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The impact of prenatal dog keeping on infant gut microbiota development

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Abstract

Introduction: Prenatal and early-life dog exposure has been linked to reduced childhood allergy and asthma. A potential mechanism includes altered early immune development in response to changes in the gut microbiome among dog-exposed infants. We thus sought to determine whether infants born into homes with indoor dog(s) exhibit altered gut microbiome development.

Methods: Pregnant women living in homes with dogs or in pet-free homes were recruited in southeast Michigan. Infant stool samples were collected at intervals between 1 week and

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AUTHOR CONTRIBUTIONS

ARP contributed to data acquisition and analyses and drafting the article: ARS performed data analyses and drafting the article; DF prepared and sequenced samples; SH and AML contributed to study design and provided statistical guidance; KJ, GW, BD, SF and KW contributed to data acquisition; NWL contributed to study design; DRO, CCJ and EMZ contributed to study design, data acquisition and drafting the article; SVL contributed to study design, provided analysis guidance and drafted the article. All authors reviewed and approved the final version of the article for intellectual content, accuracy and integrity.

CONFLICT OF INTEREST STATEMENT

SVL is a consultant, sits on the board of directors and holds stock in Siolta Therapeutics Inc. She also consults for Solarea Bio. ARP, ARS, DF, SLH, KJ, BD, SF, GRW, KW, NWL, AML, DRO, CCJ and EMZ have no disclosures related to the submitted work to report.

SUPPORTING INFORMATION

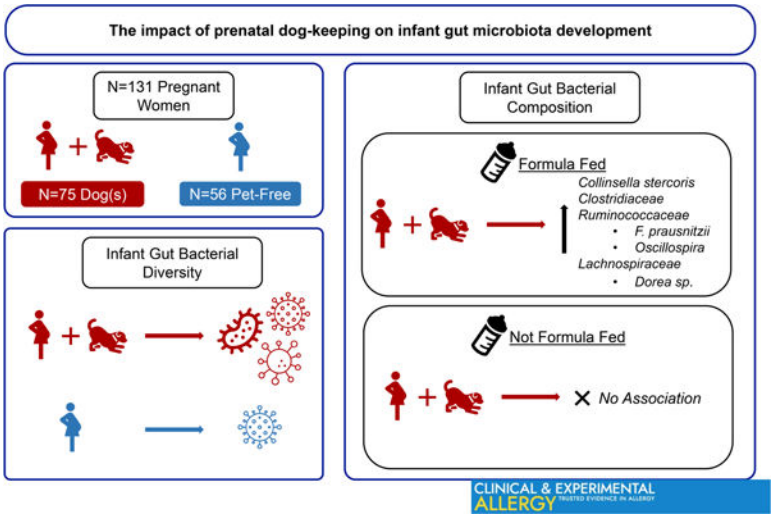
Additional supporting information can be found online in the Supporting Information section at the end of this article.

18 months after birth and microbiome was assessed using 16S ribosomal sequencing. Perinatal maternal vaginal/rectal swabs and stool samples were sequenced from a limited number of mothers. Mixed effect adjusted models were used to assess stool microbial community trajectories comparing infants from dog-keeping versus pet-free homes with adjustment for relevant covariates.

Results: Infant gut microbial composition among vaginally born babies became less similar to the maternal vaginal/rectal microbiota and more similar to the maternal gut microbiota with age-related accumulation of bacterial species with advancing age. Stool samples from dog-exposed infants were microbially more diverse (p = .041) through age 18 months with enhanced diversity most apparent between 3 and 6 months of age. Statistically significant effects of dog exposure on β-diversity metrics were restricted to formula-fed children. Across the sample collection period, dog exposure was associated with *Fusobacterium* genera enrichment, as well as enrichment of *Collinsella*, *Ruminococcus*, *Clostridaceae* and *Lachnospiraceae OTUs*.

Conclusion: Prenatal/early-life dog exposure is associated with an altered gut microbiome during infancy and supports a potential mechanism explaining lessened atopy and asthma risk. Further research directly linking specific dog-attributable changes in the infant gut microbiome to the risk of allergic disorders is needed.

GRAPHICAL ABSTRACT



Prenatal dog-keeping associates with lower rates of childhood allergic disorders. The gut microbiome was analysed longitudinally for 18 months among 75 infants whose mothers kept indoor dogs during pregnancy and 56 infants whose mother's homes were pet-free. Dog-keeping was associated with increased gut bacterial diversity. Among formula-fed children, dog keeping was associated with altered microbial composition indicating enrichment of *Collinsella stercoris*. *Ruminococcacea*, *Clostridiaceae* and *Lachnospiraceae*. These data support a potential microbial mechanism for associations between dog keeping and decreased allergy risk.

Keywords

allergy; dog; environmental microbes; gut microbiota; infant; microbial trajectory; prenatal

1 | INTRODUCTION

Vertical transfer of maternal microbes, together with environmental microbial exposures, shape the initial bacterial communities of the neonatal gastrointestinal tract.[1–3] These inceptive microbes regulate immune function[4,5] and influence trajectories of gut microbiome maturation during this critical period of development.[6] Birth cohort studies have provided evidence that early-life gut microbiome perturbations, and subsequent metabolic dysfunction, is associated with increased risk of atopy and asthma in childhood.[7–10] We previously reported that gut microbial profiles associated with protection against atopy and asthma were more likely among babies with prenatal and early life exposure to dogs.[7,8]

Indoor pet exposure during pregnancy and early childhood is associated with lower total immunoglobulin E (IgE) levels, lessened allergen sensitization, childhood wheeze and lower risk of allergic disorders including asthma.[11–21]Household pets, particularly dogs, influence household environmental microbes[22–26] and contribute to variance in infant stool microbiota.[27]

These observations are consistent with potential gut microbiome-related mechanisms linking pets to lowered risk of allergy-related disorders. However, reports directly linking indoor pet keeping to culture-independent alterations of the early-life gut microbiome are lacking. This study compares longitudinal gut microbiota taxonomic developmental trajectories among infants living in homes with indoor dogs versus pet-free homes, permitting analyses as to whether dog exposure influences initial gut microbiome development.

2 | METHODS

2.1 | Study population

The Microbes, Asthma, Allergy and Pets (MAAP) birth cohort includes 141 maternal–child pairs from southeastern Michigan enrolled to evaluate potential differences in the early-life gut microbiome between children living in homes with indoor dogs versus those living in pet-free homes. Pregnant women ages 18 through 49 years, receiving healthcare from Henry Ford Health (HFHS) obstetricians with planned delivery at selected HFHS hospitals, who intended to remain in southeast Michigan for at least 2 years post-partum were recruited during their second or third trimester of pregnancy between January 2014 and August 2016. An enrolment requirement included either: (1) living with a dog(s) kept indoors at least 12 h daily for at least 6 months prior to pregnancy with plans to keep the dog for the study's duration or (2) living in furred pet-free homes for at least 2 years prior to pregnancy with no plans to obtain pets or have contact with pets for more than 4 h weekly for the study's duration.

