Environmental and mucosal microbiota and their role in childhood asthma

Lena T. Birzele[1]*, Martin Depner[1]*, Markus J. Ege[1,2], Marion Engel[3,4], Susanne Kublik[4], Christoph Bernau[5], Georg J. Loss[1,6], Jon Genuneit[7], Elisabeth Horak[8], Michael Schloter[4], Charlotte Braun-Fahrländer[9,10], Hanna Danielewicz[11], Dick Heederik[12], Erika von Mutius[1,2], Antje Legatzki[1]

* both authors contributed equally

1   Dr. von Hauner Children's Hospital, LMU Munich, Munich, Germany

2   Member of the German Center for Lung Research (DZL), Munich, Germany

3   Research Unit Scientific Computing, Helmholtz Center Munich, Neuherberg, Germany

4   Research Unit for Environmental Genomics, Helmholtz Center Munich, Neuherberg, Germany

5   Leibniz Supercomputing Center of the Bavarian Academy of Sciences and Humanities, Garching, Germany

6   School of Medicine, Department of Pediatrics, University of California, San Diego, USA

7   Institute of Epidemiology and Medical Biometry, Ulm University, Ulm, Germany

8   Department of Pediatrics and Adolescents, Division of Cardiology and Pulmonology, Innsbruck Medical University, Innsbruck, Austria

9   Swiss Tropical and Public Health Institute, Basel, Switzerland

[10] University of Basel, Department of Epidemiology and Public Health, Basel, Switzerland

[11] Department of Pediatrics, Allergology and Cardiology, Wroclaw Medical University, Wroclaw, Poland

[12] Institute for Risk Assessment Sciences, Division of Environmental Epidemiology, University of Utrecht, Utrecht, the Netherlands

Corresponding author and address for reprint requests:

Martin Depner, PhD

Dr von Hauner Children's Hospital, LMU MunichLindwurmstrasse 4

80337 Munich

Germany

Telephone: +49-89-4400-52776

Fax: +49-89-4400-54452

E-mail: Martin.Depner@med.uni-muenchen.de

Conception and design of the GABRIELA study and sample acquisition: HD, MJE, CBF, JG, DH, EH, EvM

Design of the study and planning of experiments: LTB, MJE, ME, MS, AL, SK

Processing of samples: LTB, ME, SK

Analysis and interpretation of the data: CB, LTB, MD, MJE, AL, GL

Writing of the manuscript: MD, MJE, AL

Editing and final approval of the paper: all authors

Abbreviations:

C.I.:	Confidence Interval
GABRIELA:	Multidisciplinary Study to Identify the Genetic and Environmental Causes of Asthma in the European Community – Advanced Studies
NMDS:	Non-metric multidimensional scaling
OTU:	Operational Taxonomic Unit
aOR:	adjusted Odds Ratio
GMR	Geometric Mean Ratio
16S rRNA:	16S ribosomal RNA
SSCP:	Single strand conformation polymorphism

Abstract

Background:

High microbial diversity in the environment has been associated with lower asthma risk, particularly in children exposed to farming. It remains unclear whether this effect operates through an altered microbiome of the mucosal surfaces of the airways.

Methods:

DNA from mattress dust and nasal samples of 86 school-age children was analyzed by 454-pyrosequencing of the 16S rRNA gene fragments. Based on operational taxonomic units bacterial diversity and composition was related to farm exposure and asthma status.

Results: Farm exposure was positively associated with bacterial diversity in mattress dust samples as determined by richness ($p=8.1 * 10^{-6}$) and Shannon index ($p=1.3 * 10^{-5}$). Despite considerable agreement of richness between mattress and nasal samples, the association of richness with farming in nasal samples was restricted to a high gradient of farm exposure, i.e. exposure to cows and straw versus no exposure at all. In mattress dust the genera *Clostridium*, *Facklamia*, an unclassified genus within the family of *Ruminococcaceae* and six OTUs were positively associated with farming. Asthma was inversely associated with richness (aOR= 0.48 [0.22-1.02]) and Shannon Index (aOR=0.41 [0.21-0.83]) in mattress dust and to a lower extent in nasal samples (richness aOR 0.63[0.38-1.06], Shannon Index aOR= 0.66 [0.39-1.12]).

Conclusion:

The stronger inverse association of asthma and bacterial diversity in mattress dust as compared to nasal samples suggests microbial involvement beyond mere colonization of the upper airways. Whether inhalation of metabolites of environmental bacteria contributes to this phenomenon should be the focus of future research.

