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Maternal psychological distress before birth influences gut immunity in mid-infancy

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Abstract

Background

Maternal pre-postnatal psychosocial distress increases the risk for childhood allergic disease. This may occur through a host immunity pathway that involves intestinal secretory Immuno-globulin A (sIgA). Experimental animal models show changes in the gut microbiome and immunity of offspring when exposed to direct or prenatal maternal stress, but little is known in humans.

Objective

We determined the association between maternal depression and stress symptom trajectories and infant fecal sIgA concentrations.

Methods

1043 term infants from the Canadian Healthy Infant Longitudinal Development (CHILD) birth cohort were studied. Trajectories of maternal perceived stress and depression were based on scored-scales administered in pregnancy and postpartum. sIgA was quantified in infant stool (mean age 3.7 months) with Immundiagnostik ELISA. Linear and logistic regression were employed to test associations.

Results

Very low fecal sIgA concentrations were more common in infants of mothers in the antepartum and persistent depression trajectories (6% and 2% of women respectively). Independent of breastfeeding status at fecal sampling, infant antibiotic exposure or other covariates, the antepartum depressive symptom trajectory was associated with reduced mean infant sIgA concentrations ($\beta = -0.07$, $p < 0.01$) and a 2-fold risk for lowest quartile concentrations (OR, 1.86; 95% CI: 1.02, 3.40). This lowering of sIgA yielded a large effect size in older infants (4-8 months) – breastfed and not. No associations were seen with postpartum depressive symptoms (7% of women) or with any of the perceived stress trajectories.

Conclusion and Clinical Relevance

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Despite improved mood postpartum and independent of breastfeeding status, mothers experiencing antepartum depressive symptoms delivered offspring who exhibited lower fecal sIgA concentrations especially in later infancy. The implications of lowered sIgA concentrations in infant stool are altered microbe-sIgA interactions, greater risk for *C. difficile* colonization and atopic disease in later years.

Introduction

Psychological distress in the pregnancy and postpartum time period adversely impacts maternal wellbeing and has lasting influences on infant immunobiology. There is strong meta-analytic evidence for associations between stress during pregnancy and respiratory morbidity or atopic disease in offspring ^{1,2}. Postnatal depression on its own has been documented to be a risk factor for wheeze in three-year-old girls ³, although its association with childhood asthma wanes with increasing age ⁴.

Alongside in-utero mechanisms, the early colonization of the gut microbiome and development of gut immunity may represent a critical pathway between stressors for the infant and childhood allergic disease ⁵. Provided through breastmilk to protect newborns against pathogens before they can produce their own ^{6,7}, secretory Immunoglobulin A (sIgA) plays a vital role in gut mucosal immunity ⁸.

Plasma B cells that produce secretory IgA are absent in germ-free mice but re-appear once gut microbes are introduced ⁹. In humans, fecal sIgA levels are reduced or their pattern of binding to gut microbiota is altered in infants who develop allergic diseases ^{10,11}. Low fecal sIgA levels have been observed among infants of mothers with high-level prenatal depressive symptoms that persist into the postpartum period ¹².

Critical windows for the influence of stress and thus, timing of maternal psychological stress on the development of the infant gut microbiome and immunity remain uncertain. When young mice are directly exposed to restraint stress, intestinal levels of IgA are reduced ¹³. Changes in the gut microbial composition of human infants, such as fewer lactic acid bacteria (e.g. lactobacilli) and bifidobacteria have been observed at 1 month of age after exposure to maternal distress in the third trimester ¹⁴. Lactic acid bacteria and bifidobacteria are also reduced in the gut of adults with depression ¹⁵. More recently, Slykerman et al. ¹⁶ demonstrated the efficacy of the oral administration of a microbe *Lactobacillus rhamnosus* to women in the third trimester of pregnancy in lowering their symptoms of depression and anxiety in the postnatal period. These findings point to a possible modifiable role of the maternal microbiome during pregnancy. They also raise the question of whether maternal depressive symptoms in the last trimester before birth are an indicator of dysbiosis in mother's gut microbiome and have the capacity to influence infant gut immunity months later.

In our initial smaller-scale study of 400 infants, fecal sIgA levels were significantly lower in infants when their mothers had depressive symptom scores that exceeded a validated cut-off value for depression in the third trimester and at least 1 of 2 time points postpartum ¹². Yet, maternal depression in the perinatal period follows several trajectories of changing symptoms ¹⁷. Even though symptoms diminish postpartum in some women with depression in the third trimester, we found that postpartum symptom scores of women in this trajectory were 10 units higher than in women with low symptoms throughout the same period ¹⁸. The impact of maternal perinatal distress trajectories on infant gut immunity has not been previously evaluated. To fill this gap in knowledge, we investigated associations between maternal depressive symptoms and perceived stress trajectories (Persistent, Antepartum, Postpartum, Never) reported by Chow et al. ¹⁸ and infant fecal sIgA concentrations.

Materials and Methods

Study population

A subsample of 1043 term infants from the Vancouver, Edmonton and Winnipeg sites of the Canadian Healthy Infant Longitudinal Development (CHILD) birth cohort study were included in this quantitative study based on fecal sample availability. The birth cohort had 3455 eligible families that were being followed (See www.childstudy.ca). Pregnant mothers were enrolled into CHILD between 2008 and 2012 (mean gestational age = 27 weeks) and provided written informed consent before data collection proceeded. This study conforms to the standards of the Declaration of Helsinki and was approved by the University of Alberta (Pro00002099), University of British Columbia (H07-03120), University of Manitoba (HS10696/H2007:255), and Hamilton Integrated Research Ethics Boards (Project 07-2929). Subbarao et al.¹⁹ described the inclusion and exclusion criteria and methodology for the cohort study. Pregnant women aged greater than 18 years, who could read, write and speak English and were planning to deliver at a recruitment participating hospital were included. Children born with major congenital abnormalities, of multiple births or resulting from *in vitro* fertilization were excluded from the study.

Distress measures

Mothers reported perceived distress (stress or depressive symptoms) through scored-scales administered at several time points throughout pregnancy (recruitment and 36 weeks gestation) and postpartum (6, 12, 18, 24 months of infant age).

Center for Epidemiologic Studies Depression (CES-D)

Maternal pre and postnatal depressive symptoms were ascertained using the 20-item CES-D scale²⁰. Mothers reported how frequently they experienced depressive behaviours, cognitions and affect in the last week using scores ranging from zero (none of the time) to three (most or all the time). A total score of 16 or greater was considered to indicate clinically significant levels of depressive symptoms; however, this cut-off value does not imply that the mother had a clinical diagnosis of depression.

