5)	85 (43.8)	45 (31.2)	
Birth weight (n, %)				NS
Low (< 2500 g)	29 (8.5)	17 (8.6)	12 (8.33)	
Normal (≥ 2500 g)	312 (91.5)	180 (91.4)	132 (91.7)	
Prematurity (n, %)				NS
No (≥ 37 weeks)	312 (94.3)	181 (95.3)	131 (92.9)	
Yes (< 37 weeks)	19 (5.7)	9 (4.7)	10 (7.1)	
Treatment				< 0.001
MQ	71 (20.9)	0 (0.0)	71 (49.7)	
MQ full	68 (20.8)	68 (34.5)	0 (0.0)	
MQ split	73 (21.5)	73 (37.1)	0 (0.0)	
Placebo	72 (21.2)	0 (0.0)	72 (50.3)	
SP	56 (16.5)	56 (28.4)	0 (0.0)	
ART at baseline (n, %)				NP
No	24 (7.1)	–	24 (17.1)	
Yes	116 (34.4)	–	116 (82.9)	
CD4+ T cell counts (n, %)				NP
Lower (< 350 c/µL)	40 (12.3)	–	40 (31.2)	
Higher (≥ 350 c/µL)	88 (27.1)	–	88 (68.8)	
HIV viral load (copies/mL)				NP
< 400	21 (6.4)	–	21 (16.0)	
(400–999)	41 (12.5)	–	41 (31.3)	
(1000–9999)	48 (14.6)	–	48 (36.6)	
> 9999	21 (6.4)	–	21 (16.0)	
Placental malaria [b] (n, %)				NS
No	321 (94.1)	184 (93.4)	137 (95.1)	
Yes	20 (5.9)	13 (6.6)	7 (4.9)	
Peripheral malaria [c] (n, %)				NS
No	290 (85.0)	165 (83.8)	125 (86.8)	
Yes	51 (15.0)	32 (16.2)	19 (13.2)	
P. falciparum exposure (log_{10} MFI IgG)	5.27 [5.19; 5.34]	5.29 [5.21;5.35]	5.26 [5.18; 5.33]	0.011

For numerical variables, the median and first and third quantile, in brackets, are given. For the categorical variables the number of individuals for each group and percentages, in parentheses, are given.

[a] For the age, the Wilcoxon-Mann-Whitney test was used to compare differences between median values. For the categorical variables, the Chi-square test was used.

[b] Placental malaria was considered positive if there was any evidence of *P. falciparum* placental parasitaemia by any method.

[c] Peripheral malaria was considered positive if there was any evidence of *P. falciparum* peripheral parasitaemia by any method.

The statistical significance was considered when *p–value <0.05;* MQ, mefloquine; NS, not significant; NP, not−performed tests; SP, sulfadoxine-pyrimethamine.

