Environmental Research 131 (2014) 25–30

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Short Communication

Associations between bacterial communities of house dust and infant gut

T. Konya [a], B. Koster [a], H. Maughan [b], M. Escobar [a], M.B. Azad [c], D.S. Guttman [b], M.R. Sears [d], A.B. Becker [e], J.R. Brook [a,f], T.K. Takaro [g], A.L. Kozyrskyj [c], J.A. Scott [a,*], the CHILD Study Investigators [1]

[a] Division of Occupational and Environmental Health, Dalla Lana School of Public Health, University of Toronto, Canada
[b] Department of Cell and Systems Biology, University of Toronto, Canada
[c] Department of Pediatrics, University of Alberta, Canada
[d] Department of Medicine, McMaster University, Canada
[e] University of Manitoba, Canada
[f] Environment Canada, Canada
[g] Faculty of Health Science, Simon Fraser University, Canada

ARTICLE INFO

Article history:
Received 19 August 2013
Received in revised form
14 January 2014
Accepted 19 February 2014
Available online 15 March 2014

Keywords:
Gut microbiota
Child health
Built environment

ABSTRACT

The human gut is host to a diverse and abundant community of bacteria that influence health and disease susceptibility. This community develops in infancy, and its composition is strongly influenced by environmental factors, notably perinatal anthropogenic exposures such as delivery mode (Cesarean vs. vaginal) and feeding method (breast vs. formula); however, the built environment as a possible source of exposure has not been considered. Here we report on a preliminary investigation of the associations between bacteria in house dust and the nascent fecal microbiota from 20 subjects from the Canadian Healthy Infant Longitudinal Development (CHILD) Study using high-throughput sequence analysis of portions of the 16S rRNA gene. Despite significant differences between the dust and fecal microbiota revealed by Nonmetric Multidimensional Scaling (NMDS) analysis, permutation analysis confirmed that 14 bacterial OTUs representing the classes Actinobacteria (3), Bacilli (3), Clostridia (6) and Gammaproteobacteria (2) co-occurred at a significantly higher frequency in matched dust–stool pairs than in randomly permuted pairs, indicating an association between these dust and stool communities. These associations could indicate a role for the indoor environment in shaping the nascent gut microbiota, but future studies will be needed to confirm that our findings do not solely reflect a reverse pathway. Although pet ownership was strongly associated with the presence of certain genera in the dust for dogs (*Agrococcus*, *Carnobacterium*, *Exiguobacterium*, *Herbaspirillum*, *Leifsonia* and *Neisseria*) and cats (*Escherichia*), no clear patterns were observed in the NMDS-resolved stool community profiles as a function of pet ownership.

1. Introduction

The human gut contains approximately 10^{14} bacteria representing at least 400–500 different species (Penders et al., 2007). These bacterial commensals create an environment within the gut that both helps to protect the human from pathogenic bacteria and contributes to gut metabolic activity. In addition, there is mounting evidence that pioneer gut microbiota influence the host immune

* Corresponding author.
E-mail address: james.scott@utoronto.ca (J.A. Scott).

[1] Additional CHILD study investigators include: R. Allen (Simon Fraser University), D. Befus (University of Alberta), M. Brauer (University of British Columbia), M. Cyr (McMaster University), E. Chen (University of British Columbia), D. Daley (James Hogg iCAPTURE Centre), S. Dell (Hospital for Sick Children), J. Denburg (McMaster University), S. Elliott (University of Waterloo), H. Grasemann (Hospital for Sick Children), K. HayGlass (University of Manitoba), R. Hegele (University of Toronto), L. Holness (St. Michael's Hospital), M. Kobor (University of British Columbia), T. Kollmann (University of British Columbia), C. Laprise (Chicoutimi University Hospital), M. Larché (McMaster University), W. Lou (University of Toronto), J. Macri (McMaster University), P. Mandhane (University of Alberta), G. Miller (North Western University), R. Moqbel (University of Manitoba), T. Moraes (Hospital for Sick Children), P. Paré (University of British Columbia), C. Ramsey (University of Manitoba), F. Ratjen (Hospital for Sick Children), B. Ritchie (University of Alberta), A. Sandford (James Hogg iCAPTURE Centre), Jeremy Scott (Lakehead University), F. Silverman (University of Toronto), P. Subbarao (Hospital for Sick Children), S. Tebbutt (James Hogg iCAPTURE Centre), P. Tang (University of British Columbia), T. To (Hospital for Sick Children), and S. Turvey (University of British Columbia).