Women were required to communicate in English sufficient to provide written informed consent. The study was approved by the Henry Ford Hospital IRB.

2.2 | Data collection

Women were interviewed prenatally and at 1 week, 6 months, and 18 months post-delivery. Questionnaires included: household demographics, tobacco smoke exposure, medication

use, parental allergic history, pet keeping and questions related to their offspring's health. Mothers completed dietary calendars to track weekly changes to their infant's diet (breastfeeding, formula feeding and solid food introduction). When calendars were incomplete or missing, dietary information was utilized from questionnaires. Formula feeding included any formula use since the previous maternal contact. Breastfeeding duration was calculated as the total number of weeks of breastfeeding (whether exclusive or partial). First introduction of solid food was defined as the first week of life that any type of solid food was introduced. Pregnancy and delivery information were obtained from electronic health records. Allergen sensitization at 18 months was defined as sIgE ≥ .35 ku/L to at least one of following allergens: ragweed, dust mite, grass, cat, dog or mould mixture.

2.3 | Specimen collection

Maternal vaginal/rectal swabs (Epicentre Catch-All™) were collected by their obstetrician within 6 weeks before delivery or on the day of delivery using the standard process for Group B Streptococcus screening. Swabs were stored in RNAlater at 4°C for at least 24 h, and then transferred to −80°C.

Maternal stool was collected during the last trimester and at approximately 1 week, 6 months and 18 months post-partum.

Infant stool was collected at approximately 1 week, 1 month, 3 months, 6 months and 18 months after birth.

Soiled diapers and maternal stool specimens were transported or mailed to the laboratory in insulated containers with ice packs and then stored at −80°C.

2.4 | 16S ribosomal RNA Sequencing

2.4.1 | DNA extraction—

Punch biopsies (4 mm) (VWR International) were used to transfer ~0.3 g of frozen stool to Lysing Matrix E tubes (LME; MP Biomedicals). Vaginal/rectal swabs in RNAlater were thawed on ice and transferred to LME tubes. RNAlater was transferred into a sterile tube and centrifuged at 16,000 × g for 5 min at 4°C. Pellets were re-suspended using cetyltrimethylammonium bromide (CTAB) and transferred to the LME tube containing the swab. All prepped LME tubes were stored at −80°C. Genomic DNA was extracted using a modified CTAB buffer protocol.[7]

2.5 | Sequencing preparation, data processing and quality control

Sequencing preparation methods are described in the supplemental material. Following preparation, paired-end sequences were merged using flasH (v1.2.11), demultiplexed by barcode. Reads that contained two or more unexpected errors were discarded. Unique sequences were clustered at 97% identity into operational taxonomic units (OTU) and chimeras removed using USEARCH. USEARCH was used to map all raw quality filtered reads to the unique sequence OTUs at 97% identity. Sequences were aligned using PyNast and the alignment was filtered to remove gaps. A bacterial phylogenetic tree was built using FastTree. The OTU table was filtered to remove unaligned sequences and taxonomy was assigned using Greengenes database (v13_5). NTCs were assessed to determine potential

contamination. OTUs present in over half of NTCs were removed from samples. For OTUs present in less than half of NTCs, the highest read count was determined and subtracted from samples. To denoise the OTU table, taxa with less than 1/1000 of a percent of the total read count across all samples were removed. To normalize read depth across samples, data were rarefied 100 times to a read depth of 60,503 reads, using a multiple rarefaction algorithm.[7] This depth was chosen because it was a high rarefying depth that resulted in little sample loss (stool $N = 36$, swabs $N = 4$) and Procrustes analysis indicated the distances in this rarefied matrix were closer (lowest M_2 values) to the non-rarefied matrix compared to other rarefying depths. For timing of stool sample collection and mean number of stool samples contributed per infant, refer to Table S1 and Table 2.

2.6 | Statistical analysis

Statistical significance for main and interaction effects was set at $p < .05$. ANOVA and chi-square tests were used to compare maternal and child characteristics across groups by prenatal indoor dog keeping (yes/no). Metrics describing α-diversity were calculated using the R packages *vegan* and *picante*. Mixed effect models were used to fit stool α-diversity trajectories. Exact age at stool collection was used rather than targeted collection time (Figure S1). Best-fitting shape (linear vs. quadratic) was determined by Bayesian information criterion (BIC). Effect modification by age at time of stool sample collection, child sex, mode of delivery, household income and formula feeding were selected a priori for evaluation. The effect of dog keeping was assessed before and after adjusting for pre-specified potentially confounding covariates: maternal reported race, household income, maternal age at birth, mode of delivery (vaginal vs. caesarean-section), child sex and whether child was firstborn.

β-diversity metrics were calculated using the R packages *phyloseq* and *vegan*. Principal co-ordinates analysis (PCoA) was performed using the R package *labdsv* on baby stool samples with each β-diversity metric. The first through fourth principal co-ordinates were extracted and used as outcomes in mixed effect models to assess compositional differences by dog exposure, before and after covariate adjustment, as described previously for α-diversity. Compositional differences in cross-sectional data sets were assessed using PERMANOVA, before and after covariate adjustment.

Differences in OTU and genera abundance trajectories by age and prenatal indoor dog exposure were fit using generalized linear mixed effect models with the R package *glmmADMB*. Each taxon was first modelled with the random subject effect and a time covariate only (continuous, actual age at sample collection); negative binomial versus zero-inflated negative binomial models were compared for best fit using BIC (unless only one of the two models were able to be estimated; if neither model was able to be estimated, results were not examined). Upon determining the best-fitting model, main effects of dog exposure and interaction effects with time were obtained (with subgroup effects reported for significant interactions) with p-values rate-adjusted for false discovery ($p_{FDR} < .05$ considered significant). All models testing dog effects were adjusted for the pre-specified confounding covariates. Taxa tested included only those present in ≧10% of baby stool samples. For OTU models, interaction effects were added for potentially effect modifying

covariates, as suggested by β-diversity results. All analyses were performed using R version 3.6.1 or SAS version 9.4.