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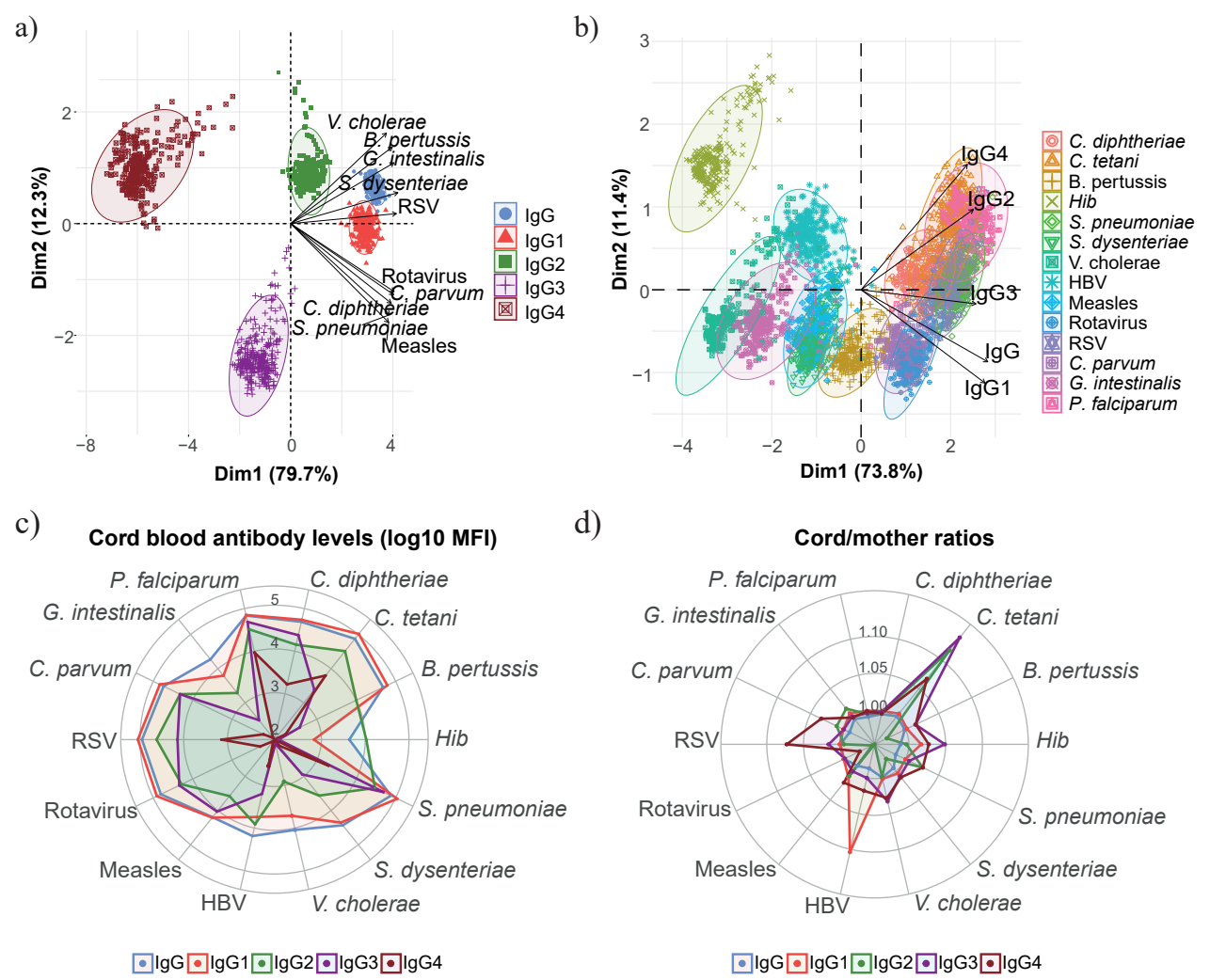
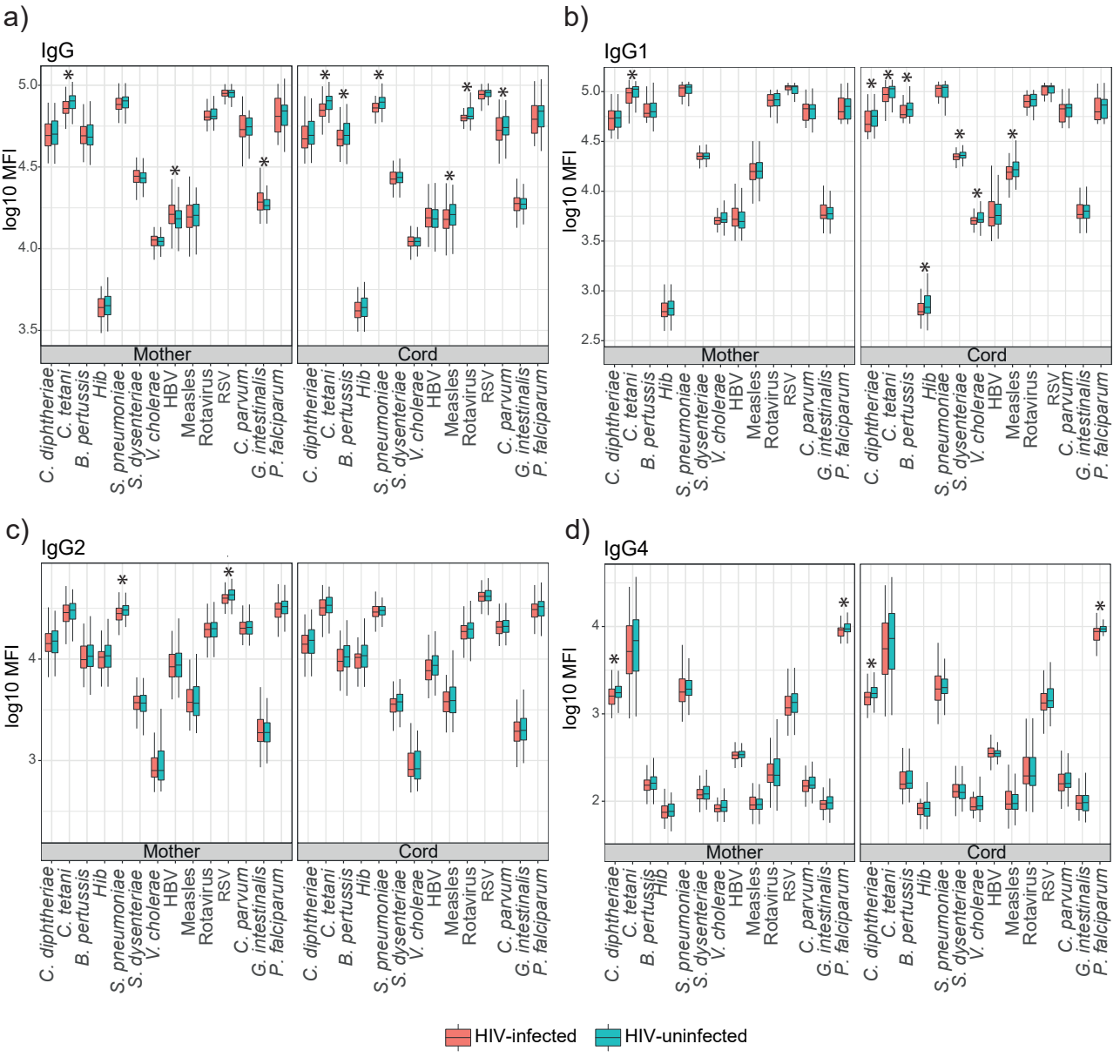
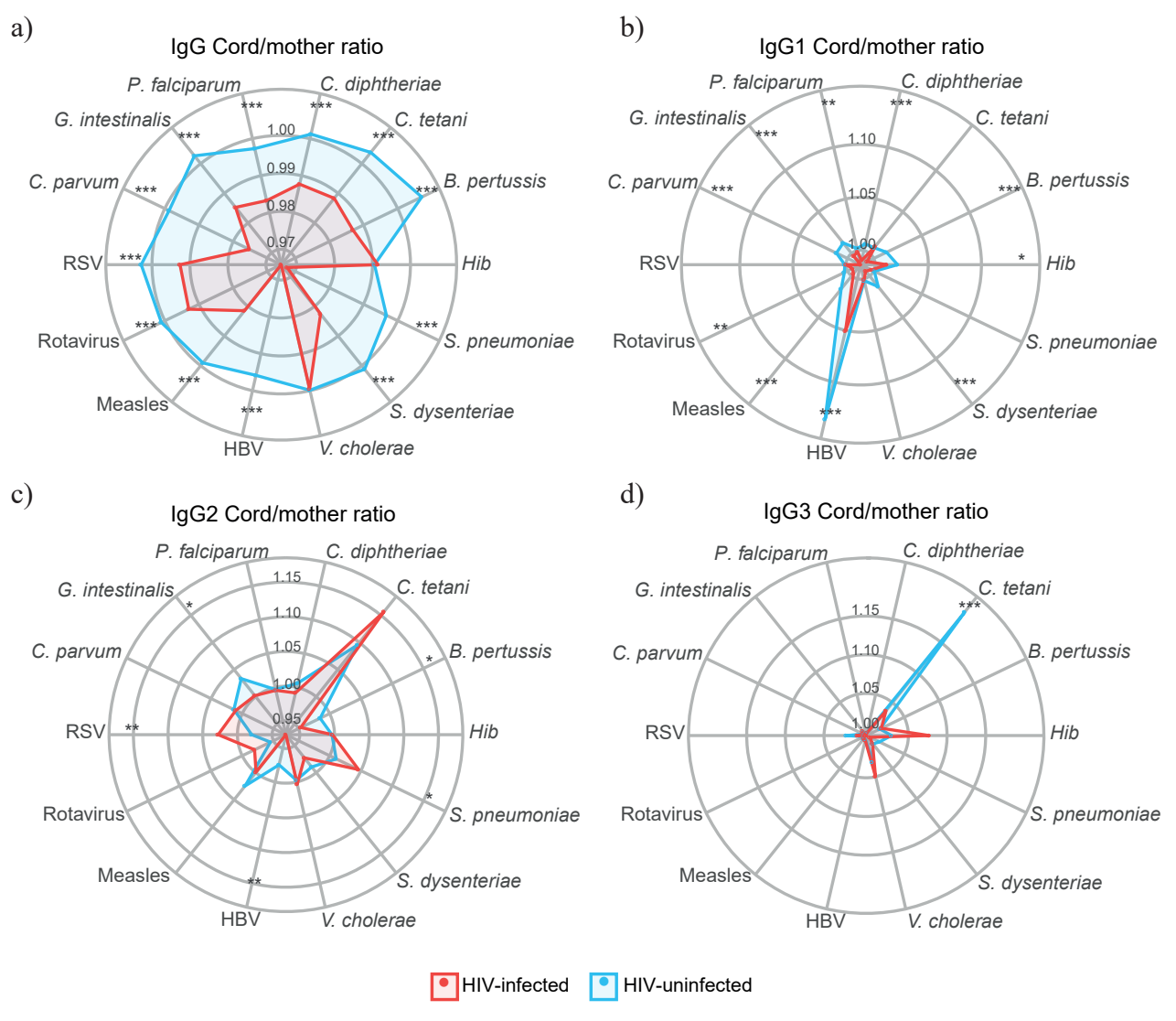
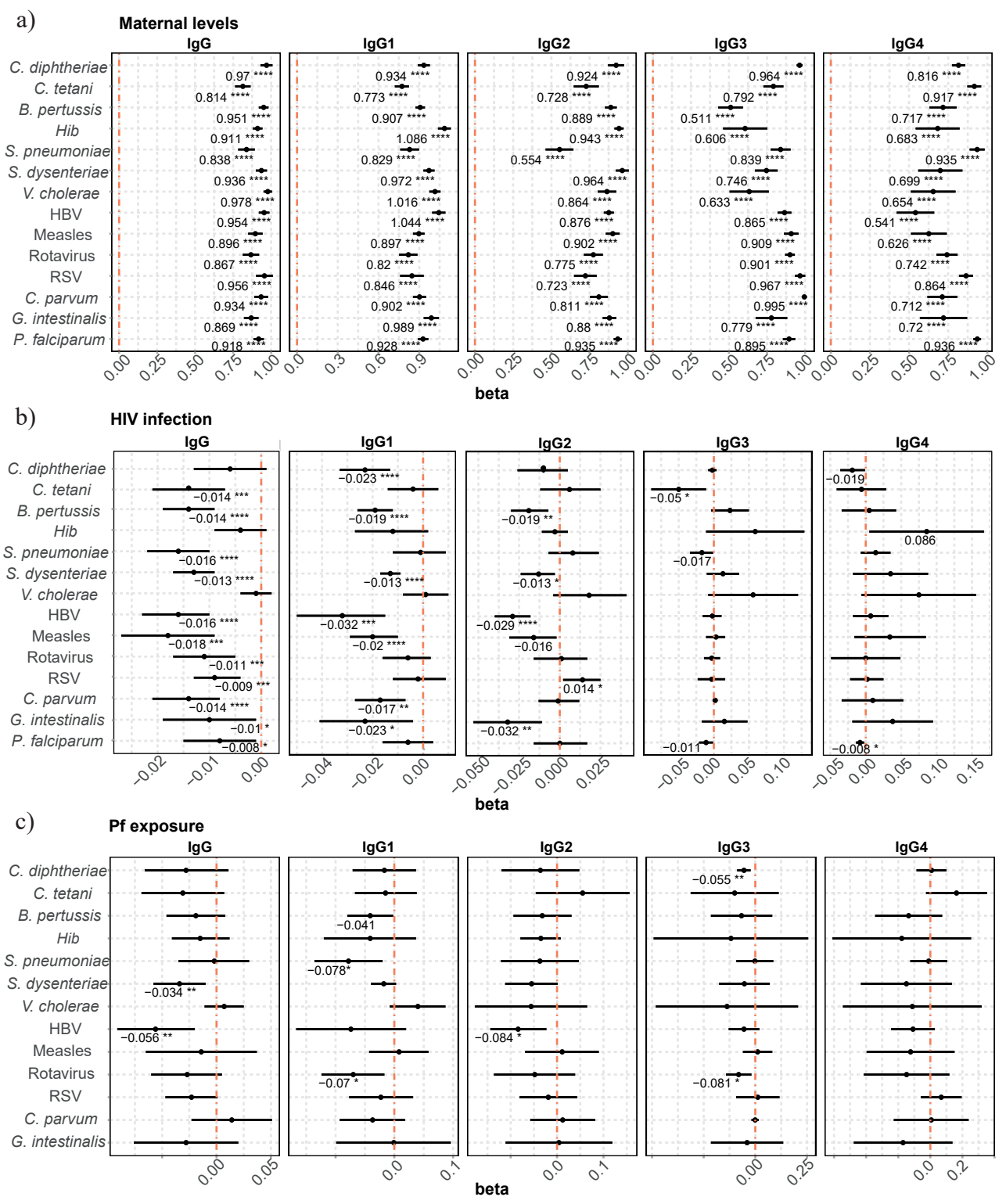
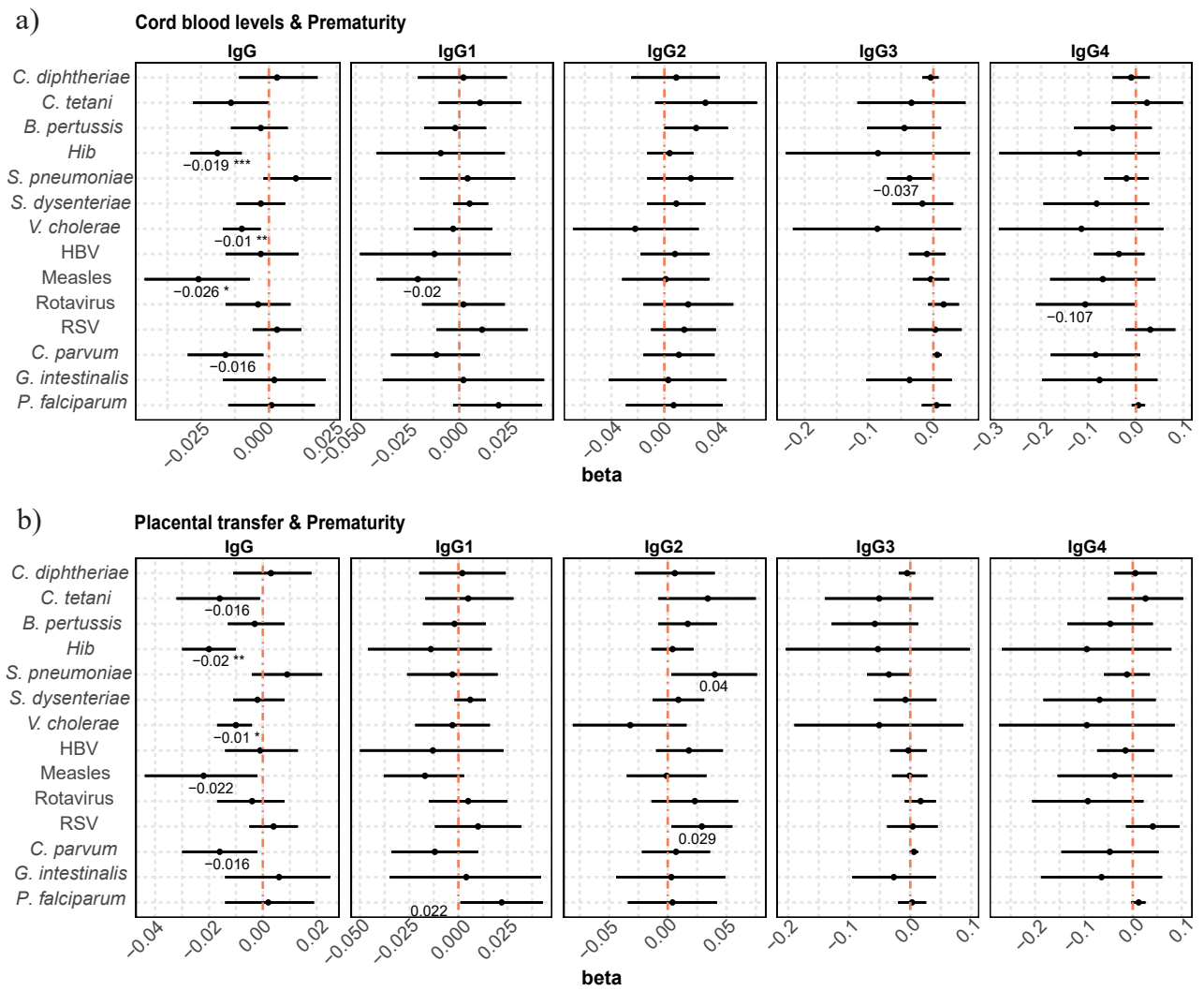
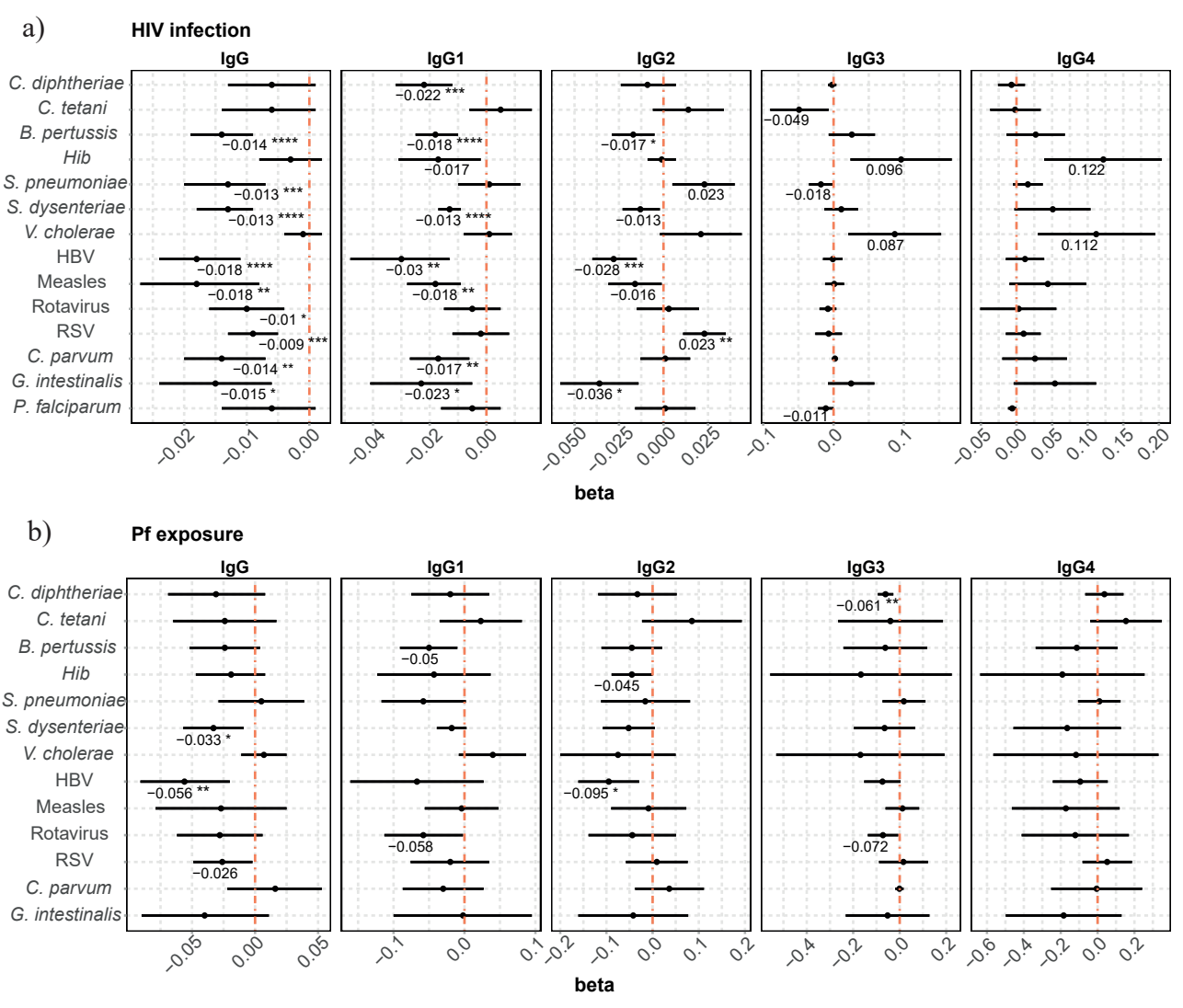