http://dx.doi.org/10.1016/j.envres.2014.02.005
0013-9351

phenotype. Numerous studies have found associations with the microbial composition of the infant gut and asthma and allergy (Sjögren et al., 2009; Johansson et al., 2011; Martin et al., 2012; Van Nimwegen et al., 2011; Abrahamsson et al., 2012; Nakayama et al., 2011). Despite much study, the origin of these bacteria remains uncertain with most researchers conjecturing a predominant pattern of vertical transmission from mother to baby during delivery and nurturing activities. Little consideration has been given to the indoor home environment itself as a possible contributor to the gut microbiota.

The infant gut has long been thought sterile inside the womb although some colonization may happen in utero (Jimenéz et al., 2005). The first environmental exposure occurs peripartum where the infant contacts mother's vaginal, fecal, and perineal microbial communities (Adlerberth et al., 2006; Orrhage et al., 1999; Koenig et al., 2011). Many exposures in the first year of life influence bacterial colonization (e.g., gestational age, mode of delivery, breastfeeding, age of weaning, number of siblings, and exposure to antibiotics) (Yap et al., 2011; Torrazza et al., 2011; Azad et al., 2013a,b). Bacteria ingested by the infant from extraneous sources are mostly transient in the intestine but some have been known to establish residency and thrive under the gut conditions (Apajalahti, 2005), suggesting that the environment may be an important reservoir supporting colonization. The impacts of environmental exposure factors, in turn, are influenced by host features such as genetics, anatomical development of the intestinal tract, peristalsis, bile acids, intestinal pH and immune responses, microbial interactions, mucosal receptors, and drug therapy (Penders et al., 2007; Apajalahti, 2005; Schwartz et al., 2012; Smith et al., 2012; Fanaro et al., 2003).

The abundance of indoor microbes has long been known (Carnelley et al., 1887) but only recently has the complexity of these communities been revealed by culture-independent methods (Rintala et al., 2012; Flores et al., 2013). The bacterial community of dust is dominated by Proteobacteria and the Gram-positive phyla Firmicutes and Actinobacteria. The most common genera are associated with human skin and gut: *Corynebacterium*, *Propionibacterium*, *Streptococcus*, *Staphylococcus*, *Lactococcus*, *Peptostreptococcus*, *and Lactobacillus* (Rintala et al., 2012). Other home characteristics also influence the dust microbiota. Homes with dogs are known to have elevated dust endotoxin (Wickens et al., 2003), implying that these non-human occupants contribute to the indoor microbial burden. Dogs also appear to modify the microbiota of their owners (Song et al., 2013) and may affect the gut microbiota during early life (Azad et al., 2013c). The question of whether house dust is a source of colonizing microbes for the infant gut has not been investigated. To address this gap, the goal in this project was to assess community-level associations between the microbiota of the infant feces and house dust collected during a home assessment of a subset of 20 children aged 3–4 months who were early participants in the Canadian Healthy Infant Longitudinal Development (CHILD) Study.

2. Materials and methods

2.1. Study design and sample collection

This descriptive study of 20 infants represents a subset of early-recruited subjects in the CHILD (Canadian Healthy Infant Longitudinal Development) Study – a national population-based birth cohort (www.canadianchildstudy.ca). Participants were enrolled in Winnipeg, Manitoba between November 2008 and August 2009. This study was approved by the University of Manitoba Human Research Ethics Board. During a home assessment at the 3-month post-partum time-point, parents completed standardized questionnaires which addressed housing characteristics and the number and types of household pets present. Parents were also asked to place a diaper liner in their child's diaper the day before the home visit and to change the liner with the diaper until stool has been deposited in the liner. Once deposited, they were instructed to place the diaper with diaper liner into a collection bag, record the time of collection, and place the sealed bag into a refrigerator until collected by the research team at the home visit. Trained research assistants collected the fecal sample from the diaper using a screw cap container with spoon (Globe Scientific, Paramus, NJ, USA) and transported to the laboratory in a cooler where it was subdivided into 40 mg aliquots that were stored at −80 °C.