3 | RESULTS

3.1 | Cohort description and participant samples

Of 141 children at 18 months of age (81 dog/60 pet-free), seven were sensitized to common inhalants (four dog vs. three pet-free), seven had parentally-reported, doctor diagnosed asthma (five dog vs. two pet-free), and 35 had reported eczema/atopic dermatitis by 18-months (24 dog vs. 11 pet-free). A total of 660 fecal samples across 134 maternal–child pairs were successfully sequenced; 131 offspring (93%) had at least one infant stool sample successfully sequenced. The number of samples successfully sequenced during each collection period are displayed in Table 1.

The analysed 131 children were similar to the full cohort and 57% of the samples were from participants residing with a dog (Table 2). The contribution of at least one infant stool sample (chi-square $p = .87$) and the number of samples sequenced per child did not differ by dog exposure (Kruskal–Wallis $p = .506$). Several factors were significantly associated with dog ownership: Mothers with dogs were less likely to self-identify as African American (10.7% vs. 28.6%, $p = .009$) and more likely to have mid-level household incomes ($40,000–$80,000; $p = .006$), and live in single family homes (90.4% vs. 64.3%, $p < .001$).

3.2 | Infant gut microbiota development over advancing chronological age

Previous studies indicate that infant gut microbiota composition changes with advancing chronological age.[8,28] We first examined relationships between gut microbiota development and infant age. The gut microbiota exhibited increasing bacterial richness and diversity and age-related compositional changes over the first 18 months of life (Figure 1A and Figure 2). Using vaginal/rectal swab and maternal stool microbiota profiles for reference, we noted that infant gut microbiota were similar to maternal vaginal/rectal microbiota at delivery, but became increasingly similar to the maternal gut microbiome with advancing age (Figure 1A [all samples] and Figure 1B [vaginally born infants with paired maternal samples]), indicating compositional maturation.

We next sought to identify bacterial genera related to chronological age. Of the 57 genera with a prevalence of >10%, 48 (84%) were significantly associated with infant age at the time of sample collection. Of these 48 genera, 29 increased and 19 decreased in relative abundance with advancing age (Figure S2, Table S3, $p_{FDR} < .05$). Twelve genera found to have the largest absolute effect size associated with advancing age are shown in Figure S2. Eleven of these genera increased in relative abundance with age and *Staphylococcus* decreased in relative abundance. These findings are consistent with previous observations of infant gut microbiota development over the first year of life and with age-related accumulation of bacterial species over this developmental period.[2,7,9,29]

3.3 | Dog-ownership and gut microbial α-diversity metrics

We examined the relationship between dog ownership and infant gut microbiota richness, evenness and diversity trajectories. For richness and evenness trajectories, linear modelling was the best fit (Table S2). The overall dog effect was not statistically significant prior to covariate adjustment (Table 3, $p = .094$ and $p = .54$ respectively) nor following full adjustment ($p = .080$, $p = .89$). Interactions between dog exposure and the pre-specified effect modifiers were non-significant for richness and evenness (Table S4).

A quadratic trajectory model best-fit Faith's phylogenetic diversity (Table S2). There were significant overall dog effects in the fully adjusted model, where diversity was on average 0.78 units higher in dog-exposed children across the observation period (Table 3; $p = .041$). Covariates including maternal race and delivery mode magnified the effect size of dog exposure, while household income and maternal age at birth diminished the effect size. However, the final multivariable model demonstrated that full covariate adjustment resulted in a 20% increase in effect size. A statistically significant dog exposure by time interaction was identified for diversity (Table S4; $p = .012$ and Figure 2). While phylogenetic diversity was greater in dog-exposed children for most of the trajectory, the curves were most divergent at 3 and 6 months (3 months: $\beta = 1.08$, $p = .006$; 6-months: $\beta = 1.63$, $p < .001$). By 18 months, the effect greatly diminished ($\beta = 0.24$, $p = .64$). Due to sparse data, estimates beyond 18 months should be interpreted with caution.

Assessment of potential confounding or effect modification by cat exposure was not possible as only dog-containing homes were allowed to have cats. We did examine the effect of cats on α-diversity trajectories from the 19 dog-containing homes that also contained a cat. Cats did not affect evenness (β (SE) = 0.024 (0.018), p-value = .19), while non-significant trends were observed for richness (β (SE) = 18.1 (9.2), p-value = .051) and phylogenetic diversity (β (SE) = 1.09 (0.56), p-value = .055).

3.4 | Dog ownership and gut microbiota composition

Several cross-sectional associations of gut microbial composition (β-diversity) to indoor dogs were observed as listed in Table S5. However, to best understand how dog exposure may impact gut microbiota composition over time, we examined β-diversity trajectories using principal co-ordinates. The first principal co-ordinate of unweighted UniFrac was positively correlated with richness: $r = .69$ at 1 week, $r = .80$ at 1 month, $r = .88$ at 3 months, $r = .89$ at 6 months and $r = .80$ at 18 months. Although dog exposure was associated with overall compositional structure in some unadjusted models, effects were non-significant after covariate adjustment (Table S6). Effects were not time-dependent (Table S4; all dog × time interaction $p \geq .058$). Other effect modifiers were non-significant except for formula-feeding on the first principal co-ordinate of unweighted UniFrac (interaction $p = .036$) and Canberra (interaction $p = .039$) distances. Specifically, the effect of dog ownership on β-diversity trajectory was apparent among formula-fed children only (Figure 3).

3.5 | Indoor dogs and specific taxa

We next identified gut bacterial taxa differing between infants living with indoor dogs versus in pet-free homes. Among 120 genera, 57 were detected in >10% of samples.

Following covariate adjustment, one genus, *Fusobacterium* (Figure 4), was significantly enriched among children living with dogs.

Of 1286 individual OTUs detected, 439 were prevalent in >10% of samples. After covariate adjustment, four were significantly related to living with a dog (Figure S3; Table S7). Specifically, *Collinsella stercoris* (OTU 93), *Ruminococcus* (OTU 1822) *Lachnospiraceae* (OTU 575) and *Clostridiaceae* (OTU 1099) were enriched in dog-exposed children.

3.6 | Dog exposure in formula-fed infants and taxa trajectories

Noting significant interaction with formula feeding in the β-diversity trajectory models, we examined individual OTU trajectories contributing to this interaction. Seventy OTUs significantly contributed to the dog by formula-feeding interaction (interaction $p_{FDR} < .05$; Table S8) with 60 exhibiting enrichment among formula-fed children. Of the four OTUs with a significant main effect of dog exposure (Table S7), *Collinsella stercoris* (OTU 93) exhibited a stronger effect of living with a dog in formula-fed versus non-formula-fed children (Table S8). Nearly all *Ruminococcaceae* and *Lachnospiraceae* OTUs having a significant dog exposure by formula-feeding interaction also showed greater enrichment with dog exposure among formula-fed children (Table S8). Many *Ruminococcaceae* OTUs were identified as *Faecalibacterium prausnitzii* or *Oscillospira*, while *Lachnospiraceae* OTUs included *Dorea* sp.