a)
Dim2 (12.3%)
Dim1 (79.7%)
V. cholerae
B. pertussis
G. intestinalis
S. dysenteriae
RSV
Rotavirus
C. parvum
C. diphtheriae
S. pneumoniae
Measles
IgG
IgG1
IgG2
IgG3
IgG4
b)
Dim2 (11.4%)
Dim1 (73.8%)
IgG4
IgG2
IgG3
IgG
IgG1
C. diphtheriae
C. tetani
B. pertussis
Hib
S. pneumoniae
S. dysenteriae
V. cholerae
HBV
Measles
Rotavirus
RSV
C. parvum
G. intestinalis
P. falciparum
c)
Cord blood antibody levels (log10 MFI)
P. falciparum
C. diphtheriae
C. tetani
B. pertussis
Hib
S. pneumoniae
S. dysenteriae
V. cholerae
HBV
Measles
Rotavirus
RSV
C. parvum
G. intestinalis
IgG IgG1 IgG2 IgG3 IgG4
d)
Cord/mother ratios
P. falciparum
C. diphtheriae
C. tetani
B. pertussis
Hib
S. pneumoniae
S. dysenteriae
V. cholerae
HBV
Measles
Rotavirus
RSV
C. parvum
G. intestinalis
1.10
1.05
1.00
IgG IgG1 IgG2 IgG3 IgG4