Vacuum collected dust samples were also collected by the research technicians at the 3 month home visit. Technicians used a clean, depyrogenated CHILD study designed aluminum collection device attached to the end of a vacuum cleaner (Model S3680, Sanitaire Canister Vac, Charlotte, NC, USA) to collect floor dust. The collection device holds two nylon DUSTREAM filters (Indoor Biotechnologies Inc, Charlottesville, VA). Technicians collected dust from a 2 m^2 area of carpeted flooring or the entire floor in the case of rooms with smooth floors. Samples for this project were obtained from residual, unused portions of vacuum cleaner-collected dust samples left over from the other dust analyses carried out in the CHILD study.

2.2. DNA extraction and amplification

Whole genome DNA was extracted from the stool aliquot and coarse dust (single DNA extraction of each matrix per subject) using the FastPrep DNA for Soil Kit (MP Biomedicals Inc, Solon, OH, USA). Bacterial 16S DNA, hypervariable regions V5-V7, was amplified through PCR using forward primer V5+791 (5′–3′ sequence: AATCAGGCGGGKAKCRAACVGGATTAGATACCCBGGTAGTCCWNRCHSTAAACGDTG) (mV5+791) and reverse primer Uni-1104 (5′–3′ sequence: AATCAGGCGGSCRTRMKGAYTTGACGTCRYCCCCDCCTTCCTCC) (V7–1104), according to Maughan et al. (2012). Each primer contained a phosphate added to the 5′ end for ligation of the Illumina adapters. The primers were also barcoded so each sample could be uniquely identified post-sequencing. Each PCR mixture (50 μL) contained 5 μL 10× Hotstart Buffer, 400 μM dNTPs, 1.5 mM $MgCl_2$, 2.5 U Hotstart Taq polymerase (Fermentas, Glen Burnie, MD, USA), 20 μg Ultrapure Bovine Serum Albumin (Ambion, Austin TX), molecular biology reagent grade water (Sigma-Aldrich, St. Louis, MO, USA), 0.16 μM primer, and 2 μL bacterial template DNA (10 ng/μL). The PCR program for stool consisted of an initial DNA denaturation step at 94 °C (4 min), followed by 18 cycles of DNA denaturation at 94 °C (0.75 min), an annealing step at 56 °C (0.5 min) and an elongation step at 72 °C (2.5 min), and was performed on the PTC-200 Peltier Thermal Cycler (MJ Research, St. Bruno, QC, Canada).

Genomic DNA from each sample was amplified using EvaGreen Mix and run in the MiniOpticon Real-Time PCR System (BioRad) to visualize the amount of amplified DNA after each cycle on the Opticon Monitor software program. An optimal cycle number was chosen for each sample showing unambiguous exponential amplification at a point prior to the plateau phase. Samples not exhibiting exponential amplification (interpreted as PCR inhibition) were subjected to a modified amplification procedure in which initial PCRs of up to 25 cycles were diluted 1:10 and re-amplified under identical PCR conditions for 12 additional cycles. Multiple PCRs (between 4 and 8 reactions, as necessary to obtain sufficient product using the minimum number of cycles) were performed for each sample (stool and dust). Negative PCR controls were included in all reactions, and uniformly yielded no product.

All final PCR products were cleaned with GENECLEAN Turbo Kit (MP Biomedicals Inc, Solon, OH, USA) and gel purified using the E-gel SizeSelect 2% agarose gel cutting system (Invitrogen, Carlsbad,

CA, USA). Fifty nanograms of cleaned/extracted product from each sample was combined and concentrated, using an Amicon Ultra-4 30 K centrifugal filter (Millipore, Billerica, MA, USA), for sequencing.

2.3. Sequencing

Sequencing was performed by the Centre for the Analysis of Genome Evolution & Function (CAGEF) at the University of Toronto using a novel Serial Illumina Sequencing (SI-Seq) method (Maughan et al., 2012). The method involved only sequencing the hypervariable V5, V6, and V7 regions of the 16S rDNA gene to create fragmented reads which were then concatenated for a final length of 144 bp, then processed through the SI-Seq analysis pipeline for de-barcoding and quality filtering, which removed reads having more than 10 sites with a Phred score less than 20 (as described in Ref. Maughan et al. (2012)). The resulting high quality FASTA-formatted sequences were denoised, cleared of chimeras, and clustered into operational taxonomic units (OTUs) using the "otu-pipe" function of USEARCH (Edgar, 2010). A representative sequence from each OTU was then compared against the SILVA reference database for taxonomic classification, and an empirically derived nucleotide identity threshold of 87% was used for OTU clustering (Maughan et al., 2012). One representative sequence from each OTU was classified according to the SILVA taxonomy by 95% identity (i.e., genus level) clustering with the SILVA database sequences formatted to SI-Seq read structure. Within each sample, OTUs with abundances lower than 0.18% were removed from the analysis based on an empirically derived misclassification/sequencing error rate (Maughan et al., 2012).