Perceived Stress Scale (PSS)

Maternal perceived stress was assessed using the 10-item PSS ²¹. The questionnaire measured how frequently mothers found their lives as stressful, unmanageable and overwhelming in the past month. Items were scored from zero (never) to four (very often) and summed, with higher scores representing greater stress levels. In the absence of a validated cut-off score for clinically significant levels of stress, the mean stress score (of 12.96) for all mothers in the CHILD study was used as a cut-off value for higher versus lower stress.

Distress Trajectories

Five mutually exclusive distress trajectories were previously identified in the CHILD cohort (Figure 1 ¹⁸), and named Persistent, Antepartum, Postpartum, Never [Moderately Low] and Never [Low]. For the current analysis, Never [Moderately Low] and Never [Low] were combined since these scores were below the cut-off value for clinical significance. In essence, the Antepartum trajectory consisted of mothers with high levels of distress primarily during the pregnancy, while women in the Postpartum trajectory reported high symptom levels predominantly after delivery. Women in the Persistent trajectory had higher reported symptoms of distress throughout pregnancy and the postpartum period.

Fecal sIgA

Infant stool samples were collected at a mean age of 3.7 months (range 2 to 8 months) and frozen as 4 aliquots. Stool aliquots did not differ statistically in mean quantity by maternal distress trajectory: Antepartum (800.9 mg), Postpartum (807.5 mg), Persistent (688.2 mg) and Never (731.8 mg). Fecal sIgA was quantified in duplicates as mg per gram of wet weight feces using the Secretory IgA ELISA (enzyme-linked immunosorbent assay) kit from Immundiagnostik, as per Urwin et al ²². The minimum detection limit of this assay was 13.4 ng/mL sIgA. Approximately 14 mg of a thawed aliquot was diluted 1:12500 in wash buffer. The diluted samples, controls, and 100µl standards were put into a microtiter plate and incubated at room temperature. The wells were aspirated and washed, and the microtiter plate was tapped on absorbent paper. 100µl of conjugate was added, and then the samples were incubated at room temperature and shaken on a horizontal mixer. After the final

washing step and adding TMB substrate, the samples were incubated in the dark for 10 minutes.

Absorption was determined with an ELISA reader at 450nm against 620nm as a reference. Standards with known concentrations allowed us to create standard curves.

Covariates

Data on covariates were obtained from CHILD study questionnaires administered during pregnancy and 3-month postpartum home visits. Women self-reported breastfeeding status at time of stool collection (exclusive, partial or no breastfeeding), date that breastfeeding was discontinued, treatment for allergy/asthma during pregnancy, use of selective serotonin reuptake inhibitors (SSRIs), personal or household smoking, and pet ownership before the 3-month home visit. Hospital birth records were the source of information on gravida status, birth mode (emergency caesarean, scheduled caesarean and vaginal delivery), and intrapartum or newborn intravenous antibiotic treatment. The latter, along with maternal report of infant oral antibiotic prescriptions, were used to construct the variable of infant antibiotic exposure between birth and 3 months of age. Prenatal tobacco smoke exposure was based on responses of mothers or other household members smoking during pregnancy; postnatal smoke exposure denoted smoking by any household member. The prevalence of study covariates was similar to that in the full CHILD cohort (Table S1). Since most covariates represented maternal behaviours downstream to socioeconomic status (SES) and breastfeeding status was highly correlated with SES in our study ($p < 0.0001$), maternal SES was not tested as separate covariate in regression models.

Statistical Analyses

Maternal distress trajectories were created using Mplus, as previously documented¹⁸. Multiple linear and logistic regression models were used to test for associations in SPSS version 24. Due to the non-normality of the sIgA data, we transformed sIgA concentrations in linear models to values exponentiated to a power of 0.1736225 using the R package “Powertransform”²³. For logistic regression, a binary variable (yes/no) indicating whether the sIgA concentrations were in the lowest quartile of the sample was used as the outcome variable. The lowest tertile was also tested in models but were not presented. Covariates were selected based on statistical significance in the model and

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based on the 10% rule where a greater than 10% change in the odds ratios with the addition of a covariate would keep the covariate in the final model. Infant age at sampling time was controlled in all regression models.

Results

Study characteristics

Table 1 shows maternal and infant characteristics of the study sample. Mean sIgA concentrations in infants were 3.79 (standard deviation (SD) 3.65), 8.06 (SD 8.1), 12.32 (SD 10.71) mg/g feces with no, partial and exclusive breastfeeding at the time of stool collection, respectively (Table S2); they declined by infant age but were consistently higher in breastfed infants, such that exclusively breastfed infants at 4-8 months of age had mean levels of 8.9 (SD 5.84) mg/g feces. Among infants no longer breastfed at age 4-8 months, breastfeeding had stopped, on average, 3.3 months ago.

Breastfeeding duration was weakly correlated with infant fecal sIgA concentrations ($r=0.2$, $p<0.0001$) in all infants; the correlation was marginally significant in older infants ($r=0.2$, $p<0.06$). Similar to the distribution of the maternal distress trajectory in the full CHILDS cohort¹⁸, 6.0% of women had antepartum, 7.4% had postpartum and 2.2% had persistent depressive symptoms (Table 1). In relation to perceived stress, 4.5% of mothers experienced antepartum, 22.1% postpartum and 6.9% persistent stress. Forty percent of mothers exclusively breastfed, 34.2% partially breastfed and 26.1% did not breastfeed their infants around the time of stool collection. Pre and postnatal SSRI antidepressant use in mothers was low in our sample ($n<40$ women). Regarding study covariates (Table 2), lack of exclusive breastfeeding, exposure to tobacco smoke and maternal history of asthma or allergy were more common among infants of mothers in the Antepartum, Postpartum and Persistent trajectories for both depressive symptoms and perceived stress. A greater percentage of infants not exclusively breastfed, of infants whose stool was collected later at 4 - 8 months of age and of infants exposed to tobacco smoke or pets had concentrations of fecal sIgA in the lowest quartile.

Maternal distress trajectories and lowest quartile infant sIgA concentrations

As shown in Table 3 and Figure 2, logistic regression analyses were conducted for the binary outcome of fecal sIgA concentrations in the lowest quartile. Crude odds ratios (OR) for lowest quartile sIgA concentrations were statistically significant for infants of mothers in both the Antepartum and Persistent depressive symptom trajectories versus the reference category of low symptom scores. When mothers experienced antepartum depressive symptoms, their infants were 2.3 times more likely to have sIgA concentrations in the lowest quartile (95% CI: 1.36, 3.89) compared to infants of

mothers in the reference group. This 2-fold greater odds of low sIgA concentrations for maternal antepartum depressive symptoms remained after individual adjustment for most covariates and the collective adjustment for breastfeeding status, infant age and antibiotic exposure (95% CI: 1.02, 3.40).