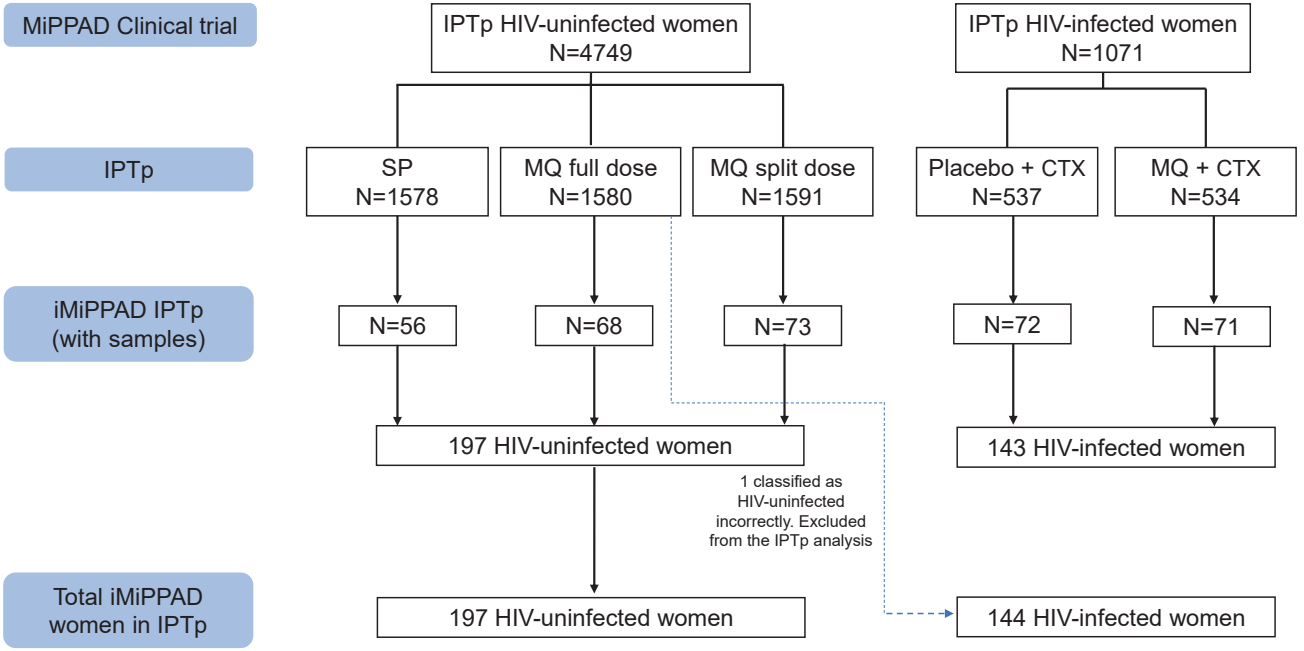

a)
IgG
log10 MFI
5.0
4.5
4.0
3.5
Mother
Cord
C. diphtheriae
C. tetani
B. pertussis
Hib
S. pneumoniae
S. dysenteriae
V. cholerae
HBV
Measles
Rotavirus
RSV
C. parvum
G. intestinalis
P. falciparum
b)
IgG1
log10 MFI
5.0
4.5
4.0
3.5
3.0
2.5
Mother
Cord
c)
IgG2
log10 MFI
4
3
Mother
Cord
d)
IgG4
log10 MFI
4
3
2
Mother
Cord
HIV-infected
HIV-uninfected