A single species control was placed in the run to determine a minimum abundance cut-off that would exclude rare taxa that may have only been present due to contamination or sequencing errors. The minimum abundance cut-off for our data was 0.18%, and data analyses were performed on the portion of the data set remaining after this cut-off was applied.

2.4. Data analysis

Summary statistical analysis was performed using Microsoft Excel. Associations between taxonomic and environmental factors were computed using Metastats (http://metastats.cbcb.umd.edu) (White et al., 2009). Nonmetric Multidimensional Scaling (NMDS) unconstrained ordination method was conducted with PC-ORD version 6 (McCune et al., 2011) to investigate patterns of covariation. NMDS was chosen due to the highly heterogeneous nature of our data and its ability to assess non-linear relationships among responses (Peck, 2010). An initial 3-dimensional NMDS was performed using the autopilot mode to find the number of axes that best represents the variation in the dataset. The process was repeated 3 times to ensure qualitatively consistent results.

Table 1
Summary of permutation test statistics for comparison of dust and gut microbial communities.

Parameter	Score
Actual value under true pairing	7.81
Estimated value under random pairing	
Mean (SD)	5.82 (0.96)
95% interval	4.24–7.62
Probability (true > random)	0.9836
P-value (1-sided)	0.0164*
P-value (2-sided)	0.0328*

* Significant p value indicates greater extent of the actual pairing than expected by random parings in the permutation test.

A modified permutation test was performed in R to assess the association between dust and stool communities. The usual application of the permutation test is similar to Student's T test, where groups are compared pair-wise. Accordingly, the permutation test permutes the labels of the two groups. In this study, we lack two groups but rather have "actual pairings" and "randomly assigned pairings", which are the agents that are permuted. Two different scoring rules were used, but our results were the same for both scoring approaches. The score (S) reported here is the square root of the sum of squares

$$S = \sqrt{\sum_{i=1}^{n} d_i^2} \quad (1)$$

where n is the number of samples, d is the number of shared OTUs between a paired dust and stool sample, and i is the index of summation.

In our test, 100,000 permutations were performed in which the pairing of the 20 stool samples to the 20 dust was done randomly. The resulting observed overlaps in OTUs were used to compute the distribution of the score under random pairing reported in Table 1. The score obtained under the true pairing is then compared to this distribution to compute a p-value.

3. Results and discussion

Infant stool and dust samples were obtained from 20 subject households. Other home characteristics were also recorded, such as the presence and types of pets present. Of the homes assessed, 65% had one or more pets (30% with dogs only, 15% with cats only, and 20% with both).

Our results revealed vastly different microbial communities in dust and stool, as expected. Both were dominated by members of the phyla Actinobacteria, Firmicutes and Proteobacteria, with dust additionally showing a large content of Cyanobacteria (Fig. 1). However, roughly one third of the "cyanobacterial" taxa (24/73) represented chloroplasts sequences from plants (e.g., *Abies*, *Agrostis*, *Cucumis*, *Lemna*, *Pisum*, *Plantanus*, *Thujopsis*, and *Trachelium*) and eukaryotic algae (e.g., *Chlorella* and *Scenedesmus*) (Supplementary Table A). Much greater total diversity was found in dust than in stool (652 vs. 108 OTUs overall), and the two communities were clearly demarcated in Nonmetric Multidimensional Scaling (NMDS) analysis (Fig. 2).

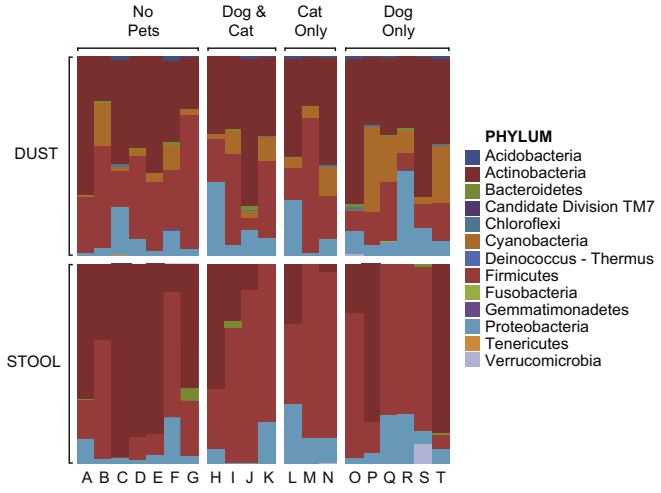

Fig. 1. Relative abundance of microbiota in dust and stool (calculated as the proportion of sequencereads) at the Phylum level by house pet status: subject households A–G lacked pets, H–K had atleast 1 dog and 1 cat, L–N had at least one cat and no dogs, and O–T had at least one dog and no cats.