Among infants of mothers in the Persistent depressive symptom trajectory (Table 3, Figure 2), the likelihood of lowest quartile sIgA concentrations was of a greater magnitude than for the Antepartum trajectory, with an OR of 3.57 (95% CI: 1.55, 8.21). This OR for lowest quartile sIgA in infants exposed to persistent maternal depressive symptoms remained statistically significant following individual adjustment with all covariates, including breastfeeding status, but not in a final model that included breastfeeding status, infant age and antibiotic exposure.

Maternal distress trajectories and mean infant sIgA concentrations

In a regression model predicting exponential-transformed infant sIgA concentrations (Table 4), unadjusted beta coefficients were statistically significant *only* for the Antepartum depressive symptom versus the reference trajectory. The negative beta coefficient indicated an inverse association, such that infants of mothers with antepartum depressive symptoms had lower fecal sIgA concentrations compared to infants of mothers with few symptoms in the perinatal period. Statistical significance of this difference was retained following adjustment for individual and combined covariates, including breastfeeding status. The final model which controlled for infant sex and age, antibiotic exposure and breastfeeding status had a lower but statistically significant beta coefficient ($\beta=-0.07$; $p<0.01$). Crude associations were seen between the Antepartum stress and the Persistent stress trajectories, and sIgA concentrations. They were of a lower magnitude and did not survive multiple covariate adjustment (Table 4).

Table 5 reports results for age-stratified regression models, based on age groupings were created according to a test of equal density of sIgA concentrations (see Figure S1). Independent of breastfeeding status, the beta coefficient for the association with maternal antepartum depressive symptoms (ie. -0.12; 95% CI: -0.23, -0.02) was statistically significant for infant sIgA concentrations at 4 - 8 months after birth. No differences were observed for other infant age groups or maternal

depression trajectories, or maternal stress trajectories following adjustment for breastfeeding. In a sensitivity analysis that adjusted models for duration of breastfeeding instead of breastfeeding status at time of stool collection, similar sIgA associations were found with antepartum depressive symptoms in infants at 4-8 months of age: -0.13 ; 95% CI: -0.24 , -0.01 . Longer duration of breastfeeding was independently but weakly associated with higher fecal sIgA concentrations in older infants (beta-coefficient, 0.03). Figure 3 displays sIgA concentrations by infant age and maternal depressive symptom trajectory, adjusted for breastfeeding status (as per equations listed in the legend). Absence of breastfeeding had the largest independent association with fecal sIgA in infants < 3 months. No statistical interactions were found between maternal distress and breastfeeding status for any age group ($p > 0.05$).

As per Table 5, Figure 3 shows the interaction with infant age, whereby maternal antepartum depressive symptoms had the largest and statistically significant association with reduced infant sIgA levels within the oldest age group. Translating these results back to the original mg/g units of sIgA measurement, the following fecal sIgA concentrations are expected for infants aged 4 - 8 months of mothers in the Antepartum trajectory versus the low symptom trajectory for each infant feeding group: i) 4.57 versus 7.69 or a reduction of 3.12 mg/g feces if exclusively breastfed, ii) 3.09 versus 5.39 or a reduction of 2.3 mg/g feces with partial breastfeeding, and iii) 1.57 versus 2.92 or a reduction of 1.35 mg/g feces if exclusively fed formula. Based on group-specific standard deviations (Table S1), these absolute reductions in sIgA are equivalent to an effect size of 0.53 , 0.25 and 0.36 SD with exclusive, partial and no breastfeeding, respectively.

Discussion

In this study of 1043 full-term infants from a general population cohort, maternal depressive symptoms in the pre-postnatal period were associated with reduced fecal sIgA concentrations in infants. Extending findings from our first study of 400 infants ¹², we observed lowest quartile fecal sIgA levels in infants of mothers with persistently high depressive symptom scores throughout the perinatal period. Novel in the present study are findings for the antepartum trajectory – mothers who had elevated third trimester depressive symptoms that declined postnatally but remained higher than in women with few symptoms during the same time period. Their infants had significantly lower mean fecal sIgA concentrations and were twice more likely to have sIgA concentrations in the lowest quartile (OR, 1.86; 95% CI: 1.02, 3.40), independent of factors which are known to modify gut microbiota, breastfeeding status at the time of stool collection and infant exposure to antibiotics. Reductions in sIgA levels were more evident at 4 to 8 months of age, such that infants born to women in the Antepartum depressive symptom trajectory had sIgA concentrations lowered by 3.12 mg/g feces for a large effect size of 0.53 SD even if they were exclusively breastfed. While our findings on infant intestinal sIgA concentrations are consistent with the experimental study of direct stress exposure on mice ¹³, this is the first human study to show an independent association between presence of maternal depressive symptoms in the third trimester and lowered gut immunity in offspring.

Unable to produce sIgA in significant amounts during the first weeks of life, newborns rely on secretory IgA and other immune factors provided by breast milk for passive immunity against pathogens ^{9, 24, 25}. Breastmilk is a well-known source of infant fecal IgA ^{25, 26} and contains human milk oligosaccharides that encourage growth of *Bifidobacterium* species in the infant gut ^{9, 27}, microbiota which stimulate infant IgA production ^{28, 29}. Mothers with postpartum depressive symptoms are less likely to exclusively breastfeed or partially breastfeed for a prolonged duration ³⁰. Early exposure to antibiotics via maternal intrapartum prophylaxis can change the abundance of infant gut microbiota that stimulate IgA levels ³¹⁻³³. While breastfeeding status and infant antibiotic exposure lowered the magnitude of the association between infant sIgA concentrations and maternal membership in the antepartum depressive symptom trajectory in our study, *they did not nullify it*. As expected in our age-

specific models, absence of breastfeeding had a larger influence on fecal sIgA levels at infant age < 3 months than at older ages. Even if it was not current in older infants, longer duration of breastfeeding since birth promoted slightly higher fecal sIgA levels. We found no statistical interaction between the maternal distress trajectory variable and breastfeeding status in the association with fecal sIgA in study infants. However, the breastfed infant may not be impervious to maternal mood, as lower sIgA levels have been reported in the breast milk of mothers who experience anxiety³⁴. This observation may partially explain our findings in older breastfed infants.