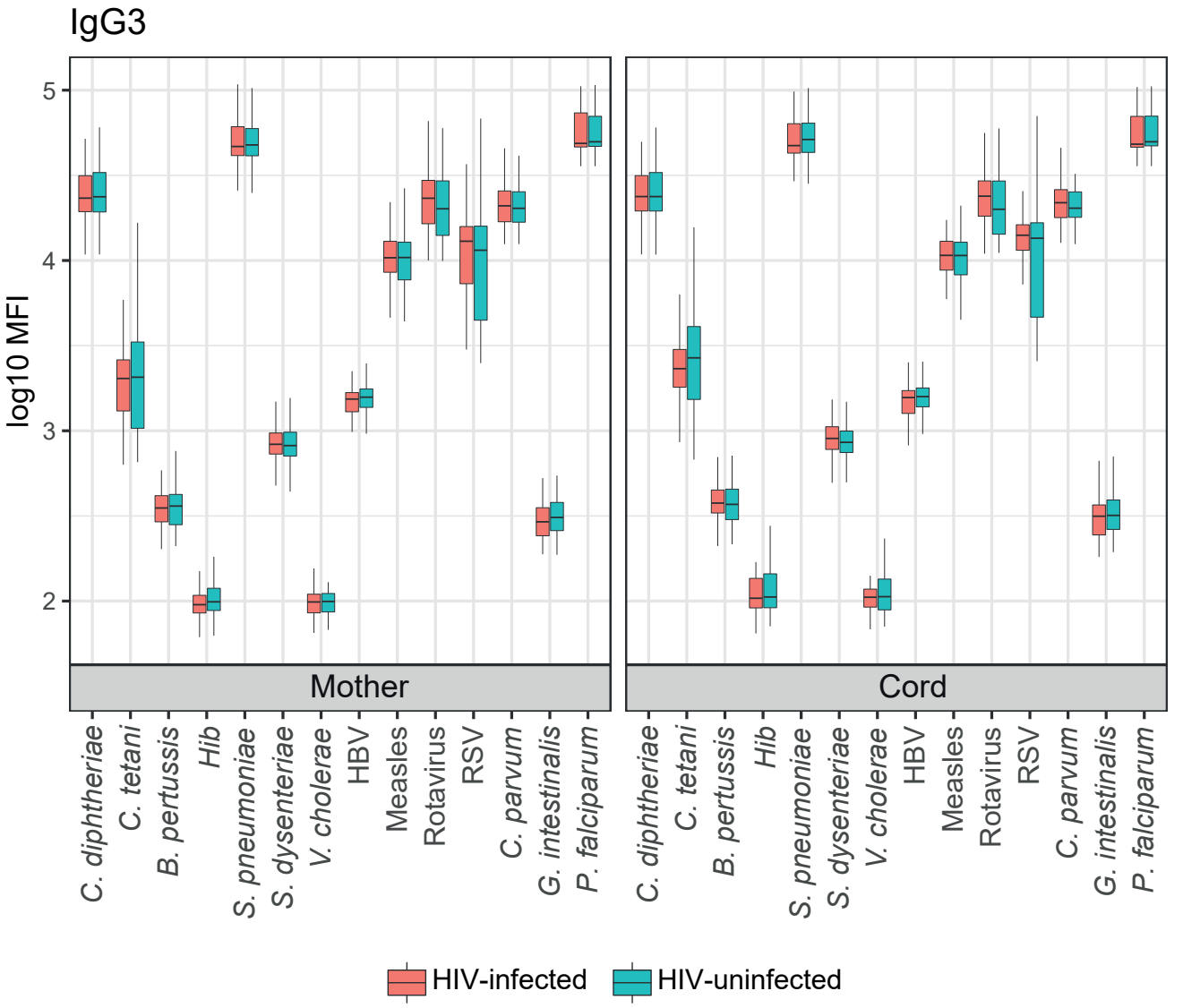

a)
IgG Cord/mother ratio
P. falciparum
C. diphtheriae
G. intestinalis
C. tetani
C. parvum
B. pertussis
RSV
Hib
Rotavirus
S. pneumoniae
Measles
S. dysenteriae
HBV
V. cholerae
1.00
0.99
0.98
0.97
b)
IgG1 Cord/mother ratio
1.10
1.05
1.00
c)
IgG2 Cord/mother ratio
1.15
1.10
1.05
1.00
0.95
d)
IgG3 Cord/mother ratio
HIV-infected
HIV-uninfected