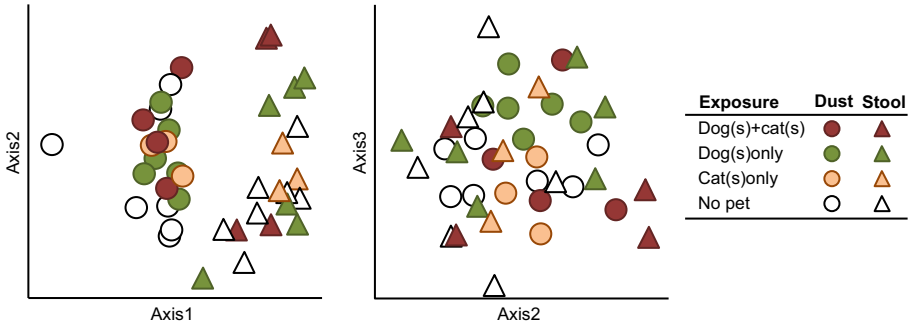

Fig. 2. Three-dimensional NMDS ordination plots of axis 1 vs. 2 (left) and axis 2 vs. 3 (right) showing microbial communities of dust (circles) andstool (triangles) coded according to the presence (shaded) or absence (unshaded) of a pet in thehome and its type: dog/cat (red), dog only (green), cat only (orange).

Table 2
OTUs contributing significantly to dust–fecal community overlap.

Genbank#	# Pairs shared[a]	Phylum	Class	Order	Family	Genus
EU763194	2	Actinobacteria	Actinobacteria	Actinobacteridae	Bifidobacteriaceae	*Bifidobacterium*
EU767611	2	Actinobacteria	Actinobacteria	Actinobacteridae	Bifidobacteriaceae	*Bifidobacterium*
AY850360	1	Actinobacteria	Actinobacteria	Actinobacteridae	Bifidobacteriaceae	*Bifidobacterium*
EU776066	1	Firmicutes	Bacilli	Bacillales	Planococcaceae	*Planomicrobium*
EF398803	5	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	*Streptococcus*
DQ447791	1	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	*Lactococcus*
EU771602	3	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unknown
EU762821	2	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unknown
GQ422725	1	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	*Veillonella*
CP000312	1	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unknown
DQ455971	1	Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	Unknown
DQ113670	1	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	*Faecalibacterium*
CU928162	5	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Escherichia*
DQ676994	1	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Escherichia*

[a] Denotes the number of infant–dust pairs that shared the particular OTU (total pairs=20).

Pet ownership strongly associated with the occurrence of certain genera in the dust separately for dogs (*Agrococcus*, *Carnobacterium*, *Exiguobacterium*, *Herbaspirillum*, *Leifsonia* and *Neisseria* – all common genera of naturally occurring outdoor soil, water and food bacteria with potential to be tracked into homes on dog paws) and cats (*Escherichia* – a genus of mammalian gut commensals whose enhanced presence may be a consequence of indoor feline toileting accommodations), but not for both types of pets in combination (Supplementary Table B).

The plot of NMDS axis 1 vs. 2 (Fig. 2, left) clearly separated dust communities from stool communities, indicating that axis 1 largely corresponded to sample type, as expected. In this plot, no patterning was observed within dust and stool communities according to overall pet ownership or cat ownership; however, the stool communities of those infants in homes where a dog was present appeared to form two distinctive subgroupings. The basis for these subgroupings was uncertain, but may relate to information not collected (e.g., sex of pets, neutered status, breed, etc.). A plot of NMDS axis 2 vs. 3 (Fig. 2, right) lacked any clear separation of samples or subject groupings.