4 | DISCUSSION

We observed 131 infants over the first 18 months of life that exhibited gut microbial developmental trajectories consistent with published studies describing increasing richness and progression towards and adult-like gut microbiome.[2,30] These microbial developmental trajectories indicated that compared to infants living in homes without furred pets, those living with dogs had elevated gut bacterial phylogenetic diversity over the first 18 months of life. Early life represents a critical period of immunological development when low diversity or inappropriate assembly of the gut microbiota is associated with adverse respiratory health outcomes.[31–33] Our results lend support to hypotheses linking dog exposure to decreased risks of atopy, allergy and asthma[11,12,16,18,34,35] through an impact of dog exposure on the developing gut microbiome.

For phylogenetic diversity, we observed a statistically significant dog exposure by time interactions with the most robust associations at 3 and 6 months of age; a common period of complementary food introduction also known to influence the repertoire of local and circulating microbial metabolic products known to shape immune function and development of atopy and asthma.[7,9,36–38] Whether diversification of the gut microbiota, specifically at this key point in development results in differences in the functional pathways or metabolic output of the gut microbiome or associated effects on immune function merits further study.

Several taxa enriched in infants living with dogs compared to pet-free homes included *Collinsella stercoris*, *Ruminococcus sp.*, *Lachnospiraceae sp.* and *Clostridiaceae sp.* Gut *Ruminococcus* enrichment was also reported in 3–4-month-old infants exposed to furry pets.[39] We previously reported *Ruminococcaceae*, *Lachnospiraceae* and *Clostridiaceae*

enrichment in the gut of mice orally supplemented with house dust collected from dog-containing homes and resulting in microbiota restructuring associated with protection against allergic airway inflammation.[40] This effect appeared mediated, in part, by alteration of the serum metabolome including increased concentrations of circulating anti-inflammatory polyunsaturated fatty acids that reduced bone marrow-derived dendritic cell activation and inflammatory cytokine production in vitro.[41] Thus suggesting that dog exposure enriches gut microbes that can down-regulate responses to inflammatory stimuli.

The impact of dog cohabitation on gut microbiota was greatest among formula-fed infants, revealing enrichment of OTUs such as *Collinsella stercoris* (OTU 93) *and Dorea sp.* (OTUs 1015). Both have been identified in breast milk[42] with *Collinsella* abundance highest in the gut of actively breastfeeding infants.[2] Whereas breast milk contains bacterial communities[43] capable of colonizing the infant gut[44–47] and influencing community composition,[2,47] formula fails to provide these microbial exposures. Perhaps, in the absence of breast milk, dogs provide an alternative source of environmental microbes for the developing gut microbiome of formula-fed infants. Supporting this concept, *Dorea* was found to be depleted in stool from dust mite-sensitized, 4–5-year-old children with allergic rhinitis compared to healthy controls with *Dorea* relative abundance negatively correlated to fecal IgE levels.[48] *Dorea* is also underrepresented in the gut microbiota food-allergic children.[49]

Faecalibacterium prausnitzii was also enriched in the formula-fed infants living with a dog. *F. prausnitzii* is an abundant and key inhabitant of the human gut capable of blocking nuclear factor kB activation and enhancing anti-inflammatory, antigen-specific T cell proliferation and IL-10 expression.[50,51] Its loss from the gut microbiota is characteristic of infants at high risk of developing allergies and asthma in childhood and in adults with chronic inflammatory conditions such as inflammatory bowel disease.[7,8,52] Exposure of formula-fed infants to dogs in early life may serve to increase the presence of this species in early infancy.

Few studies have examined the relationship between household pets and the infant gut microbial composition and prior studies were limited by small sample size, lack of pet-free controls and/or examination at a single time point.[39,53,54] Our inclusion of infants from pet-free homes allowed comparison of the impact of dog-associated exposures on early gut microbiota trajectories. Frequent longitudinal stool collection also allowed us to determine whether the impact of dog on the developing gut microbiome was associated with specific windows of microbial divergence while detailed surveys permitted adjustment for key, potentially confounding covariates. However, unmeasured, residual confounding is possible and should be further explored in larger studies. To the best of our knowledge, this study is the first to conduct longitudinal analyses examining the impact of dog exposure and the interaction of dog exposure and other early-life factors on infant gut microbiota trajectories.

Our study has several limitations. The limited cohort size and assessment of non-high-risk infants for less than 2 years limits the statistical power to directly assess whether the observed dog-attributable microbes are linked to differences in relevant allergic outcomes and also limits statistical power to assess potential associations among some subgroups. MAAP participants assessments at age 5–8 years are ongoing and may provide an

opportunity to address some of these potential associations. Although low DNA yields from the vaginal/rectal swabs contributed to a high sequencing failure rate, this limitation did not impact analyses relating dog keeping to the infant gut microbiome since these samples were used solely as adult microbiota references to compare with the infant's developing gut microbiome. Our study allowed dog-containing homes to also contain cats, whereas cats were not allowed in pet-free homes. This precluded the ability to examine confounding or effect modification by cat exposure. Although, cats within dog-containing homes did not significantly alter α-diversity trajectories, a trend for enhanced richness and phylogenetic diversity was observed. The possibility that cats, or other furred pets could have partially contributed to the altered gut microbiota of dog-exposed infants was not excluded. Finally, while fungi or viruses may play an important role as inhabitants of the gut microbiome and influence immune development, we were limited in our study to only 16 S ribosomal sequencing of gut bacteria.

5 | CONCLUSIONS

Prenatal dog-keeping associates with altered infant gut microbial community trajectories including higher microbial diversity, particularly at 3 and 6 months of age. The strongest dog-associated compositional effects were observed among formula-fed infants and involved enhancement of key immunomodulatory gut bacteria. These data support ongoing hypotheses positing that associations of dog exposure and decreased allergy may be related to early-life gut microbiome development. Additional, appropriately scaled future studies are needed to definitively link dog-attributable changes in the infant gut microbiome to effects on immune development that causally explain lowered risk of allergy among dog-exposed infants.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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DATA AVAILABILITY STATEMENT

The Raw sequence read data that support the findings of this study been submitted to the European Nucleotide Archive under accession number PRJEB50482. Additional supplementary and supporting data are available within this article and can be made available from the corresponding author [EMZ], upon reasonable request.