Introduction

Living on a working farm is characterized by intensive contact of the farmers and their families with animals and plants. The farm environment offers various specific habitats and niches for microbes; e.g., animals, manure, feeding and bedding material are responsible for a higher microbial diversity at farms compared to non-farm environments (1). Also the indoor environment of farm dwellings is characterized by a higher microbial diversity as shown in mattress dust samples (2, 3). It remains, however, unclear whether the environmental microbiome affects the nasal microbiome. A key function of the nose is filtering inhaled air (4). Therefore, the nose may harbor microbes originating from the surrounding environment in addition to its local microflora.

A high diversity of environmental microorganisms has been found inversely associated with asthma in rural populations (5). Growing up on traditional farms consistently showed a reduced risk of childhood asthma (reviewed in (6, 7)). Different farm exposures such as contact with cows and straw, which obviously harbor a set of different microbes, have been identified as protective factors for asthma (8). Furthermore, several studies have demonstrated an association between specific environmental microbes or their cell wall components and a reduced risk of allergic diseases including childhood asthma (2, 9, 10). In addition, experimental mouse studies suggest protective effects of several bacterial strains isolated from the farm environment (11-13).

The aim of the current study was a comprehensive analysis of the bacterial exposure related to farming using a cultivation independent approach based on directly extracted DNA and high throughput sequencing of amplified 16S rRNA gene fragments.

Diversity and composition of bacterial microbiota of mattress dust and nasal swab samples of the same children were characterized and related to farming and asthma status.

Methods

Study design and subjects

The cross-sectional Multidisciplinary Study to Identify the Genetic and Environmental Causes of Asthma in the European Community – Advanced Studies (GABRIELA) included children 6-12 years of age from five rural areas in Europe (8) and was approved by the ethics committees of the participating universities and the regional data protection authorities.

From the Austrian arm of the GABRIELA study a stratified random sample of 102 children was drawn for microbial analyses (Figure E1). Children living on a farm run by the family were defined as farm children. Non-farm children were defined as children not living on a farm and not exposed to farm environments or cow's milk directly bought from a farm (8, 14). As a more specific farm-related exposure, contact to cow and straw during the last 12 months versus no contact to either was defined.

Asthma was defined as either parent-reported wheeze during the last 12 months, a positive answer to the question "Did your child ever use an asthma spray?" or a doctor's diagnosis of asthma at least once or of wheezy bronchitis more than once (8, 14). Medication was based as intake of any medication in the 4 weeks prior to nasal sampling.

Sample collection and processing

Mattress dust was collected by the parents of the participating children using a standardized dust collection protocol (see Supporting Information) (15). Nasal samples were collected by trained field workers using sterile dry cotton-headed swabs (MASTASWAB MD 559, MAST Diagnostica GmbH, Germany). The samples were collected between May and July 2007 (Figure E2). The two different specimens from each child were obtained on average within 2 days. After arrival at the study center the samples were stored at –80°C until further processing.

Mattress dust DNA was extracted from 30 mg dust using the FastDNA SPIN Kit for Soil (MP Biomedicals GmbH, Germany) including a bead beating step according the protocol of the manufacturer. Nasal swab DNA was extracted with the QIAmp DNA Mini Kit (Qiagen GmbH, Germany) using an adapted protocol (16).

At the beginning of this procedure a 20 seconds bead beating step with 50 mg acid-washed UV-treated glass beads (Sigma-Aldrich GmbH, Germany) was included. With every extraction run a negative control was processed. Extracted DNA was stored at -20°C until further processing.

The V3–V5 region of the bacterial 16S rRNA gene was amplified with the primers 357F (5'-CCTACGGGAGGCAGCAG-3') and 926R (5'-CCGTCAATTCMTTTRAGT-3') (17), tagged with sequencing adapters and barcodes (18) using the Fast Start High Fidelity PCR System (Roche Diagnostics Deutschland GmbH, Germany), (see Supporting Information). After purification of the amplicons with Agencourt AMPure XP beads (Beckmann Coulter GmbH, Germany) concentrations were determined using Quant-iT PicoGreen (Life Technologies). Subsequently the amplicons were pooled for sequencing at equimolar concentrations.