Despite the clear qualitative differences between dust and stool communities revealed by NMDS, certain OTUs co-occurred at a significantly higher frequency in co-located dust and stool samples than in randomly paired dust and stool samples (Table 1). Notable OTUs contributing to the dust–stool community overlap spanned 4 mainly anaerobic classes: Actinobacteria (3 OTUs of *Bifidobacterium* spp.), Bacilli (1 OTU each of *Lactococcus*, *Streptococcus* and *Planomicrobium*), Clostridia (1 OTU each of *Veillonella* and *Faecalibacterium* in addition to 4 unknown OTUs), and Gammaproteobacteria (2 OTUs of *Escherichia*) (Table 2).

The strong tendency towards oxygen sensitivity amongst the overlapping OTUs implies that these microbes probably originate from the gut and are unlikely to undergo amplification in the environment, although they may remain viable. For example, oxygen sensitivity is strongly species-dependent in the genus *Bifidobacterium* whose members range from obligate anaerobes to microaerophiles, whereas members of the family Lachnospiraceae tend to be obligate anaerobes. When present in dust, these microbes have arisen elsewhere, likely through household activities such as diaper changing and possibly aerosolization of fecal bacteria through “toilet spray” (Flores et al., 2011; Gerba et al., 1975; Barker et al., 2005). In addition, some gut-borne lactic acid bacteria (especially members of the genus *Lactococcus* and certain Bifidobacteria) proliferate naturally on some organic materials under suitable conditions (e.g., dairy products, sauerkraut, dough) (Leroy et al., 2004). Thus, a wide range of normal household activities may result in the aerosolization of these taxa and their subsequent deposition and accumulation in dust. Culture studies have recovered members of both anaerobic and aerobic bacteria from house dust (Baldwin et al., 1957), suggesting the possibility that propagules remain viable in dust for extended periods of time. This phenomenon has been well-studied in spore-forming enteric bacteria (e.g., *Clostridium difficile*) (Russell et al., 1999). From aerobic to anaerobic bacteria, then, house dust could contribute inoculum to the nascent gut microbiota when children are exposed normally to dust and fomites. Early work using culture-based methods suggested the possibility that intermediate environmental bacterial reservoirs may play a role in the transfer of gut bacteria between parents and babies (Bettelheim et al., 1974; Bettelheim and Lennox-King, 1976; Bettelheim et al., 1983), however the lack of comprehensive measures of microbial community composition prevented the confirmation of these findings simultaneously across a wide taxonomic range. Our previous report on the gut microbiota of this group of infants in relation to delivery mode and breast feeding showed the taxonomic composition of fecal specimens to be

comparable to other recent studies, and the influences of perinatal factors to be similar (Azad et al., 2013a,b).

In the present study, the community composition of house dust contained the same dominant bacterial phyla found in other sequence-based studies (Flores et al., 2013; Taubel et al., 2009). Likewise, in our study, the influence of pets corresponded with what has been shown (Fujimura et al., 2010).

For some time it has been suggested that the presence of a dog or cat in the home is protective against the development of atopy (Ownby et al., 2002). In an effort to determine the mechanism responsible for this effect, a very recent study examined the interaction between the gut microbiome and house dust microbiome in two homes – one with a dog and the other (control) lacking a dog (Fujimura et al., 2014). Consistent with ours and other studies, these workers showed a number of differences between the microbiota of dust according to the presence of a dog in the form of enrichment for certain taxa (Fujimura et al., 2010). Using a murine model, this interesting study further showed that gavage exposure to house dust modified the composition of cecal microbiota according to the presence of a dog in the home. Furthermore, the dog-modified mouse gut microbiota protected against a pro-inflammatory airway challenge. By selectively enriching control dust with taxa cultured from the dog-associated house and repeating the gavage and challenge procedures, the workers were able to deduce that *Lactobacillus johnsonii* (as represented by OTUs in the clade containing *L. gasseri*) was primarily responsible for the anti-inflammatory effect (Fujimura et al., 2014). Conceivably a similar mechanism may contribute to the beneficial anti-inflammatory or anti-atopic effects of pet ownership.

Our results support the long-standing hypothesis that humans and house dust each, in turn, serve as reservoirs and receptors of so-called "old friend" microbes – harmless microbes present throughout mammalian evolution that are thought to influence immune regulation (Kozyrskyj et al., 2011). In the process, this interaction may leave a lingering microbial imprint on the home. The demonstration of such a reciprocal pathway would have important implications on our understanding of the influence of environment on health. For example, sedentary families might be more likely to transmit their gut microbes to their children in a strictly vertical manner, whereas the children of families who change residences may be influenced by the microbial communities of prior home occupants. Exposure to dust from other environments, such as daycares, may similarly be influential.