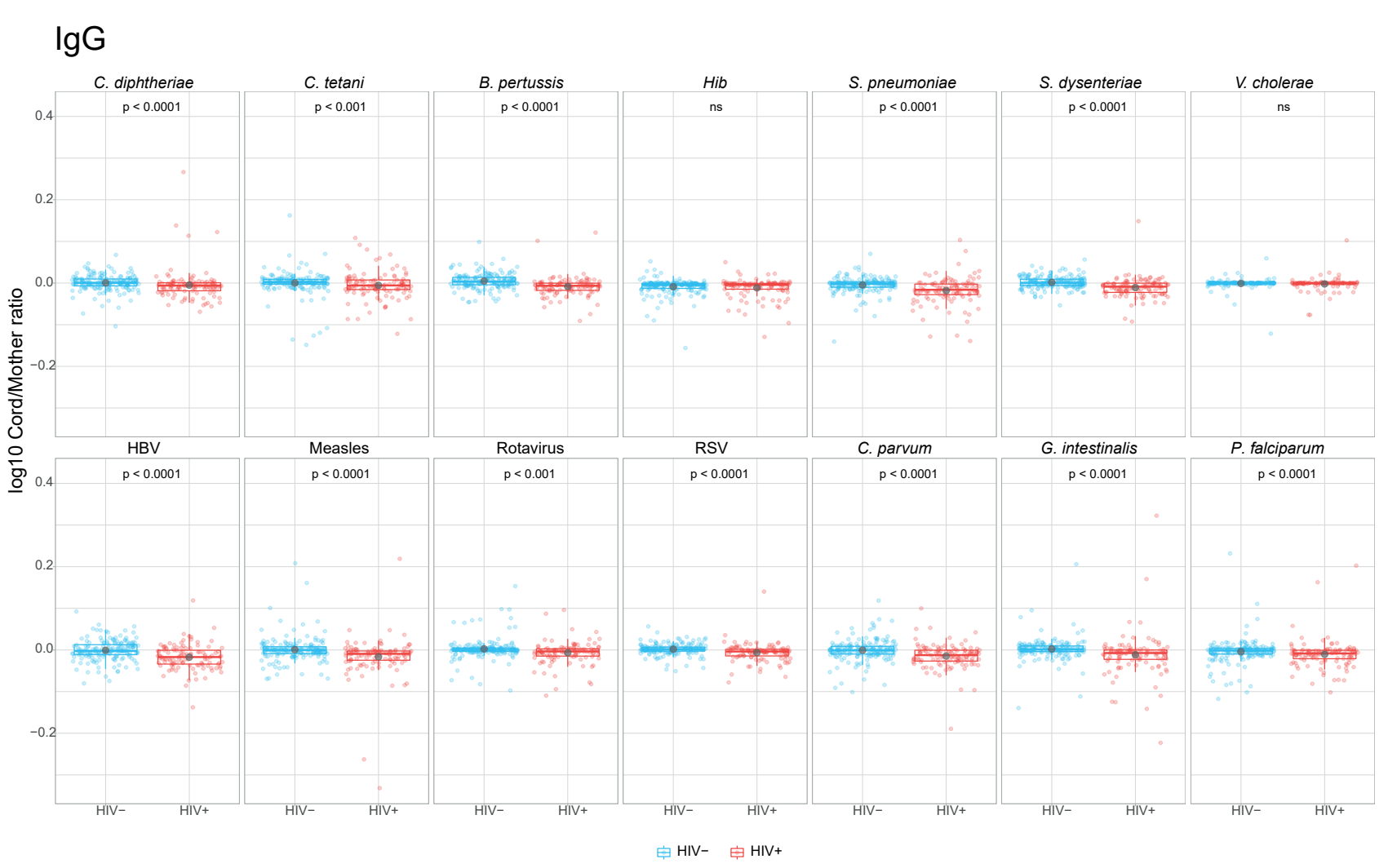

a)
Maternal levels
IgG
IgG1
IgG2
IgG3
IgG4
C. diphtheriae
C. tetani
B. pertussis
Hib
S. pneumoniae
S. dysenteriae
V. cholerae
HBV
Measles
Rotavirus
RSV
C. parvum
G. intestinalis
P. falciparum
0.97 ****
0.814 ****
0.951 ****
0.911 ****
0.838 ****
0.936 ****
0.978 ****
0.954 ****
0.896 ****
0.867 ****
0.956 ****
0.934 ****
0.869 ****
0.918 ****
0.934 ****
0.773 ****
0.907 ****
1.086 ****
0.829 ****
0.972 ****
1.016 ****
1.044 ****
0.897 ****
0.82 ****
0.846 ****
0.902 ****
0.989 ****
0.928 ****
0.924 ****
0.728 ****
0.889 ****
0.943 ****
0.554 ****
0.964 ****
0.864 ****
0.876 ****
0.902 ****
0.775 ****
0.723 ****
0.811 ****
0.88 ****
0.935 ****
0.964 ****
0.792 ****
0.511 ****
0.606 ****
0.839 ****
0.746 ****
0.633 ****
0.865 ****
0.909 ****
0.901 ****
0.967 ****
0.995 ****
0.779 ****
0.895 ****
0.816 ****
0.917 ****
0.717 ****
0.683 ****
0.935 ****
0.699 ****
0.654 ****
0.541 ****
0.626 ****
0.742 ****
0.864 ****
0.712 ****
0.72 ****
0.936 ****
0.00 0.25 0.50 0.75 1.00
0.0 0.3 0.6 0.9
beta
b)
HIV infection
IgG
IgG1
IgG2
IgG3
IgG4
C. diphtheriae
C. tetani
B. pertussis
Hib
S. pneumoniae
S. dysenteriae
V. cholerae
HBV
Measles
Rotavirus
RSV
C. parvum
G. intestinalis
P. falciparum
−0.014 ***
−0.014 ****
−0.016 ****
−0.013 ****
−0.016 ****
−0.018 ***
−0.011 ***
−0.009 ***
−0.014 ****
−0.01 *
−0.008 *
−0.023 ****
−0.019 ****
−0.013 ****
−0.032 ***
−0.02 ****
−0.017 **
−0.023 *
−0.019 **
−0.013 *
−0.029 ****
−0.016
0.014 *
−0.032 **
−0.05 *
−0.017
−0.011
−0.019
0.086
−0.008 *
−0.02 −0.01 0.00
−0.04 −0.02 0.00
−0.050 −0.025 0.000 0.025
−0.05 0.00 0.05 0.10
−0.05 0.00 0.05 0.10 0.15
beta
c)
Pf exposure
IgG
IgG1
IgG2
IgG3
IgG4
C. diphtheriae
C. tetani
B. pertussis
Hib
S. pneumoniae
S. dysenteriae
V. cholerae
HBV
Measles
Rotavirus
RSV
C. parvum
G. intestinalis
−0.034 **
−0.056 **
−0.041
−0.078*
−0.07 *
−0.084 *
−0.055 **
−0.081 *
0.00 0.05
0.0 0.1
0.0 0.1
0.00 0.25
0.0 0.2
beta

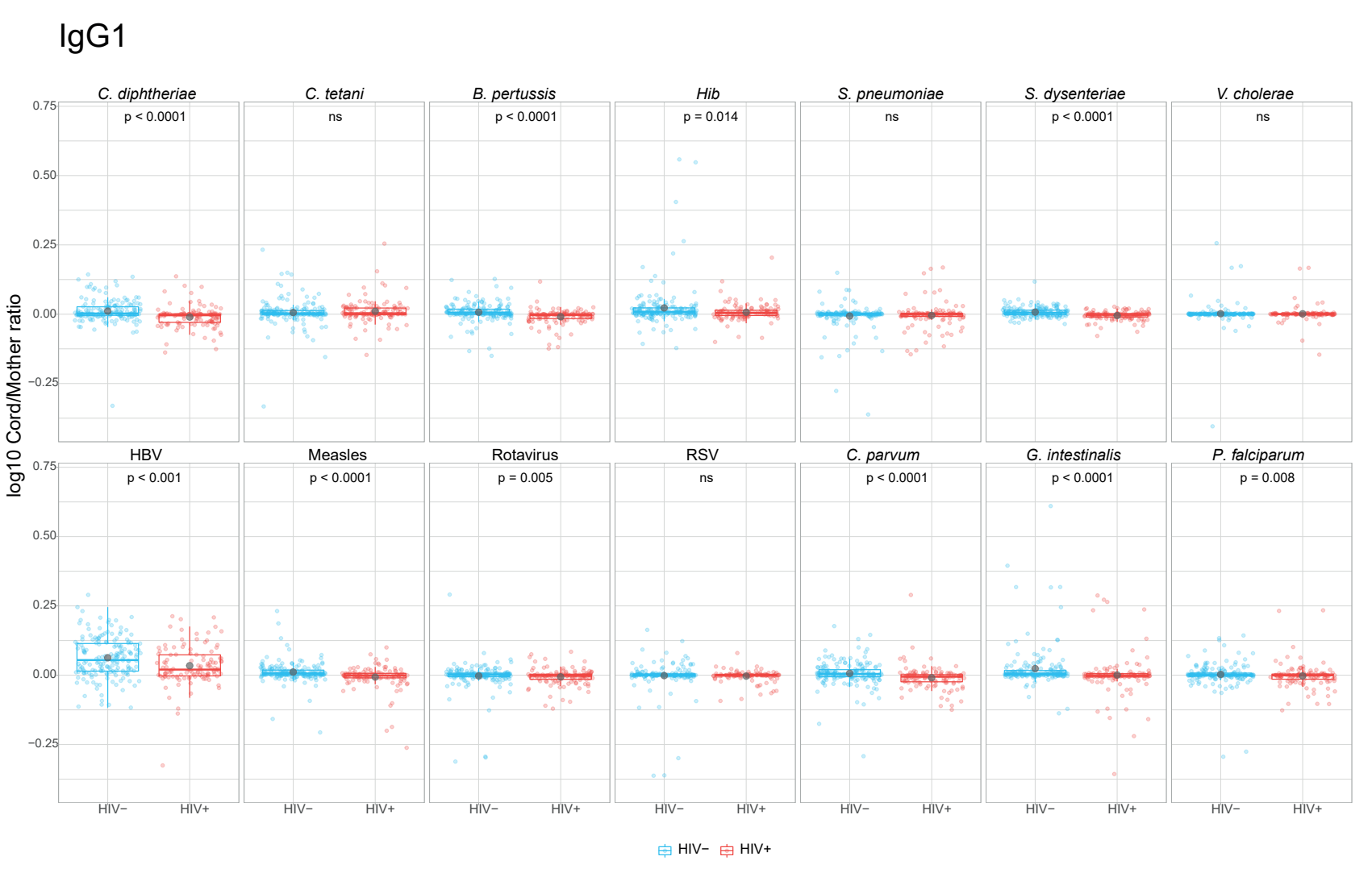

a)
Cord blood levels & Prematurity
IgG
IgG1
IgG2
IgG3
IgG4
C. diphtheriae
C. tetani
B. pertussis
Hib
S. pneumoniae
S. dysenteriae
V. cholerae
HBV
Measles
Rotavirus
RSV
C. parvum
G. intestinalis
P. falciparum
−0.019 ***
−0.01 **
−0.026 *
−0.02
−0.016
−0.037
−0.107
−0.025
0.000
0.025
−0.050
−0.04
0.00
0.04
−0.2
−0.1
0.0
−0.3
0.1
beta
b)
Placental transfer & Prematurity
IgG
IgG1
IgG2
IgG3
IgG4
−0.016
−0.02 **
−0.01 *
−0.022
0.04
0.029
0.022
−0.04
−0.02
0.00
0.02
0.05