Unidirectional sequencing of the 16S rRNA gene fragments starting from the reverse primer 926R was performed using 454-GS FLX Titanium (Roche Diagnostics Deutschland GmbH, Germany) protocol. Raw data were processed by the 454 GS Run Processor v2.8 for shotgun reads. Denoising and removal of chimera was achieved by Ampliconnoise (19), resulting in about 400 bp long fragments. Following, files from all mattress dust and nasal samples were concatenated and sequences clustered together in operational taxonomic units (OTUs) using UCLUST (20) applying the de-novo picking method in QIIME version 1.8 (21). OTUs were defined as clusters of the respective 16S rRNA sequences with at least 97% sequence similarity. For taxonomy assignment, OTUs were aligned against the SILVA database version111 NR. OTUs classified as *Chloroplast* were excluded from further analysis as well as singletons.

Negative extraction controls were performed for both extraction protocols and evaluated (see Table E1 in the online supplement). A negligible read count close to zero validated the experiment. Following filtering children with less than 1,000 sequences in either the mattress dust or nasal samples were excluded, resulting in 86 children (Figure E1). Unclassified sequences were excluded from downstream analyses on phylum and genus level.

Data analysis

Alpha diversity, i.e. the diversity of the community within one sample, was calculated as number of OTUs (richness). In addition the Shannon index was calculated, which combines information on number and proportional abundance of taxa.

Differences in α-diversity between mattress dust and nasal swab were calculated by geometric means ratios, and agreement between richness in mattress dust and the nose on an individual level was assessed by a Bland-Altman plot.

Descriptive analyses were based on relative abundance of the main taxa, which were defined as being represented in at least 1% of all reads of all children. The remaining taxa (less than 1% relative abundance) were combined in one variable termed "rare group'. In addition, all samples were rarefied to 1,252 sequences, the minimum sequence number of all mattress dust and nasal swab samples. This process was performed in 1000 replicates per sample and subsequently averaged for α-diversity analysis. α-diversity indices such as species richness and Shannon index were calculated with the R package vegan (22). Survey weighted Wilcoxon tests were used to compare α-diversity between exposure groups. Moreover, presence of common genera and OTUs prevalent in at least 10% of the children was defined as the occurrence of the respective taxon in the rarefied sample.

To assess β-diversity, i.e. the diversity of the community between samples, we used the UniFrac measure (23) representing dissimilarity on a phylogenetic level and the Bray-Curtis dissimilarity index on a non-phylogenetic level. Survey-weighted regression models were used to relate differences in relative abundances or presence of single taxa to farm exposure and asthma status. Association analyses with asthma are adjusted for farming if not further specified. Correction for multiple comparisons was done by the Bonferroni method or the false discovery rate (FDR) as indicated. A mediation analysis was performed by multiplying the effect estimates of the single paths and tested for significance on the basis of a bootstrapped standard error.

All statistical analyses were performed with R 3.02 (24) and the phyloseq package (25) if not further specified. Survey-weighted analyses were applied to account for the stratified sampling design of the GABRIELA study (8, 14) by the R package "survey".

Results

Study population

The analyzed study population of 86 children did not differ from the original Austrian part of the GABRIELA study population with respect to farm exposure, asthma, and general demographic variables except for parental education (Table E2).

Bacterial community composition in mattress dust and nasal samples

Of all 1,730,479 reads, 212,030 reads from mattress dust and 29,179 reads from nasal samples aligned to *Chloroplast* sequences with one predominant OTU (>90% abundance in both sample types) being classified within the plant family *Pinaceae*. These were considered contaminants and

excluded leaving 618,635 reads from mattress dust and 870,635 reads from nasal samples for further analysis.

The mean number of reads was 7,193 for mattress dust and 10,120 for nasal samples (Figure E3). Rarefaction analysis showed an adequate saturation except for richness in mattress dust samples (Figure E4).

The two sampling sources separated well by phylogenetic and non-phylogenetic distance measures (Figure E5). On the phylum level, 0.48% of the mattress dust reads and 0.05% of nasal reads were unclassified; on the genus level the proportions were slightly higher (2.72% and 0.47%, respectively).