It is noteworthy that a statistically significant difference in fecal sIgA concentrations was only seen at 4 - 8 months of infant age with the antepartum depressive symptom trajectory, both in breastfed and non-breastfed infants, and independent of breastfeeding duration. This age-relevant finding points to prenatal pathways that influence future sIgA production since at this later age, an infant's own intestinal production represents a more significant contribution to sIgA levels than at an earlier age^{6, 7}. Candidate pathways are those related to maternal stress hormones. Glucocorticoids, which are transferred to a greater extent to the fetus when women experience psychological distress during pregnancy³⁵, have the capacity to lower intestinal IgA concentrations in young mice¹³. Specifically, animal models of repeated restraint stress have shown that corticosteroids lower the transcytosis of secretory IgA in enterocytes, thereby reducing sIgA secretion into the gut lumen³⁶. Stress can also stimulate the enteric nervous system to release catecholamines and neuropeptides, such as neuropeptide Y and somatostatin that modulate the production of intestinal sIgA³⁶. In humans, higher maternal scores for depressive symptom but not for perceived stress scales have shown an association with elevated evening cortisol levels during pregnancy³⁷; this apparent lack of a stress effect, as measured by the perceived stress scale, on maternal prenatal cortisol levels may explain the null association between maternal stress trajectories and offspring fecal sIgA concentrations in our study.

Maternal vaginal and intestinal microbiota, the primary sources of microbes for newborns, are altered in stressed mothers during pregnancy^{38, 39}. Hence, maternal distress before birth has the capacity for continual influence on the infant via their gut microbes. Zijlmans et al.¹⁴ reported that when stress/anxiety and salivary cortisol levels were elevated among women in the third trimester, the gut microbiota of their infants was persistently depleted of lactic acid bacteria and bifidobacteria from 4

until 16 weeks after birth. Slykerman et al. ¹⁶ demonstrated the efficacy of prenatal administration of the probiotic *L. rhamnosus* in reducing postpartum stress and depression, which provides indirect evidence for an influence of the pregnancy microbiome on symptoms of distress in the postpartum period. Also as reported by Kukkonen et al. ¹⁰, reinforcing mother-infant transmission of microbiota with post birth administration of a maternal probiotic to the infant contributes to elevated fecal IgA levels in offspring after birth. In our study, maternal depressive symptoms which are maintained at subclinical levels in the postpartum period may impose continual stress on infant production of intestinal IgA or lower breast milk sIgA in an environment which is already depleted of stimulatory microbiota, such as the lactic acid bacteria. Both maternal and infant sources of sIgA bind to gut microbiota, which is driven by specific microbial species and rises with infant age ⁴⁰. Microbiota-sIgA binding is essential to prevent overgrowth by one species and altered binding has been associated with asthma development ^{11, 41}.

The strengths of this study were the large subsample of 1043 infants taken from a nationally-representative prospective birth cohort. Multiple variable regression analyses strengthened the validity of reported associations because of our ability to control for several covariates, including infant age. However, we could not fully adjust for many covariates in our age-specific analyses due to an inadequate sample size. In addition, our Persistent depressive symptom trajectory consisted of only 23 mother-infant pairs, which likely explained the marginal statistical significance following collective adjustment with several covariates, despite the fact that lowest quartile sIgA concentrations were 2.6 times more likely among these infants. Finally, sIgA concentrations per wet weight of feces are subject to volume changes in the intestine. However, our study sIgA concentrations by feeding method were in a similar range as those reported by others using an alternate kit, where concentrations were also per wet weight of feces ^{42, 43}. Importantly, the quantity of stool we collected (more liquid stool yielded smaller amounts) did not differ in infants of mothers with and without antenatal depressive symptoms.

In conclusion, we found an inverse association between mothers that experience perinatal depressive symptoms and infant fecal sIgA concentrations. While reductions in sIgA levels with increasing depressive symptoms were partially explained by breastfeeding status, differences between

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membership in the antepartum depressive and low symptom trajectories were independent of breastfeeding and other covariates. Reported associations appear to be age-specific, as sIgA levels in the later months were more affected than the earlier months. The health implications of lowered sIgA concentrations in infant stool are compromised immune maturation and resultant altered microbe-sIgA interactions ¹¹, short-term risk for *Clostridioides difficile* colonization ⁴⁴, and allergic and gastrointestinal symptoms ¹⁴, and allergic conditions in later years ¹⁰. By optimizing sIgA immune development in the infant, we may be able to prevent some of these risks.

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Conflicts of Interest: None to declare

Data Availability: Data available on request from the authors.

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Table 1. Maternal and infant characteristics in subsample (N=1043)

Characteristics	N (%)
Depressive symptom trajectories	
Never	880 (84.4)
Antepartum	63 (6.0)
Postpartum	77 (7.4)
Persistent	23 (2.2)
Perceived stress trajectories	
Never	694 (66.5)
Antepartum	47 (4.5)
Postpartum	230 (22.1)
Persistent	72 (6.9)
Gravida	
Primigravida	378 (37.0)
Multigravida	643 (63.0)
Maternal asthma or allergy during pregnancy	
No	386 (37.8)
Yes	636 (62.2)
Birth mode	
Vaginal	772 (75.5)
Scheduled Caesarean	100 (9.8)
Emergency Caesarean	151 (14.8)
Breastfeeding status at stool collection	
Exclusive Breastfeeding	409 (39.7)
Partial	352 (34.2)
No current breastfeeding	269 (26.1)
Antibiotics exposure to infant up to 3 months	
No	487 (48.6)
Yes	515 (51.4)
Prenatal Selective Serotonin Reuptake Inhibitors	
No	1009 (96.7)
Yes	34 (3.3)
Postnatal Selective Serotonin Reuptake Inhibitors	

No	997 (95.6)
Yes	46 (4.4)
Prenatal smoke exposure	
No	933 (91.4)
Yes	88 (8.6)
Postnatal smoke exposure	
No	847 (82.6)
Yes	179 (17.4)
Pets at home	
No	551 (53.9)
Yes	471 (46.1)
Infant sex	
Male	545 (53.4)
Female	476 (46.6)
Infant age at stool collection (months)	
Mean (SD)	3.7 (1.1)
Infant fecal sIgA (mg/g feces)	
Median (IQR)	6.1 (2.9 – 11.0)

Table 2. Distribution of potential covariates for distress trajectories and lowest quartile concentrations of sIgA