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Key Messages

- Prior studies indicate prenatal and early-life dog exposure associates with lower IgE production and allergy.
- Infants of mothers that lived with dogs during pregnancy exhibit an altered gut microbiome.
- An altered infant gut microbiome may explain associations of early-life dog exposure on allergy-related outcomes.

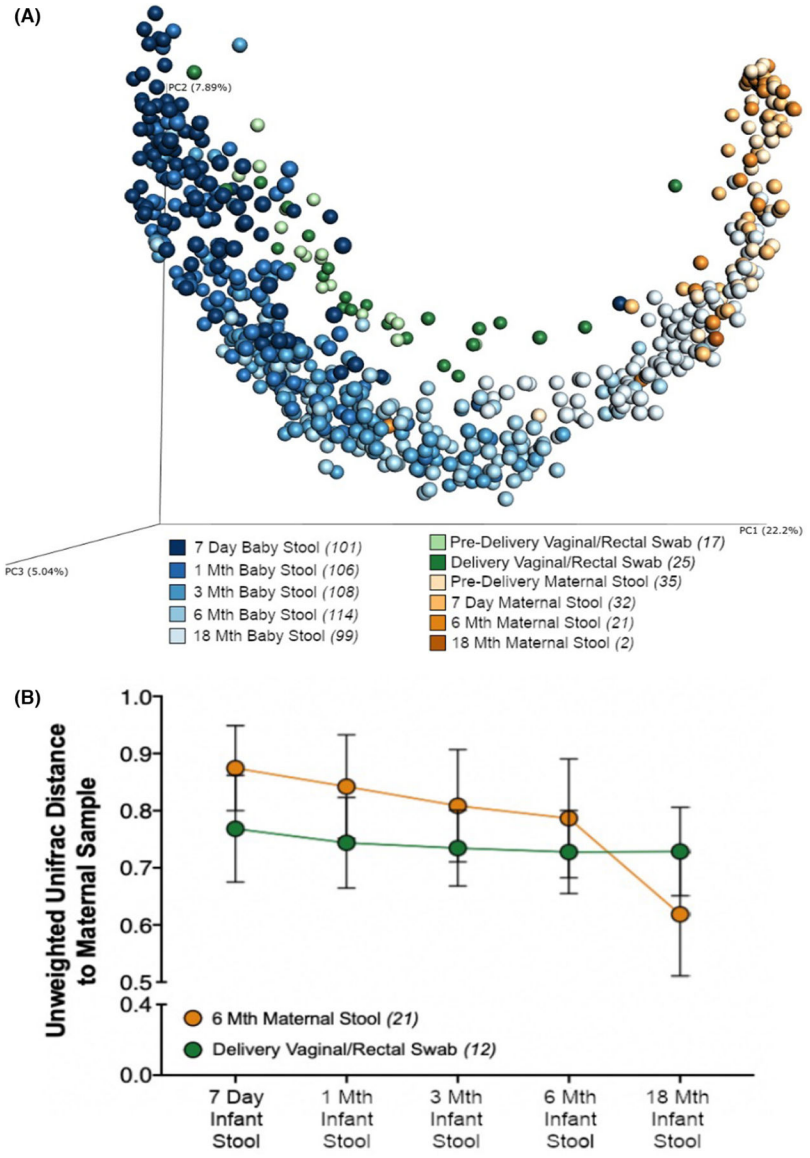

FIGURE 1.

PCoA based on an unweighted Unifrac dissimilarity matrix showing bacterial community composition of infant stool samples (7 days and 1, 2, 6 and 18 months), maternal stool samples (pre-delivery, 7 days, 6 and 18 months), and maternal vaginal/rectal swabs (pre-delivery and delivery) (A). Mean unweighted Unifrac distance of delivery maternal vaginal/rectal swabs or 6 months maternal stool to paired, vaginally born infant stool samples at each time point (B).