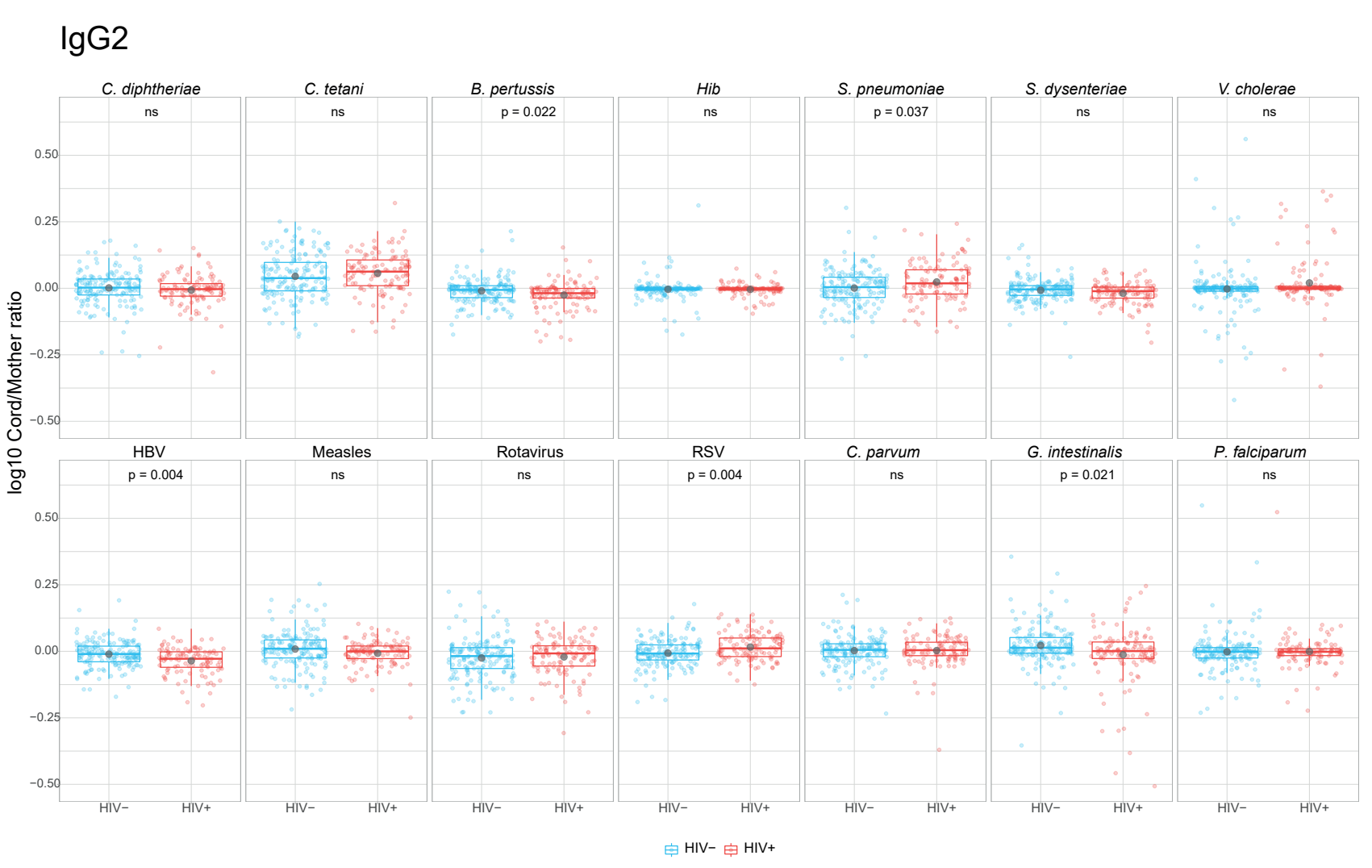

a)
HIV infection
IgG
IgG1
IgG2
IgG3
IgG4
C. diphtheriae
C. tetani
B. pertussis
Hib
S. pneumoniae
S. dysenteriae
V. cholerae
HBV
Measles
Rotavirus
RSV
C. parvum
G. intestinalis
P. falciparum
−0.014 ****
−0.013 ***
−0.013 ****
−0.018 ****
−0.018 **
−0.01 *
−0.009 ***
−0.014 **
−0.015 *
−0.022 ***
−0.018 ****
−0.017
−0.013 ****
−0.03 **
−0.018 **
−0.017 **
−0.023 *
−0.017 *
0.023
−0.013
−0.028 ***
−0.016
0.023 **
−0.036 *
−0.049
0.096
−0.018
0.087
−0.011
0.122
0.112
beta
b)
Pf exposure
IgG
IgG1
IgG2
IgG3
IgG4
C. diphtheriae
C. tetani
B. pertussis
Hib
S. pneumoniae
S. dysenteriae
V. cholerae
HBV
Measles
Rotavirus
RSV
C. parvum
G. intestinalis
−0.033 *
−0.056 **
−0.026
−0.05
−0.058
−0.045
−0.095 *
−0.061 **
−0.072
beta


MiPPAD Clinical trial
IPTp HIV-uninfected women
N=4749
IPTp HIV-infected women
N=1071
IPTp
SP
N=1578
MQ full dose
N=1580
MQ split dose
N=1591
Placebo + CTX
N=537
MQ + CTX
N=534
iMiPPAD IPTp
(with samples)
N=56
N=68
N=73
N=72
N=71
197 HIV-uninfected women
143 HIV-infected women
1 classified as
HIV-uninfected
incorrectly. Excluded
from the IPTp analysis
Total iMiPPAD
women in IPTp
197 HIV-uninfected women
144 HIV-infected women


IgG3
log10 MFI
5
4
3
2
Mother
Cord
C. diphtheriae
C. tetani
B. pertussis
Hib
S. pneumoniae
S. dysenteriae
V. cholerae
HBV
Measles
Rotavirus
RSV
C. parvum
G. intestinalis
P. falciparum
HIV-infected
HIV-uninfected