Bacterial mattress dust microbiota were dominated by the phyla *Firmicutes* (53.2%), *Proteobacteria* (16.8%), *Actinobacteria* (16.0%), and *Bacteroidetes* (10.9%), whereas in nasal samples *Proteobacteria* (44.8%), *Firmicutes* (37.1%), *Actinobacteria* (12.3%), and *Bacteroidetes* (4.1%) prevailed (Figure 1A). On the genus level, 23 main genera (genera with > 1% relative abundance on average) were observed in mattress dust, led by *Streptococcus* with 15.5% (Figure 1B). For the nasal samples 12 main genera were detected (Figure 1B) with *Moraxella* prevailing at 34.2%. Common to both sample sources were the genera *Streptococcus*, *Staphylococcus*, *Corynebacterium*, *Propionibacterium*, *Prevotella*, *Peptinophilus*, *Neisseria*, *Actinobacillus*, *Porphyromonas* and *Moraxella*. Most of the other main genera detected in the mattress dust were also present in the nose, however at frequencies below 1% on average. These genera defined as 'rare' accounted for 32.9% in mattress dust and 14.7% in the nose. The corresponding variable correlated with richness (mattress dust, r=0.77; nose, r=0.89). Mattress dust showed a higher α-diversity compared to nasal samples (richness: GMR=3.42; Shannon index GMR=2.56). On an individual level, richness values of mattress dust and nasal samples agreed reasonably (Figure 2).

Farm exposure and bacterial communities

As already suggested by Figure 2, α-diversity was significantly higher in mattress dust of farm children compared to non-farm children (richness p=8.1*10^{-6}; Shannon-Index p=1.3*10^{-5}, Figure 3A). Similarly, exposure to cow and straw was associated with higher bacterial α-diversity in mattress dust (Figure 3B). In nasal samples α-diversity was not associated with farming but with exposure to cow and straw (Figure 3A and B).

In mattress dust, farming was significantly associated with relative abundance of the 'rare' genera, a not further classified genus of the family *Ruminococcaceae* (Figure 4A) and six OTUs with sequence similarities to taxa previously related to cow feces (Table 1). In addition, farming was associated with the presence of the genera *Clostridium* and *Facklamia* and one OTU (OTU2597) belonging to the family of *Ruminococcaceae* (Table 1). For exposure to cow and straw similar associations emerged (Figure 4B, Table E3).

For the nasal samples, no significant association of farm exposures with relative abundance was seen except for a weak association of *Streptococcus* with cow/straw exposure (Figure 4). When not correcting for multiple testing, farming was associated with the presence of the genus *Prevotella* (aOR=4.84 [1.39-16.89], p=0.016), an unclassified genus of the *Ruminococcaceae* family (aOR=4.05

[1.22-13.43], p=0.025), and the *Ruminococcaceae* OTU 2597 (aOR=7.57 [1.94-29.55], p=4.6 *10^{-3}).
Also with respect to its relative abundance this OTU was related to farming (beta=0.37 [0.14-0.59],
p=1.9 *10^{-3}) thereby mirroring the associations observed in mattress dust.

Asthma and bacterial communities

Asthma was inversely associated with richness and Shannon index in mattress dust (aOR=0.48 [0.22-
1.02], p=0.060; aOR=0.41 [0.21-0.83], p=0.015, respectively) and nasal samples (aOR=0.63 [0.38-
1.06], p=0.087; aOR=0.66 [0.39-1.12], p=0.129, respectively); the effects of nasal and mattress
diversity were independent of each other (Figure 5) and not confounded by medication or atopy
status (Table E4). Exclusion of atopic controls increased the effect size only in nasal swabs (Table E4).

Richness in mattress dust (p=0.028), but not in nasal swabs (p=0.430) mediated the protective farm
effect on asthma as shown by the indirect effect of the path from farm on asthma via richness.

Among specific genera, inverse associations of asthma with the relative abundance of bacteria
belonging to the genus *Prevotella* in the nasal swabs (aOR=0.44 [0.21-0.93], p=0.0354) and with the
rare group (less than 1% relative abundance) in mattress dust (aOR=0.45 [0.24 0.84], p=0.014)
emerged, though the latter might be a proxy for bacterial diversity.

A presence vs. absence analysis on genus and OTU level revealed several candidates for asthma
protection both in mattress dust and nasal samples (Table E5). All significant hits for the specific taxa
require confirmation in larger population as they were above the FDR.

Discussion

This analysis revealed differences in the microbial composition and diversity between mattress dust
and nasal samples. Despite agreement of richness between mattress and nasal samples on an
individual level, growing up on a farm was related to bacterial diversity and specific taxa indicative of
animal exposure primarily in mattress dust samples. In contrast, asthma was associated with
bacterial diversity in both mattress dust and nasal samples. In mattress dust, bacterial richness
operated as an intermediate factor in the association of farm exposure and asthma.