Covariates N (%)	Depressive symptoms				Perceived stress				sIgA concentrations	
	Antepartum	Postpartum	Persistent	p-value	Antepartum	Postpartum	Persistent	p-value	Lowest quartile	p-value
Infant sex										
Male	23 (4.2)	41 (7.5)	10 (1.8)	0.06	23 (4.2)	127 (23.3)	32 (5.9)	0.32	127 (23.3)	0.14
Female	38 (8.0)	35 (7.4)	13 (2.7)		24 (5.0)	96 (20.2)	39 (8.2)		130 (27.3)	
Infant age										
<3 months	13 (4.1)	24 (7.6)	10 (3.2)	0.54	13 (4.1)	73 (23.1)	28 (8.9)	0.66	70 (22.2)	0.01
≥3 and <4 months	32 (7.0)	35 (7.7)	8 (1.8)		22 (4.8)	102 (22.3)	29 (6.3)		104 (22.8)	
≥4 and ≤8 months	18 (6.7)	18 (6.7)	5 (1.9)		12 (4.4)	55 (20.4)	15 (5.6)		87 (32.2)	
Gravida										
Primigravida	23 (6.1)	18 (4.8)	9 (2.4)	0.10	13 (3.4)	83 (22.0)	18 (4.8)	0.08	98 (25.9)	0.67
Multigravida	38 (5.7)	58 (9.0)	14 (2.2)		34 (5.3)	140 (21.8)	53 (8.2)		159 (24.7)	
Maternal asthma/ allergy										
No	20 (5.2)	19 (4.9)	6 (1.6)	0.05	14 (3.6)	68 (17.6)	23 (6.0)	0.01	91 (23.6)	0.57
Yes	42 (6.6)	56 (8.8)	16 (2.5)		33 (5.2)	157 (24.7)	46 (7.2)		160 (25.2)	
Birth mode										
Vaginal	44 (5.7)	50 (6.5)	18 (2.3)	0.50 ^a	33 (4.3)	162 (21.0)	57 (7.4)	0.66	194 (25.1)	0.75
Scheduled caesarean	7 (7.0)	10 (10.0)	2 (2.0)		7 (7.0)	26 (26.0)	5 (5.0)		23 (23.0)	
Emergency caesarean	10 (6.6)	16 (10.6)	3 (2.0)		7 (4.6)	36 (23.8)	9 (6.0)		41 (27.2)	
Breastfeeding status^b										
Exclusive	15 (3.7)	28 (6.8)	3 (0.7)	<0.01^a	13 (3.2)	89 (21.8)	14 (3.4)	<0.01	39 (9.5)	<0.001
Partial	24 (6.8)	20 (5.7)	8 (2.3)		20 (5.7)	70 (19.9)	26 (7.4)		82 (23.3)	
None	21 (7.8)	29 (10.8)	11 (4.1)		13 (4.8)	69 (25.7)	29 (10.8)		135 (50.2)	
Antibiotics exposure										
No	22 (4.5)	34 (7.0)	11 (2.3)	0.23	26 (5.3)	96 (19.7)	31 (6.4)	0.23	129 (26.5)	0.46
Yes	38 (7.4)	42 (8.2)	11 (2.1)		20 (3.9)	124 (24.1)	38 (7.4)		126 (24.5)	
Prenatal smoke										
No	45 (4.8)	62 (6.6)	17 (1.8)	<0.001	37 (4.0)	202 (21.7)	50 (5.4)	<0.001	221 (23.7)	0.03
Yes	17 (19.3)	13 (14.8)	5 (5.7)		10 (11.4)	22 (25.0)	19 (21.6)		30 (34.1)	
Postnatal smoke										
No	40 (4.7)	54 (6.4)	12 (1.4)	<0.001	31 (3.7)	179 (21.1)	41 (4.8)	<0.001	197 (23.3)	0.01
Yes	23 (12.8)	19 (10.6)	9 (5.0)		15 (8.4)	47 (26.3)	26 (14.5)		58 (32.4)	
Pets at home										
No	32 (5.8)	35 (6.4)	11 (2.0)	0.68	26 (4.7)	132 (24.0)	26 (4.7)	0.04	121 (22.0)	0.02
Yes	31 (6.6)	38 (8.1)	10 (2.1)		20 (4.2)	93 (19.7)	41 (8.7)		134 (28.5)	

^aFisher's Exact Test ^bat time of stool collection

Table 3. Logistic regression for associations between distress trajectories and lowest quartile concentrations of fecal sIgA

Lowest quartile sIgA levels OR (95% CI) (Never as reference)	Depressive symptoms			Perceived stress		
	Antepartum	Postpartum	Persistent	Antepartum	Postpartum	Persistent
Crude for depressive symptoms	2.30 (1.36 – 3.89)	0.93 (0.53 – 1.62)	3.57 (1.55 – 8.21)	1.83 (0.99 – 3.41)	1.07 (0.75 – 1.51)	1.52 (0.90 – 2.57)
Adjusted for infant age at stool collection	2.28 (1.35 – 3.88)	0.94 (0.54 – 1.65)	3.71 (1.61 – 8.59)	1.86 (1.00 – 3.47)	1.08 (0.77 – 1.54)	1.58 (0.93 – 2.68)
Adjusted for maternal asthma or allergy	2.25 (1.32 – 3.82)	0.90 (0.50 – 1.60)	3.31 (1.41 – 7.76)	1.85 (0.99 – 3.44)	1.03 (0.72 – 1.47)	1.43 (0.83 – 2.46)
Adjusted for prenatal smoke exposure	2.08 (1.21 – 3.59)	0.86 (0.48 – 1.54)	3.14 (1.34 – 7.40)	1.72 (0.92 – 3.23)	0.99 (0.69 – 1.41)	1.29 (0.74 – 2.25)
Adjusted for postnatal smoke exposure	2.15 (1.26 – 3.66)	0.96 (0.54 – 1.70)	2.73 (1.13 – 6.56)	1.76 (0.94 – 3.30)	1.01 (0.71 – 1.44)	1.33 (0.76 – 2.32)
Adjusted for breastfeeding status at stool collection	1.67* (0.93 – 3.00)	0.70 (0.38 – 1.28)	2.63 (1.05 – 6.60)	1.51 (0.77 – 3.00)	0.90 (0.62 – 1.32)	0.92 (0.51 – 1.65)
Adjusted for antibiotics exposure	2.49 (1.46 – 4.26)	0.93 (0.53 – 1.63)	3.20 (1.37 – 7.50)	1.85 (0.99 – 3.45)	1.09 (0.77 – 1.55)	1.49 (0.87 – 2.55)
Adjusted for pets	2.29 (1.35 – 3.88)	0.97 (0.55 – 1.72)	2.98 (1.24 – 7.14)	1.92 (1.02 – 3.58)	1.06 (0.75 – 1.51)	1.40 (0.81 – 2.43)
Adjusted for age, breastfeeding, antibiotics	1.86 (1.02 – 3.40)	0.73 (0.40 – 1.34)	2.57 (0.99 – 6.65)	1.59 (0.80 – 3.16)	0.95 (0.64 – 1.39)	0.96 (0.53 – 1.75)