To our knowledge, this is the first modern indication of an association between the microbiota of household dust and stool from the inhabitants of the house, and suggests a role for the indoor environment in shaping the nascent gut microbiota and/or vice versa. Our results are preliminary and, as such, neither imply health significance nor do they resolve the distribution pathway (s) responsible for the community similarities we observed, but it remains to be confirmed by future studies that our findings do not solely reflect a reverse pathway, i.e. the impact of the human occupant on the house dust microbiome. Despite these limitations, it is clear that further investigation of these community associations may yield valuable insight into the interactions between environment and health.

Acknowledgments

We thank the study families and research teams of the Canadian Healthy Infant Longitudinal Development (CHILD) Study, AllerGen NCE for supporting pilot dust analyses, and the Canadian Institutes of Health Research (CIHR) for funding the *Sy*MBIOTA team. The CHILD Study is supported by the Canadian Institutes of Health Research, the AllerGen Network of Centres of Excellence, the Childhood Asthma Foundation, Health Canada, Environment Canada, and Canada Mortgage and Housing Corporation. Professor L. Sly (University of Queensland) kindly provided comments and suggestions on an earlier version of this manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2014.02.005.

References

Abrahamsson, T.R., et al., 2012. Low diversity of the gut microbiota in infants with atopic eczema. J. Allergy Clin. Immunol. 129, 434–440.
Adlerberth, I., et al., 2006. Reduced enterobacterial and increased Staphylococcal colonization of the infantile bowel: an effect of hygienic lifestyle? Pediatr. Res. 59, 96–101.
Apajalahti, J., 2005. Comparative gut microflora, metabolic challenges, and potential opportunities. J. Appl. Poult. Res. 14, 444–453.
Azad, M.B., et al., 2013a. Gut microbiota diversity and atopic disease: does breastfeeding play a role? J. Allergy Clin. Immunol. 131, 247248.
Azad, M.B., et al., 2013b. Gut microbiota in Canadian infants: profiles by mode of delivery and infant diet at 4 months. Can. Med. Assoc. J. 185, 385–394.
Azad, M.B., et al., 2013c. Infant gut microbiota and the hygiene hypothesis of allergic disease: impact of household pets and siblings on microbiota composition and diversity. Allergy Asthma Clin. Immunol. 9, 15.
Baldwin, H.S., et al., 1957. Aerobic bacteria in house dust. J. Allergy 28, 179191.
Barker, J., et al., 2005. The potential spread of infection caused by aerosol contamination of surfaces after flushing a domestic toilet. J. Appl. Microbiol. 99, 339–347.
Bettelheim, K.A., Lennox-King, S.M.J., 1976. The acquisition of *Escherichia coli* by newborn babies. Infection 4, 174–179.
Bettelheim, K.A., et al., 1974. Spread of *Escherichia coli* colonizing new-born babies and their mothers. J. Hyg. 73, 383–387.
Bettelheim, K.A., et al., 1983. Relationship of an epidemic strain of *Escherichia coli* 0125.H21 to other serotypes of *E. coli* during an outbreak situation in a neonatal ward. Zentralbl. Bakteriol. Mikrobiol. Hyg. I. Abt. Orig. A 253, 509–514.
Carnelley, T., et al., 1887. The carbonic acid, organic matter, and micro-organisms in air, more especially of dwellings and schools. Philos. Trans. R. Soc. Lond. Ser. B 178, 61–111.
Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460–2461.
Fanaro, S., et al., 2003. Intestinal microflora in early infancy: composition and development. Acta Paediatr. 441, 48–55.
Flores, G.E., et al., 2011. Microbial biogeography of public restroom surfaces. PLoS ONE 6, e28132.
Flores, G.E., et al., 2013. Diversity, distribution and sources of bacteria in residential kitchens. Environ. Microbiol. 15, 588–596.
Fujimura, K.E., et al., 2010. Man's best friend? The effect of pet ownership on house dust microbial communities. J. Allergy Clin. Immunol. 126 (2), 410–412.
Fujimura, K.E., et al., 2014. House dust exposure mediates gut microbiome *Lactobacillus* enrichment and airway immune defense against allergens and virus infection. Proc. Natl. Acad. Sci. USA (Early Edition) 111 (2), 805–810 (10.1073/pnas.1310750111).
Gerba, C.P., et al., 1975. Microbiological hazards of household toilets: droplet production and the fate of residual organisms. Appl. Microbiol. 30, 229–237.
Jimenéz, E., et al., 2005. Isolation of commensal bacteria from umbilical cord blood of health neonates born by Cesarean section. Curr. Microbiol. 51, 270–274.
Johansson, M.A., et al., 2011. Early colonization with a group of Lactobacilli decreases the risk for allergy at five years of age despite allergic heredity. PLoS ONE 6 (8), e23031.
Koenig, J.E., et al., 2011. Succession of microbial consortia in the developing infant gut microbiome. Proc. Natl. Acad. Sci. USA 108 (1), 4578–4585.
Kozyrskyj, A.L., et al., 2011. Early life exposures: impact on asthma and allergic disease. Curr. Opin. Allergy Clin. Immunol. 11, 400–406.
Leroy, F., et al., 2004. Lactic acid bacteria as functional starter cultures for the food fermentation industry. Trends Food Sci. Technol. 15, 67–78.
Martin, R., et al., 2012. Early life: gut microbiota and immune development in infancy. Benef. Microbes 1 (4), 367–382.
Maughan, H., et al., 2012. Analysis of the cystic fibrosis lung microbiota via Serial Illumina Sequencing of bacterial 16S rRNA hypervariable regions. PLoS ONE 7, e45791.
McCune, B., et al., 2011. PC-ORD. Multivariate Analysis of Ecological Data, Version 6, MjM Software. Gleneden Beach, Oregon, USA.
Nakayama, J., et al., 2011. Aberrant structures of fecal bacterial community in allergic infants profiled by 16S rRNA gene pyrosequencing. FEMS Immunol. Med. Microbiol. 63, 397–406.
Orrhage, K., et al., 1999. Factors controlling the bacterial colonization of the intestine in breastfed infants. Acta Paediatr. 430, 47–57.
Ownby, D.R., et al., 2002. Exposure to dogs and cats in the first year of life and risk of allergic sensitization at 6 to 7 years of age. J. Am. Med. Assoc. 288, 963–972.