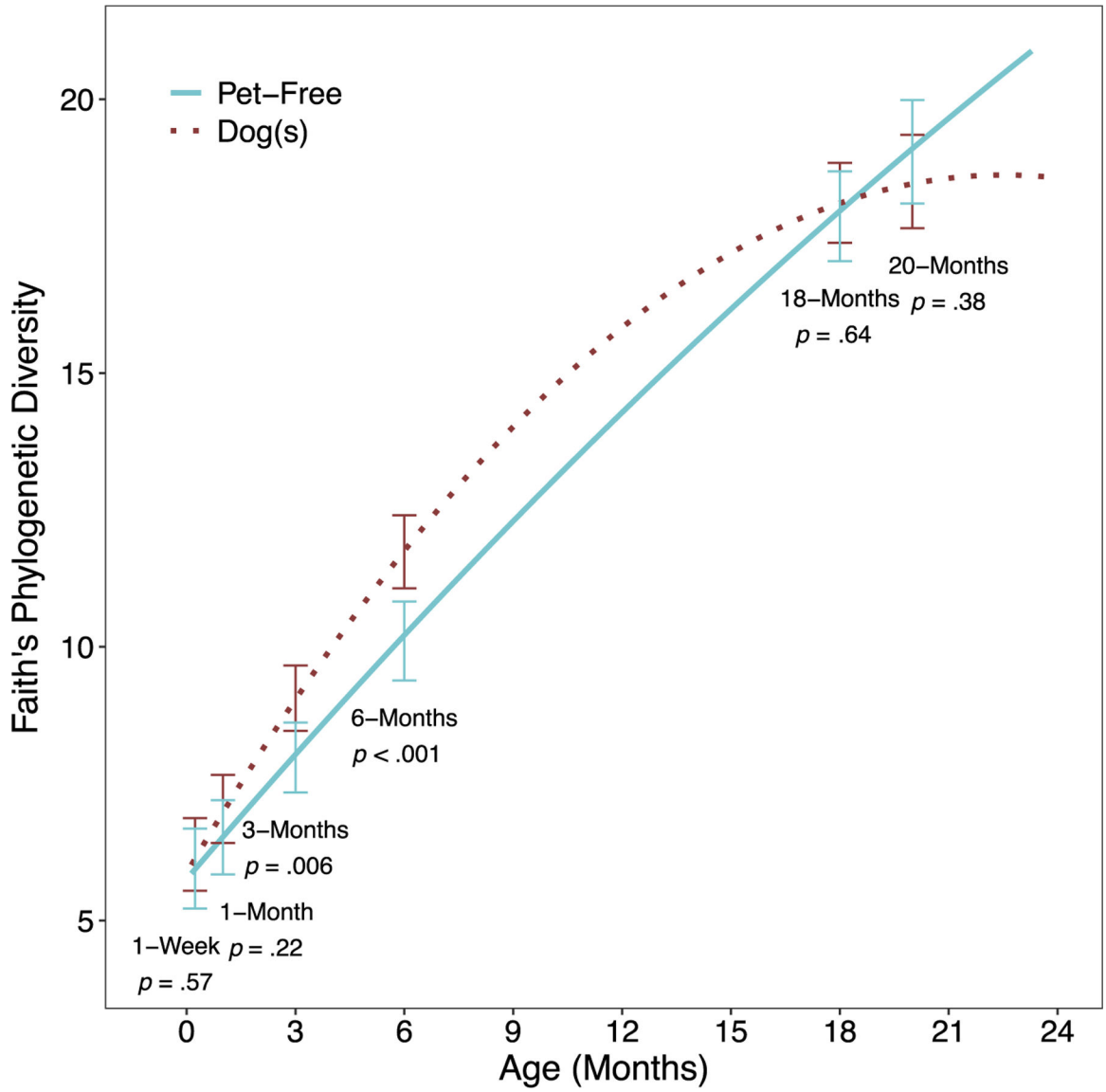

FIGURE 2.

Fitted Faith's phylogenetic diversity trajectories by prenatal indoor dogs. Model adjusts for maternal race, household income, maternal age at birth, mode of delivery, child sex and first-born child, and includes a term for the dog × time interaction, as this was statistically significant ($p = .012$).

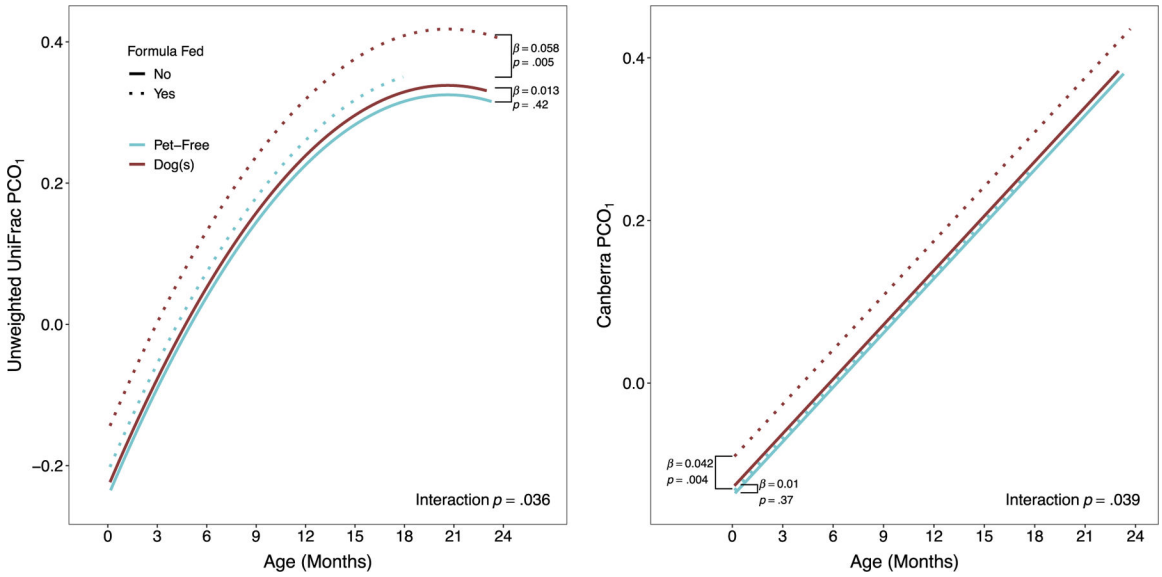

FIGURE 3.

Trajectories of unweighted UniFrac and Canberra metrics (first principal co-ordinate), by prenatal dog exposure and type of feeding. Brackets show the difference between dog-exposed and unexposed children, among children who are formula fed (dotted lines) versus not (solid lines).