*2.47 (1.35-4.52) when adjusted for breastfeeding duration instead

Table 4. Multiple linear regression for associations of distress trajectories and transformed sIgA

Transformed sIgA Beta Coefficients (95% CI) (Never as Reference)	Depressive symptoms			Perceived stress		
	Antepartum	Postpartum	Persistent	Antepartum	Postpartum	Persistent
Crude	-0.12 (-0.19, -0.06)	-0.01 (-0.07 – 0.04)	-0.05 (-0.19 – 0.01)	-0.08 (-0.15, -0.01)	-0.01 (-0.05 – 0.02)	-0.06 (-0.12 – 0.00)
Adjusted for infant age at stool collection	-0.12 (-0.18, -0.06)	-0.02 (-0.07 – 0.04)	-0.06 (-0.19 – 0.00)	-0.08 (-0.15, -0.01)	-0.01 (-0.05 – 0.02)	-0.07 (-0.13, -0.01)
Adjusted for infant sex	-0.12 (-0.19, -0.06)	-0.01 (-0.07 – 0.04)	-0.05 (-0.18 – 0.02)	-0.08 (-0.15, -0.01)	-0.01 (-0.05 – 0.02)	-0.06 (-0.12 – 0.00)
Adjusted for maternal asthma or allergy	-0.12 (-0.18, -0.06)	-0.02 (-0.07 – 0.04)	-0.05 (-0.18 – 0.03)	-0.08 (-0.15, -0.01)	-0.01 (-0.04 – 0.03)	-0.06 (-0.12 – 0.00)
Adjusted for breastfeeding status at stool collection	-0.07* (-0.13, -0.02)	0.02 (-0.03 – 0.07)	-0.02 (-0.12 – 0.07)	-0.05 (-0.11 – 0.01)	0.01 (-0.03 – 0.04)	0.00 (-0.05 – 0.05)
Adjusted for prenatal smoke	-0.12 (-0.18, -0.05)	-0.01 (-0.07 – 0.05)	-0.07 (-0.17 – 0.03)	-0.08 (-0.15, -0.01)	0.00 (-0.04 – 0.03)	-0.05 (-0.11 – 0.01)
Adjusted for postnatal smoke	-0.12 (-0.18, -0.06)	-0.02 (-0.08 – 0.04)	-0.03 (-0.16 – 0.05)	-0.08 (-0.15, -0.01)	-0.01 (-0.04 – 0.03)	-0.05 (-0.11 – 0.01)
Adjusted for antibiotics exposure	-0.13 (-0.19, -0.07)	-0.01 (-0.07 – 0.05)	-0.05 (-0.18 – 0.02)	-0.08 (-0.16, -0.01)	-0.02 (-0.05 – 0.02)	-0.06 (-0.12 – 0.00)
Adjusted for pets	-0.12 (-0.18, -0.06)	-0.02 (-0.08 – 0.04)	-0.04 (-0.16 – 0.04)	-0.08 (-0.16, -0.01)	-0.01 (-0.05 – 0.02)	-0.06 (-0.12 – 0.00)
Model with significant covariates (age, breastfeeding, sex, postnatal smoke, antibiotics, pets)	-0.08 (-0.14, -0.02)	0.01 (-0.04 – 0.06)	0.01 (-0.09 – 0.11)	-0.06 (-0.12 – 0.01)	0.00 (-0.04 – 0.03)	0.00 (-0.05 – 0.06)
Final model (sex, age, breastfeeding, antibiotics)	-0.07 (-0.13, -0.02)	0.02 (-0.04 – 0.06)	-0.02 (-0.12 – 0.07)	-0.05 (-0.12 – 0.01)	0.00 (-0.04 – 0.03)	-0.01 (-0.06 – 0.05)

Note: sIgA concentrations were transformed (sIgA concentration to the power of 0.1736225) so that the distribution was normal for multiple linear regression. * -0.12 (-0.19, -0.06) when adjusted for duration of breastfeeding instead

Table 5. Age-specific beta coefficients of transformed infant fecal sIgA for different maternal distress trajectories (adjusted for breastfeeding status)

Beta (95% CI)	Age category	Antepartum	Postpartum	Persistent
CES-D trajectories	<3 months	-0.08 (-0.21 – 0.04)	0.05 (-0.04 – 0.14)	0.00 (-0.14 – 0.14)
	≥3 and <4 months	-0.04 (-0.11 – 0.04)	0.00 (-0.07 – 0.07)	0.00 (-0.14 – 0.14)
	≥4 and ≤8 months	-0.12 (-0.23, -0.02)	-0.01 (-0.11 – 0.09)	-0.15 (-0.36 – 0.06)
PSS trajectories	<3 months	-0.05 (-0.18 – 0.07)	-0.01 (-0.07 – 0.05)	0.04 (-0.05 – 0.13)
	≥3 and <4 months	-0.04 (-0.13 – 0.05)	0.02 (-0.03 – 0.06)	-0.02 (-0.1 – 0.07)
	≥4 and ≤8 months	-0.08 (-0.2 – 0.05)	-0.01 (-0.08 – 0.05)	-0.07 (-0.19 – 0.04)

Age-stratified linear regression equations (references are Depression_(low level) and Breast feeding_(exclusive)): **1) among age < 3 months:** $(sIgA)^{0.17} = 1.51 - 0.08 * \text{Depression}_{(\text{antepartum})} - 0.0001 * \text{Depression}_{(\text{persistent})} + 0.05 * \text{Depression}_{(\text{postpartum})} - 0.34 * \text{BF}_{(\text{none})} - 0.11 * \text{BF}_{(\text{partial})}$, **2) among age 3 - <4 months:** $(sIgA)^{0.17} = 1.47 - 0.04 * \text{Depression}_{(\text{antepartum})} - 0.001 * \text{Depression}_{(\text{persistent})} + 0.002 * \text{Depression}_{(\text{postpartum})} - 0.25 * \text{BF}_{(\text{none})} - 0.11 * \text{BF}_{(\text{partial})}$, **3) among age 4-8 months:** $(sIgA)^{0.17} = 1.42 - 0.12 * \text{Depression}_{(\text{antepartum})} - 0.15 * \text{Depression}_{(\text{persistent})} - 0.01 * \text{Depression}_{(\text{postpartum})} - 0.22 * \text{BF}_{(\text{none})} - 0.09 * \text{BF}_{(\text{partial})}$

Figure Legends

Figure 1. Maternal depressive symptom (CES-D score) and perceived stress (PSS score)