Peck, J.E., 2010. Multivariate Analysis for Community Ecologists: Step-by-Step Using PC-ORD, MjM Software Design. Gleneden Beach, Oregon, USA.
Penders, J., et al., 2007. Gut microbiota composition and development of atopic manifestations in infancy: the KOALA birth cohort. Gut 56, 661–667.
Rintala, H., et al., 2012. Microbial communities associated with house dust. Adv. Appl. Microbiol. 78, 75–120.
Russell, A.D., et al., 1999. Bacterial resistance to antiseptics and disinfectants. J. Hosp. Infect. 43, S57–S68.
Schwartz, S., et al., 2012. A metagenomic study of diet-dependent interaction between gut microbiota and host in infants reveals differences in immune response. Genome Biol. 13, r32.
Sjögren, Y.M., et al., 2009. Altered early infant gut microbiota in children developing allergy up to 5 years of age. Clin. Exp. Allergy 29, 518–526.
Smith, P., et al., 2012. Host genetics and environmental factors regulate ecological succession of the mouse colon tissue-associated microbiota. PLoS ONE 7, e30273.
Song, S.J., et al., 2013. Cohabiting family members share microbiota with one another and with their dogs. eLife 2, e00458.
Taubel, M., et al., 2009. The occupant as a source of house dust bacteria. J. Allergy Clin. Immunol. 124 (4), 834–840.
Torrazza, R.M., et al., 2011. The developing intestinal microbiome and its relationship to health and disease in the neonate. J. Perinatol. 31, S29–S34.
Van Nimwegen, F.A., et al., 2011. Mode and place of delivery, gastrointestinal microbiota, and their influence on asthma and atopy. J. Allergy Clin. Immunol. 128, 948–955.
White, J.R., et al., 2009. Statistical methods for detecting differentially abundant features in clinical metagenomic samples. PLoS Comput. Biol. 5, e1000352.
Wickens, K., et al., 2003. Determinants of endotoxin levels in carpets in New Zealand homes. Indoor Air 13, 128–135.
Yap, G.C., et al., 2011. Evaluation of stool microbiota signatures in two cohorts of Asian (Singapore and Indonesia) newborns at risk of atopy. BMC Microbiol. 11, 193.