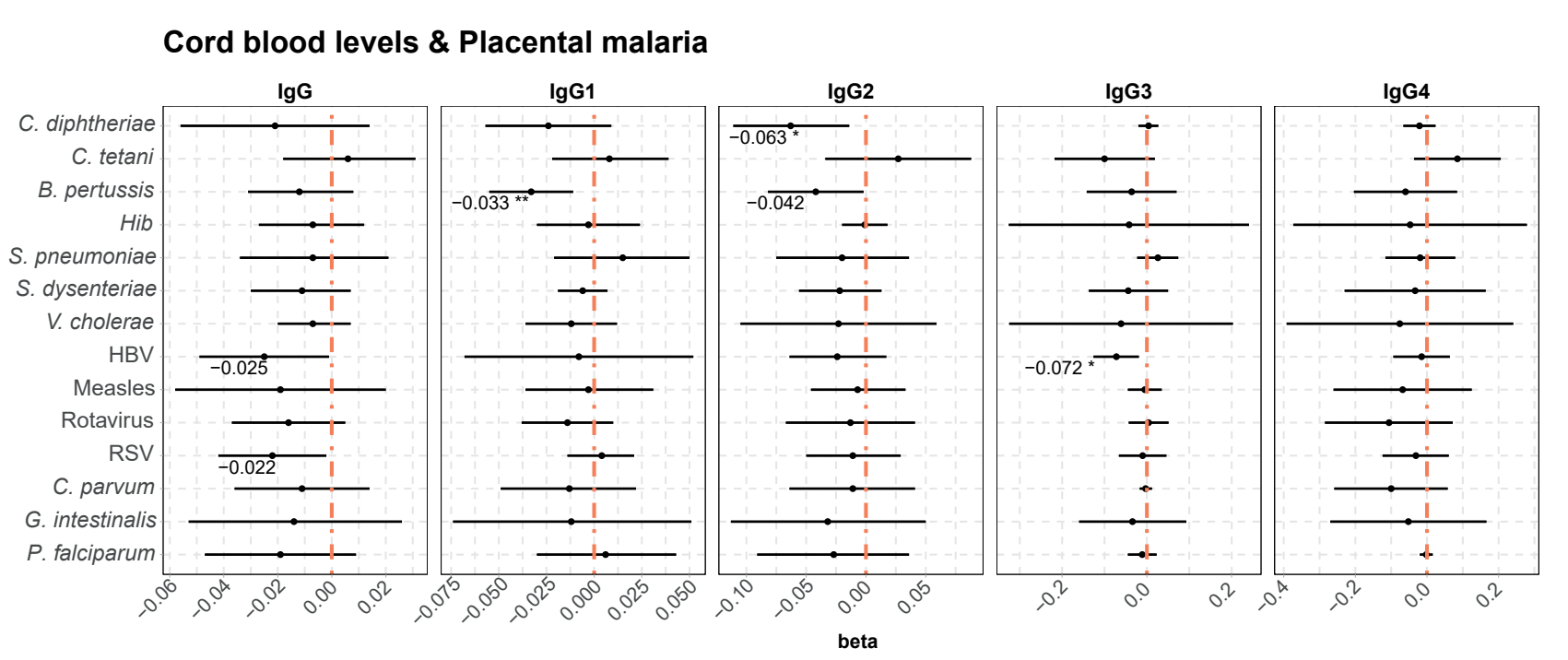

IgG
C. diphtheriae
p < 0.0001
C. tetani
p < 0.001
B. pertussis
p < 0.0001
Hib
ns
S. pneumoniae
p < 0.0001
S. dysenteriae
p < 0.0001
V. cholerae
ns
HBV
p < 0.0001
Measles
p < 0.0001
Rotavirus
p < 0.001
RSV
p < 0.0001
C. parvum
p < 0.0001
G. intestinalis
p < 0.0001
P. falciparum
p < 0.0001
log10 Cord/Mother ratio
0.4
0.2
0.0
−0.2
HIV−
HIV+
HIV− HIV+

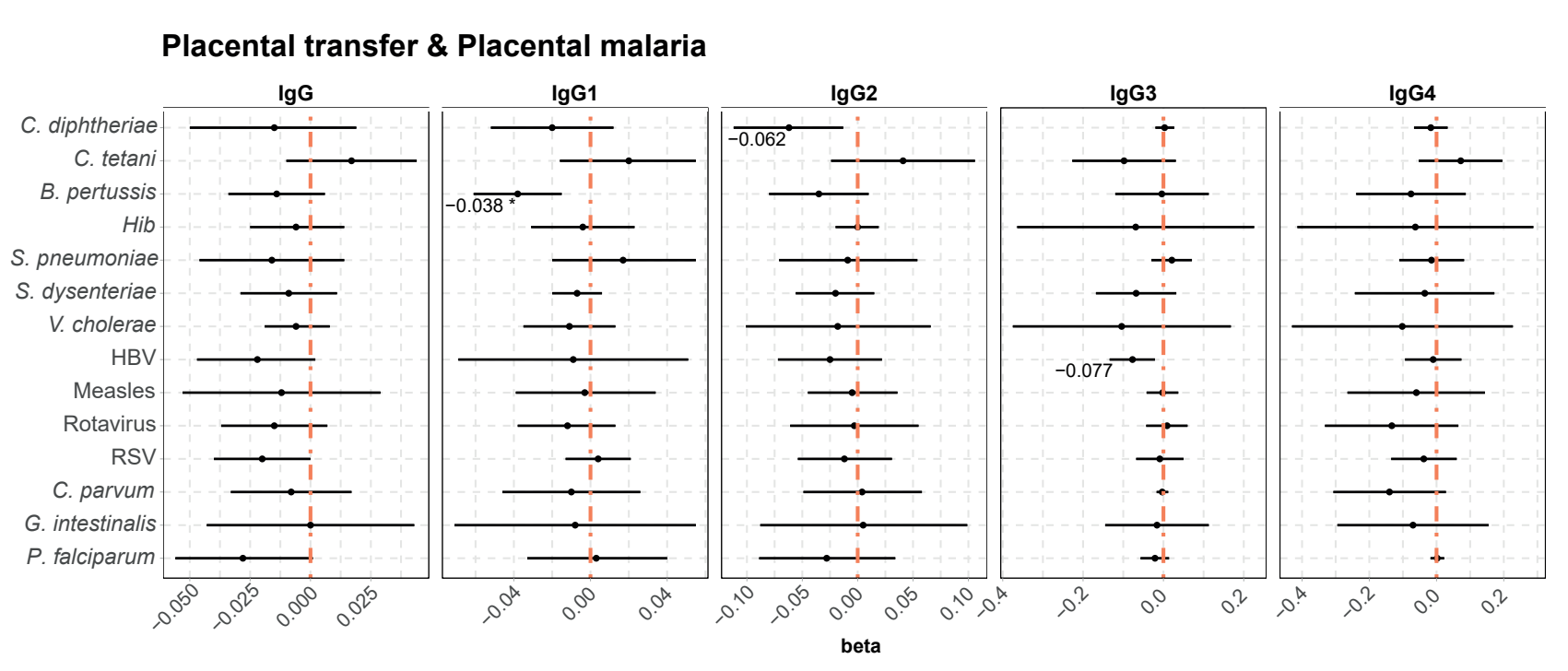

IgG1
C. diphtheriae
p < 0.0001
C. tetani
ns
B. pertussis
p < 0.0001
Hib
p = 0.014
S. pneumoniae
ns
S. dysenteriae
p < 0.0001
V. cholerae
ns
HBV
p < 0.001
Measles
p < 0.0001
Rotavirus
p = 0.005
RSV
ns
C. parvum
p < 0.0001
G. intestinalis
p < 0.0001
P. falciparum
p = 0.008
log10 Cord/Mother ratio
0.75
0.50
0.25
0.00
−0.25
HIV−
HIV+
HIV− HIV+


IgG2
C. diphtheriae
C. tetani
B. pertussis
Hib
S. pneumoniae
S. dysenteriae
V. cholerae
HBV
Measles
Rotavirus
RSV
C. parvum
G. intestinalis
P. falciparum
ns
p = 0.022
p = 0.037
p = 0.004
p = 0.021
log10 Cord/Mother ratio
0.50
0.25
0.00
−0.25
−0.50
HIV−
HIV+


IgG3
C. diphtheriae
C. tetani
B. pertussis
Hib
S. pneumoniae
S. dysenteriae
V. cholerae
HBV
Measles
Rotavirus
RSV
C. parvum
G. intestinalis
P. falciparum
ns
p < 0.001
log10 Cord/Mother ratio
1
0
−1
HIV−
HIV+


IgG4
C. diphtheriae
C. tetani
B. pertussis
Hib
S. pneumoniae
S. dysenteriae
V. cholerae
HBV
Measles
Rotavirus
RSV
C. parvum
G. intestinalis
P. falciparum
ns
log10 Cord/Mother ratio
1
0
−1
HIV−
HIV+


Cord blood levels & Placental malaria
IgG
IgG1
IgG2
IgG3
IgG4
C. diphtheriae
C. tetani
B. pertussis
Hib
S. pneumoniae
S. dysenteriae
V. cholerae
HBV
Measles
Rotavirus
RSV
C. parvum
G. intestinalis
P. falciparum
−0.025
−0.022
−0.033 **
−0.063 *
−0.042
−0.072 *
−0.06
−0.04
−0.02
0.00
0.02
−0.075
−0.050
−0.025
0.000
0.025
0.050
−0.10
−0.05
0.00
0.05
−0.2
0.0
0.2
−0.4
−0.2
0.0
0.2
beta


Placental transfer & Placental malaria
IgG
IgG1
IgG2
IgG3
IgG4
C. diphtheriae
C. tetani
B. pertussis
Hib
S. pneumoniae
S. dysenteriae
V. cholerae
HBV
Measles
Rotavirus
RSV
C. parvum
G. intestinalis
P. falciparum
−0.062
−0.038 *
−0.077
−0.050
−0.025
0.000
0.025
−0.04
0.00
0.04
−0.10
−0.05
0.00
0.05
0.10
−0.4
−0.2
0.0
0.2
beta