Indoor dust samples in general are a mix of microbial species originating from the house occupants
and from the outside environment (26-28). The main genera from our analysis, i.e. *Staphylococcus,*
Propionibacterium, *Corynebacterium* and *Streptococcus* are typical for human skin (29, 30). Most
likely the house occupants shed these microbes throughout the indoor environment including the
mattresses, where they eventually were detected. There is also strong evidence for outdoor
environmental bacteria in the mattress dust samples such as *Sphingomonas,* which is known from
samples of soil, water and sediments (31). Further, *Acinetobacter* and *Lactococcus* were previously
detected in raw milk (32, 33) and isolated from cow sheds (11). We also detected a *Chloroplast* OTU
related to the plant family *Pinaceae*, a group of trees and scrubs native to northern temperate
regions. As Chloroplast DNA of *Pinaceae* is inherited via pollen (34) and our sample collection
occurred during the pollen season of *Pinaceae* (35) we take this OTU as evidence for transfer of

particles from the outside environment to the mattress dust.

Though this *Pinaceae Chloroplast* OTU was occasionally detected in nasal samples as well, the main nasal taxa were local bacteria such as *Moraxella* and *Streptococcus*, which are well known from clinical experience and previous studies (36-38), particularly in children (38).

This predominance of local bacteria may explain the less pronounced signature of environmental microbes in nasal samples. Nevertheless, mattress dust and nasal samples agreed reasonably with respect to bacterial richness as illustrated by Figure 2 though mucosal samples are characterized by a large inter-individual variability (39). Nasal samples also reflect other exposures beyond the home environment; however, the frequent clearance of the nose may result in a shorter time of residence of airborne bacteria thereby constraining the effect of the environmental exposure quantitatively. This may explain the generally less pronounced associations of nasal microbiota with environmental determinants in the present analysis.

In contrast, mattress dust is a long-term reservoir for settled material reflecting continuous exposure also to an outer environment. Indeed, transfer of bacteria from animal sheds to the children's mattresses was shown before (3). Bacterial dispersal from the outdoor to the indoor environment by airflow or direct transport via the child and other family members (by hair or cloths) or pets (by fur or paws) most likely account for the increased bacterial diversity in homes of farm children and the detection of farm- and animal-specific taxa in these samples.

When performing a BLAST search for the mattress dust OTUs that were associated with farming or cow/straw exposure we found nearly all hits to be known from previous farm or cow related samples (Table 1, Table E3).

In this regard, the association of farming with the genera *Clostridium* and *Facklamia* is notable since representatives of the genus *Clostridium* are commonly found in soil, sewage and intestines of animals and humans, whereas *Facklamia* is known particularly from milk and cow samples (40, 41). *Trichococcus* was associated with current cow/straw exposure and was previously isolated from raw milk (42), dairy waste (43), and sewage fluent (44).

More interestingly, an association of farming with bacteria from a not further classified genus of the family *Ruminococcaceae* emerged and was even paralleled in nasal samples by a more prevalent occurrence of OTU 2597 in farm children. This OTU belongs also to the family *Ruminococcaceae*, which is typically found in the mammalian gut, also of cows and humans. In another study on airborne microbes in a rural area of the USA, the family of *Ruminococcaceae* was identified as an indicator for cow feces (45).

The identification of these animal- and feces-specific taxa supports the notion that the identified microorganisms are specific for the farm exposure and may originate from animal sheds. Alternatively, the detection of fecal microorganisms, which are also known from human studies, may point towards another important body surface where host-microbial interaction is quite intensive, i.e. the intestinal mucosa. As the gut microbiome is highly diverse, the detected signal by bacterial richness or rare taxa might also be a proxy for intestinal microorganisms.

Anyway, the pronounced gradient in bacterial diversity between farm and non-farm children we detected in this study is paralleled by findings in a comparable study including school children from rural areas of 5 European countries (5). In that study, microbial diversity was assessed in mattress

dust by the gel-based single strand conformation polymorphism (SSCP-PCR) method. Now we rediscovered this gradient in bacterial diversity between farm and non-farm children by a more elaborate method offering a much higher resolution of bacterial diversity down to an OTU level. In that earlier study (5), the higher bacterial diversity of farm children also partially explained their lower asthma prevalence. Hence we tested whether this effect was also seen in the GABRIELA population and, more importantly, whether it was related to nasal microbiota, as well.