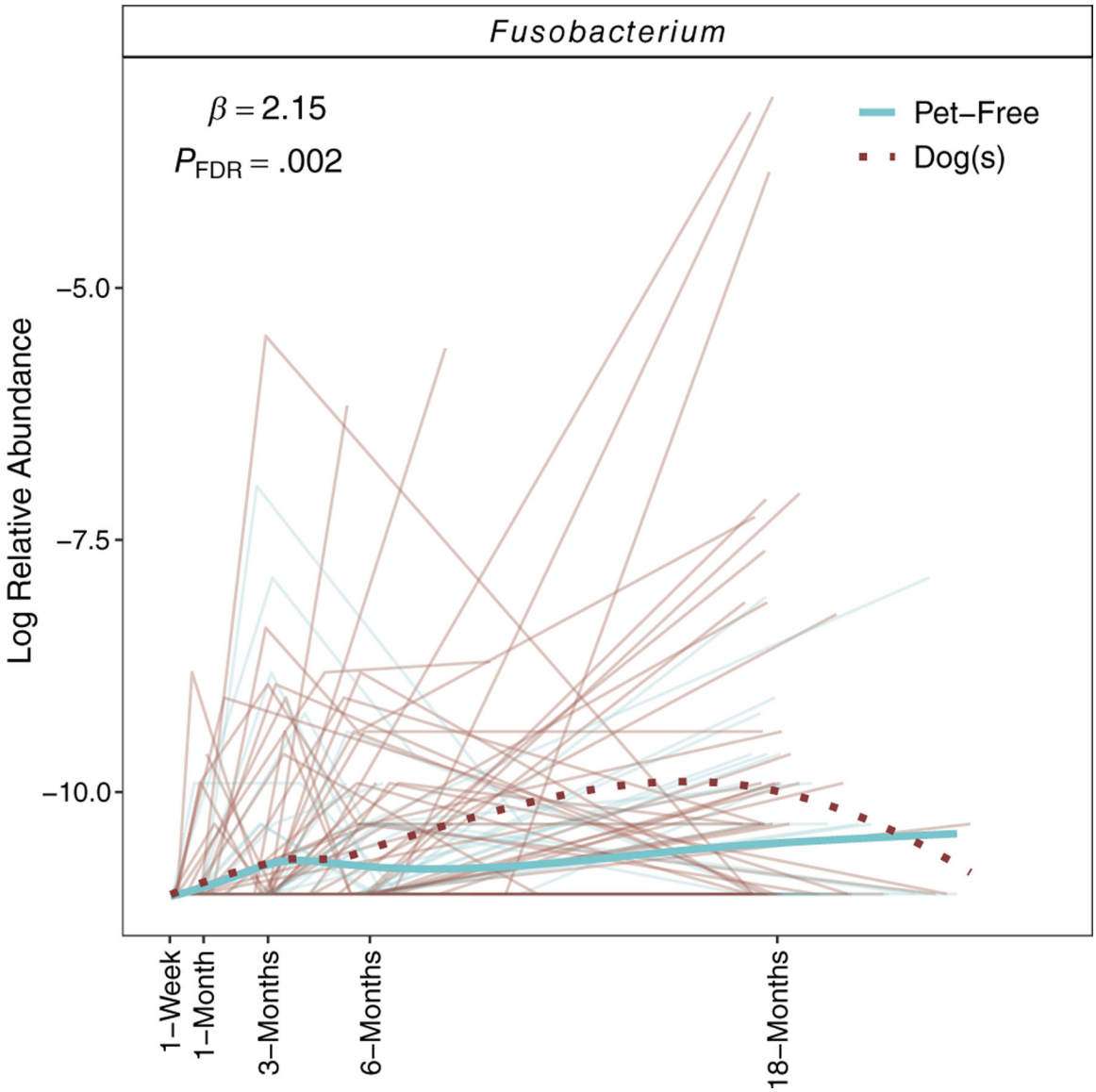

FIGURE 4.

Fusobacterium was the only genus significantly associated with prenatal dog exposure ($p_{FDR} < .05$), after covariate adjustment. Estimates shown represent the difference in log-transformed mean abundance, comparing infants living with dogs versus pet-free homes.

TABLE 1

Description of 660 samples across 134 maternal–child pairs with microbiome data.

			Observed collection time in days, with respect to baby date of birth (*N* = sequenced samples)						
Subject	**Sample**	**Sample collection time (Number sent for sequencing)**	***N***	**Min**	**Q1**	**Median**	**Mean**	**Q3**	**Max**
Mother	Stool	Pregnancy (36)	35	−65	−27	−20	−21	−10	−1
		1 Week (33)	32	6	13	22	24	27	95
		6 Months (21)	21	163	175	188	188	193	258
		18 Months (2)	2	532	534	535	535	536	538
	Vaginal/rectal swab	Pre-delivery (52)	17	−45	−25	−21	−20	−10	−1
		Delivery (102)	25	0	0	0	0	0	0
Baby	Stool	1 Week (119)	101	4	7	8	8	9	14
		1 Month (122)	106	18	27	31	35	38	79
		3 Months (119)	108	81	90	95	99	99	166
		6 Months (123)	114	153	173	181	193	194	332
		18 Months (103)	99	516	532	544	560	566	720

TABLE 2

Characteristics of the overall MAAP cohort and those with infant stool microbiota measurements.

Covariate	Statistic	Level	Overall cohort (*N* = 141)	Has baby stool microbiota (*N* = 131)	Indoor dog(s) during pregnancy (*N* = 131)		*p*-Value
					No *N* = 56	Yes *N* = 75	
African American mother[a]	*N*(Col %)	No	112 (79.4%)	107 (81.7%)	40 (71.4%)	67 (89.3%)	.009[b]
	N(Col %)	Yes	29 (20.6%)	24 (18.3%)	16 (28.6%)	8 (10.7%)	
Maternal-reported marital status	*N*(Col %)	No	19 (13.5%)	16 (12.2%)	6 (10.7%)	10 (13.3%)	.651[b]
	N(Col %)	Yes	122 (86.5%)	115 (87.8%)	50 (89.3%)	65 (86.7%)	
Household income	*N*(Col %)	<$40 K	21 (14.9%)	20 (15.3%)	14 (25%)	6 (8%)	.006[b]
	N(Col %)	$40 K–$80 K	33 (23.4%)	32 (24.4%)	7 (12.5%)	25 (33.3%)	
	N(Col %)	>$80 K	64 (45.4%)	60 (45.8%)	28 (50%)	32 (42.7%)	
	N(Col %)	Refused/do not know	23 (16.3%)	19 (14.5%)	7 (12.5%)	12 (16%)	
Highest level of maternal education	*N*(Col %)	HS diploma or less	22 (15.7%)	19 (14.6%)	8 (14.3%)	11 (14.9%)	.493[b]
	N(Col %)	Some college, Associate's degree or tech school	40 (28.6%)	37 (28.5%)	13 (23.2%)	24 (32.4%)	
	N(Col %)	Bachelor's degree	40 (28.6%)	38 (29.2%)	20 (35.7%)	18 (24.3%)	
	N(Col %)	Advanced degree	38 (27.1%)	36 (27.7%)	15 (26.8%)	21 (28.4%)	
Lives in single family home	*N*(Col %)	No	30 (21.7%)	27 (20.9%)	20 (35.7%)	7 (9.6%)	<.001[b]
	N(Col %)	Yes	108 (78.3%)	102 (79.1%)	36 (64.3%)	66 (90.4%)	
First-born child	*N*(Col %)	No	90 (63.8%)	82 (62.6%)	40 (71.4%)	42 (56%)	.071[b]
	N(Col %)	Yes	51 (36.2%)	49 (37.4%)	16 (28.6%)	33 (44%)	
Mode of delivery	*N*(Col %)	Vaginal	96 (68.1%)	89 (67.9%)	41 (73.2%)	48 (64%)	.264[b]
	N(Col %)	C-section	45 (31.9%)	42 (32.1%)	15 (26.8%)	27 (36%)	
Mother ever diagnosed with eczema	*N*(Col %)	No	122 (86.5%)	112 (85.5%)	47 (83.9%)	65 (86.7%)	.66[b]
	N(Col %)	Yes	19 (13.5%)	19 (14.5%)	9 (16.1%)	10 (13.3%)	
Mother ever diagnosed with asthma	*N*(Col %)	No	95 (67.4%)	90 (68.7%)	37 (66.1%)	53 (70.7%)	.575[b]
	N(Col %)	Yes	46 (32.6%)	41 (31.3%)	19 (33.9%)	22 (29.3%)	
Mother tobacco smoker at pre-delivery	*N*(Col %)	No	131 (93.6%)	122 (93.8%)	55 (98.2%)	67 (90.5%)	.137[c]
	N(Col %)	Yes	9 (6.4%)	8 (6.2%)	1 (1.8%)	7 (9.5%)	
Household tobacco smoke at pre-delivery	*N*(Col %)	No	125 (91.9%)	117 (92.1%)	53 (96.4%)	64 (88.9%)	.185[c]