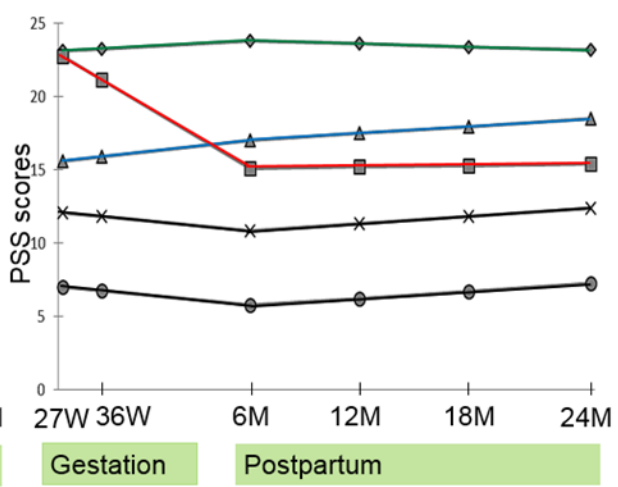
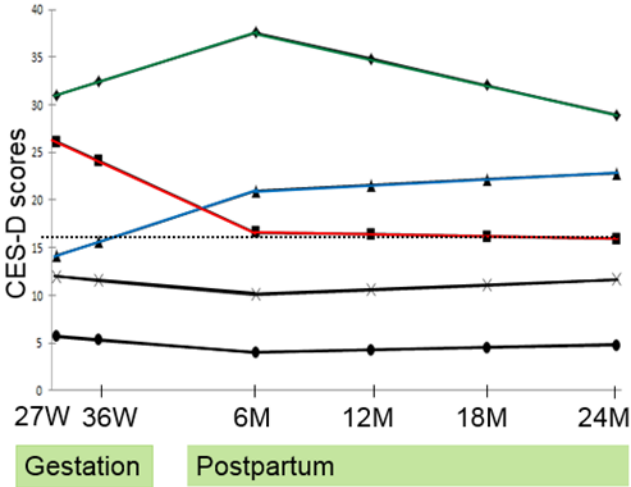
trajectories for the CHILD birth cohort. Adapted from Chow et al (2018). The two trajectories (parallel black lines) for ‘Never low’ and ‘Never moderate low’ represented women with unchanging low symptom scores in the pre and postnatal periods that fell below the cut-off for clinical significance. They were combined for this paper to increase sample size in the reference group since symptom scores always remained below the cut-off value.

Figure 2. Odds ratios from the crude and adjusted logistic regression models for associations

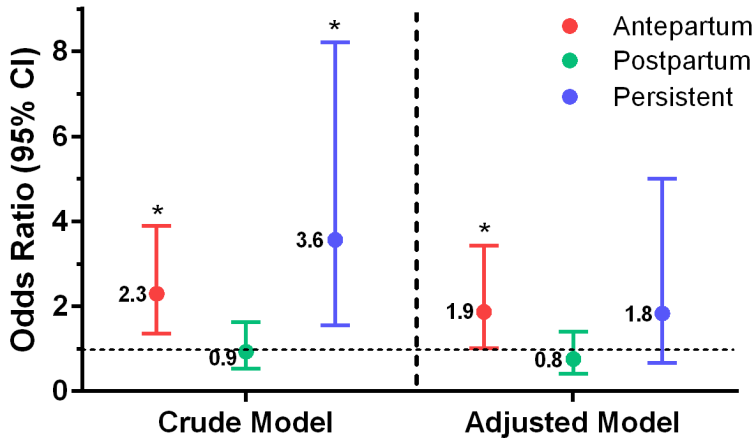
between depressive symptom trajectories and lowest quartile concentrations of fecal sIgA. The Antepartum group had 63, Postpartum 77 and Persistent 23 mother-infant pairs.

Figure 3. Secretory IgA by maternal depression and child age.

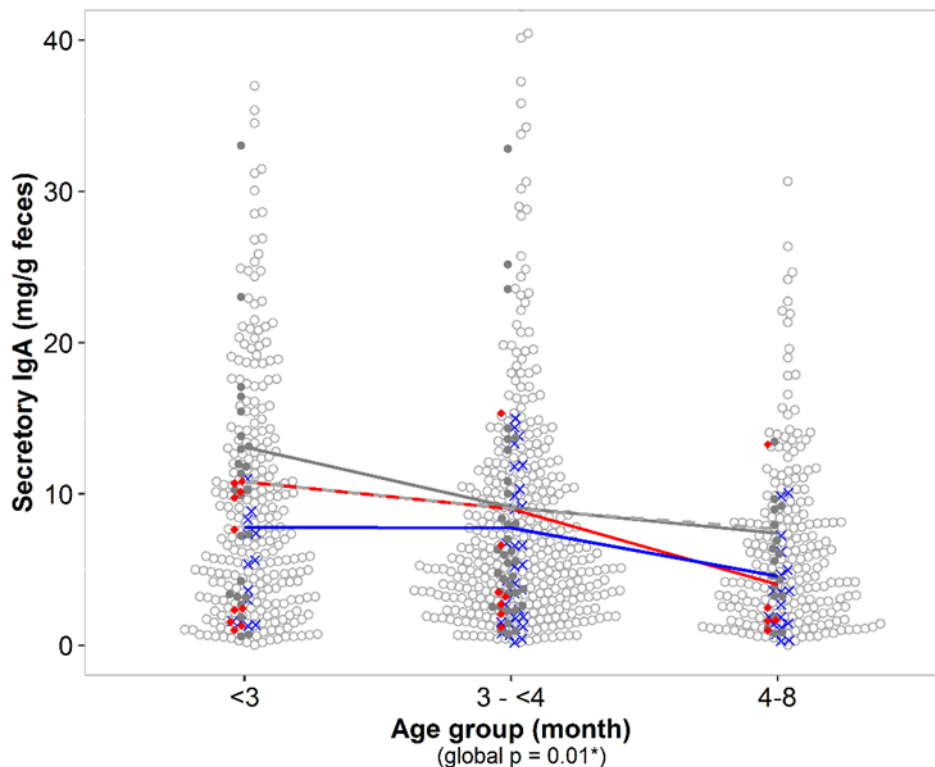
Age-stratified linear regression equations (references are Depression_(low level) and Breast feeding_(exclusive)): **1) among age < 3 months:** $(sIgA)^{0.17} = 1.51 - 0.08 * \text{Depression}_{(\text{antepartum})} - 0.0001 * \text{Depression}_{(\text{persistent})} + 0.05 * \text{Depression}_{(\text{postpartum})} - 0.34 * \text{BF}_{(\text{none})} - 0.11 * \text{BF}_{(\text{partial})}$, **2) among age 3 - <4 months:** $(sIgA)^{0.17} = 1.47 - 0.04 * \text{Depression}_{(\text{antepartum})} - 0.001 * \text{Depression}_{(\text{persistent})} + 0.002 * \text{Depression}_{(\text{postpartum})} - 0.25 * \text{BF}_{(\text{none})} - 0.11 * \text{BF}_{(\text{partial})}$, **3) among age 4-8 months:** $(sIgA)^{0.17} = 1.42 - 0.12 * \text{Depression}_{(\text{antepartum})} - 0.15 * \text{Depression}_{(\text{persistent})} - 0.01 * \text{Depression}_{(\text{postpartum})} - 0.22 * \text{BF}_{(\text{none})} - 0.09 * \text{BF}_{(\text{partial})}$