Covariate	Statistic	Level	Overall cohort (*N* = 141)	Has baby stool microbiota (*N* = 131)	Indoor dog(s) during pregnancy (*N* = 131)		*p*-Value
					No *N* = 56	Yes *N* = 75	
	N(Col %)	Yes	11 (8.1%)	10 (7.9%)	2 (3.6%)	8 (11.1%)	
Child sex	*N*(Col %)	Male	68 (48.6%)	63 (48.1%)	24 (42.9%)	39 (52%)	.30[b]
	N(Col %)	Female	72 (51.4%)	68 (51.9%)	32 (57.1%)	36 (48%)	
Antibiotic use in first 6 months of life	*N*(Col %)	No	115 (89.8%)	114 (89.8%)	47 (88.7%)	67 (90.5%)	.733[b]
	N(Col %)	Yes	13 (10.2%)	13 (10.2%)	6 (11.3%)	7 (9.5%)	
Antifungal use in first 6 months of life	*N*(Col %)	No	121 (94.5%)	120 (94.5%)	50 (94.3%)	70 (94.6%)	.99[c]
	N(Col %)	Yes	7 (5.5%)	7 (5.5%)	3 (5.7%)	4 (5.4%)	
Maternal age at birth (years)	*N*		141	131	56	75	.952[d]
	Mean		31	31.05	31.02	31.07	
	SD		4.98	4.92	4.68	5.13	
Number of previous pregnancies	*N*		141	131	56	75	.072 [e]
	Mean		1.52	1.51	1.73	1.36	
	SD		1.32	1.35	1.31	1.36	
Number of previous live births	*N*		141	131	56	75	.093 [e]
	Mean		0.98	0.96	1.11	0.85	
	SD		0.99	1	1	0.98	
Number of children in the Home at pre-delivery	*N*		141	131	56	75	.555 [e]
	Mean		0.99	0.99	1.04	0.96	
	SD		1.1	1.11	1.11	1.12	
Breastfeeding duration (weeks)	*N*		125	124	51	73	.26 [e]
	Mean		30.63	30.69	33.8	28.51	
	SD		24.46	24.55	25.47	23.83	
Formula feeding duration (weeks)	*N*		127	126	52	74	
	Mean		30.00	30.01	25.06	33.49	.065 [e]
	SD		22.88	22.98	23.30	22.25	
Formula feeding at 1 week	*N*(Col %)	No	90 (67.2%)	88 (67.2%)	41 (73.2%)	47 (62.7%)	.203[b]
	N(Col %)	Yes	44 (32.8%)	43 (32.8%)	15 (26.8%)	28 (37.3%)	
Formula feeding at 1 month	*N*(Col %)	No	83 (66.9%)	82 (66.7%)	38 (74.5%)	44 (61.1%)	.120[b]
	N(Col %)	Yes	41 (33.1%)	41 (33.3%)	13 (25.5%)	28 (38.9%)	

Covariate	Statistic	Level	Overall cohort (*N* = 141)	Has baby stool microbiota (*N* = 131)	Indoor dog(s) during pregnancy (*N* = 131)		*p*-Value
					No *N* = 56	Yes *N* = 75	
Formula feeding at 3 months	*N*(Col %)	No	73 (58.4%)	72 (58.1%)	32 (61.5%)	40 (55.6%)	.505[b]
	N(Col %)	Yes	52 (41.6%)	52 (41.9%)	20 (38.5%)	32 (44.4%)	
Formula feeding at 6 months	*N*(Col %)	No	53 (44.9%)	53 (45.3%)	21 (43.8%)	32 (46.4%)	.779[b]
	N(Col %)	Yes	65 (55.1%)	64 (54.7%)	27 (56.3%)	37 (53.6%)	
Formula feeding at 18 months	*N*(Col %)	No	106 (96.4%)	106 (96.4%)	41 (95.3%)	65 (97%)	.643[c]
	N(Col %)	Yes	4 (3.6%)	4 (3.6%)	2 (4.7%)	2 (3.0%)	
First solid food introduction (weeks)	*N*		123	122	50	72	.074[d]
	Mean		20.72	20.75	21.92	19.94	
	SD		6	6.01	4.43	6.82	
Number of sequenced baby stool samples	*N*		131	131	56	75	.506[e]
	Mean		4.03	4.03	3.91	4.12	
	SD		1.03	1.03	1.18	0.90	

[a] If multiracial with at least one part Black, assigned to Black category.

[b] Calculated by Chi-square test.

[c] Calculated by Fisher's exact test.

[d] Calculated by ANOVA.

[e] Calculated by Kruskal–Wallis test.

TABLE 3

Association between dog exposure and infant gut α-diversity trajectories, before and after covariate adjustment.

Model	*N*	β^a	SE	*p*-Value	Percent change in unadjusted β
Richness (Linear model)					
Unadjusted	131	10.0	6.0	.094	N/A
Adjusted for maternal race	131	11.6	6.1	.058	16.0%
Adjusted for household income	131	7.0	6.1	.25	−30.0%
Adjusted for maternal age at birth	131	9.7	5.8	.098	−3.0%
Adjusted for mode of delivery	131	11.2	5.9	.059	12.0%
Adjusted for child sex	131	10.4	6.0	.082	4.0%
Adjusted for first-born child	131	10.4	6.0	.088	4.0%
Fully adjusted (all of the above)	131	11.1	6.3	.080	11.0%
Evenness (Linear model)					
Unadjusted	131	0.007	0.0122	.54	N/A
Adjusted for maternal race	131	0.0082	0.0126	.52	9.3%
Adjusted for household income	131	−0.001	0.0128	.94	−113.3%
Adjusted for maternal age at birth	131	0.007	0.0122	.56	−6.7%
Adjusted for mode of delivery	131	0.0089	0.0122	.47	18.7%
Adjusted for child sex	131	0.0084	0.0122	.49	12.0%
Adjusted for first-born child	131	0.006	0.0123	.63	−20.0%
Fully adjusted (all of the above)	131	0.002	0.0136	.89	−74.7%
Faith's phylogenetic diversity (Quadratic model)					
Unadjusted	131	0.65	0.36	.071	N/A
Adjusted for maternal race	131	0.73	0.37	.050	12.3%
Adjusted for household income	131	0.53	0.37	.15	−18.5%
Adjusted for maternal age at birth	131	0.63	0.35	.074	−3.1%
Adjusted for mode of delivery	131	0.72	0.35	.042	10.8%
Adjusted for child sex	131	0.68	0.36	.061	4.6%
Adjusted for first-born child	131	0.69	0.36	.058	6.2%
Fully adjusted (all of the above)	131	0.78	0.38	.041	20.0%

[a]Average difference in specified α-diversity metric (comparing dog-exposed vs. pet-free), throughout early life, based on linear mixed effect models.