— Persistent — Antepartum — Postpartum — Never --- CES-D cut-off



Secretory IgA by Age and Depression



*Lines: Age-stratified multivariable linear regression with transformed sIgA to the power of 0.17
*from the non-stratified model, **significant among infants 4-8 months of age only*

Supporting Information

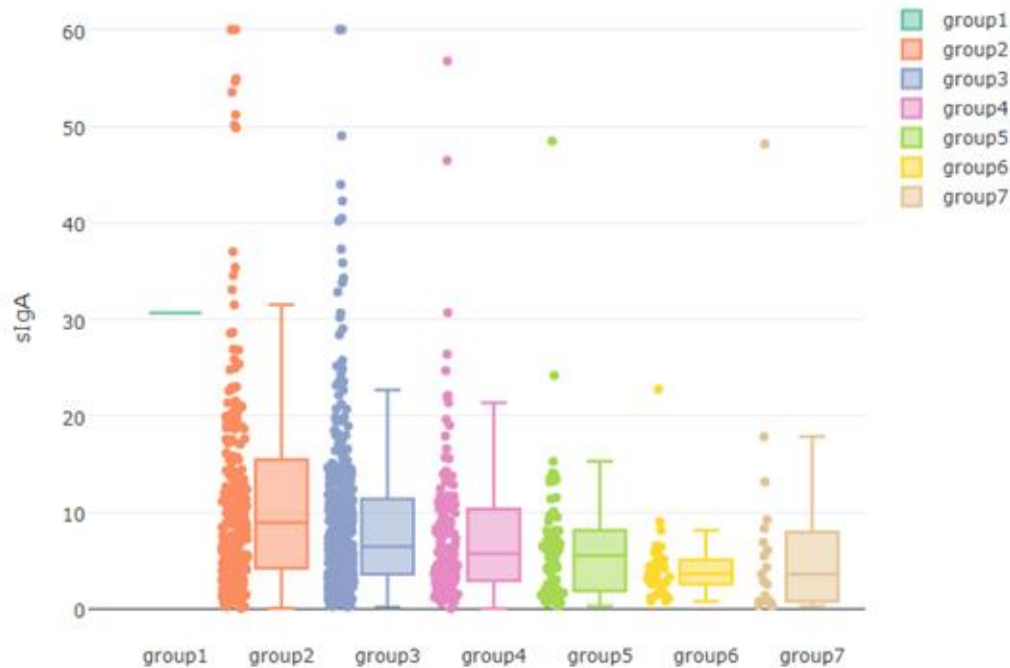
Table S1. Covariates in Study Population versus Full CHILD Cohort

Characteristics	CHILD cohort		Study population	
	N	%	N	%
Child age at 3-month stool collection (Median)	3.25		3.32	
Child sex				
Male	1726	52.7	557	53.4
Female	1550	47.3	486	46.6
Maternal asthma				
No	2490	78.1	796	78.0
Yes	697	21.9	224	22.0
Maternal postsecondary degree				
No	746	23.7	281	27.8
Yes	2407	76.3	729	72.2
Maternal prenatal smoking				
No	2897	90.9	907	88.8
Yes	290	9.1	115	11.3
Prenatal furry pet exposure (dog and cat)				
No	1621	52.8	475	48.0
Yes	1451	47.2	515	52.0
Having older siblings				
No	1772	53.8	573	55.0
Yes	1519	46.2	469	45.0
Birth mode				
Vaginal	2405	74.8	771	75.4
Scheduled caesarean	346	10.8	100	9.8
Emergency caesarean	466	14.5	151	14.8
Direct infant antibiotics use at 3 months				
No	2750	92.0	885	90.8
Yes	239	8.0	90	9.2
Breastfeeding at 3-month stool collection*				
Exclusive	796	47.7	409	39.7
Partial	532	31.9	352	34.2
Zero	340	20.4	269	26.1

*Study population vs. CHILD

Table S2. Secretory IgA by Child Age and Breastfeeding Status

Age	Measure	Breastfeeding Status			Overall
		None	Partial	Exclusive	
<3 months	Median	2.34	7.53	10.59	7.68
	(IQR)	(1.29 - 4.31)	(3.65 - 11.99)	(6.84 - 17.57)	(3.19 - 13.42)
	Mean (SD)	3.49 (3.31)	9.66 (8.41)	14.26 (12.17)	10.48 (10.66)
ñ3 and <4 months	Median	3.23	5.35	9.11	5.97
	(IQR)	(1.82 - 4.77)	(3.22 - 10.01)	(5.74 - 13.82)	(3.21 - 10.88)
	Mean (SD)	4.00 (3.83)	7.47 (6.72)	11.73 (10.25)	8.42 (8.53)
ñ4 and á8 months	Median	2.64	5.24	7.57	4.71
	(IQR)	(1.37 - 4.75)	(2.55 - 9.51)	(4.57 - 11.66)	(2.15 - 8.67)
	Mean (SD)	3.79 (3.71)	7.70 (9.27)	8.90 (5.84)	6.79 (7.47)
Overall	Median	2.88	5.74	9.83	6.07
	(IQR)	(1.54 - 4.73)	(3.16 - 10.38)	(5.89 - 14.5)	(2.91 - 11.03)
	Mean (SD)	3.79 (3.65)	8.06 (8.10)	12.32 (10.71)	8.62 (9.09)



- group 2 is different than all other groups
- groups 3, 4 and 5 are the same distributions
- groups 3, 4 and 5 are different than group 6
- group 3 is different than group group 7
- groups 4, 5 and 6 are the same as group 7

Figure S1. Results of test of equal densities for infant fecal secretory IgA in different age groups. Group 1 had infants with stool collected at 1 month (infant was not included in the main analysis), group 2 <3 months, group 3 ≥ 3 and <4 months, group 4 ≥ 4 and <5 months, group 5 ≥ 5 and <6 months, group 6 ≥ 6 and <7 months and group 7 ≥ 7 and ≤ 8 months.