Indeed, in the present analysis α−diversity was inversely associated with asthma occurrence also after adjustment for the farm exposure. Of note, the effect was not only seen in mattress dust but also independently in nasal samples, albeit with a lower effect size (Figure 5). An important difference of these effects between both sampling sites is illustrated by the mediation analysis: only richness in mattress dust operated as an intermediate factor in the association of farming with asthma, whereas richness in nasal swabs was hardly related to farming but still contributed to a lower asthma risk.

One may speculate that diversity of the nasal microbiome is influenced by other factors, which independently of the farm exposure lower the risk of asthma. For nasal microbiome obviously airborne bacteria might be relevant, but also skin bacteria might play a role since we found typical skin in the nasal samples abundantly (Figure 1). Ultimately, a host factor itself may impact on the microbial diversity of the nasal cavity. This might be governed by the host immune system, which again might be shaped by genetic as well as environmental factors such as (viral) infections possibly transmitted by siblings in the classical sense of the hygiene hypothesis.

Since bacteria trigger a rather limited number of different receptors of the innate immune system, microbial diversity has been interpreted as a proxy for a cocktail of specific environmental bacteria (5). Therefore we assessed also individual bacterial genera and OTUs for associations with asthma. The inverse association of asthma with the genus *Prevotella* in nasal samples is interesting as this genus produces immunomodulatory metabolites such as short chain fatty acids (46), which have been implied in asthma protection in mouse models (47). In addition, *Prevotella* may alleviate detrimental effects of other airway bacteria such as *Haemophilus* (48).

However, the detection of individual bacterial taxa with asthma-protective potential (Table E5) is an exploratory approach, as these protective effects did not withstand correction for multiple testing. This is most likely limited by the sample size of this pilot study and warrants replication in larger studies in the future. The moderate sample size and the stratification for farm exposure also precluded a more detailed analysis of further exposures beyond farming. Moreover, this study was performed in healthy children attending school. Very few of them (5.8%) were taking any drug at time of nasal sampling thereby rendering specific medication effects difficult.

In addition, the present analyses were based on extracted DNA. As microbial DNA is not necessarily derived from living organisms but can persist freely or associated to cell wall particles of dead organisms for years (49) the detected taxa do not unambiguously represent living or active bacteria.

On the other side, the in-depth analysis of samples from two rather different sampling sites, but related to the same individuals, is a major strength of this study. Samples were taken only two days apart and processed following identical protocols except for DNA extraction. This situation provided the unique opportunity to study the microbiome of the upper airways in the context of the pertinent environmental microbiota.

Taken together, we found environmental, or more precisely farm-related, bacteria in mattress dust and to a lower extent in nasal microbiota. The effect of microbial exposure on asthma was stronger in mattress dust as compared to nasal samples. This may seem unexpected as the nasal cavity as part of the upper airway mucosa is likely to reflect interactions of environmental microorganisms with the host mucosa more adequately. This paradox thus challenges the concept of colonization of the (upper) airways by asthma-protective bacteria. Other hypotheses such as asthma-protection by colonization of the intestine by beneficial microorganisms or inhalation of microbial metabolites with immunomodulatory properties may come to the focus of future research.

Acknowledgement

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Table 1: Genera and OTUs from mattress dust significantly associated with farming. Listed are only associations significant (p<0.05) after Bonferroni correction.

	Taxa (further classification)	Effect	pValue	Sample site of BLAST hit[a]
Relative abundance		**Beta (95%-C.I.)**		
	Genus			
	rare group	0.97 (0.57-1.36)	$6.3 *10^{-6}$	n/a
	g_unclassified (f_*Ruminococcaceae*)	0.98 (0.42-1.55)	$1.0 *10^{-3}$	n/a
	OTU			
	OTU12986 (g_*unclassified*/ f_*Ruminococcaceae*)	1.08 (0.67-1.48)	$1.2 *10^{-6}$	Bovine reproductive tract/ fecal sample from *Bos taurus*; gayal (*Bos frontalis*) breeding farm/ cattle herd feces/ fecal sample in taurus (neonatal calf)
	OTU9879 (g_ *Planococcus/*f_*Planococcaceae*)	1.01 (0.62-1.40)	$2.7 *10^{-6}$	bioaerosol emitted from wastewater treatment plant/ *Planomicrobium stacke-brandtii* - strain collection/ Chandra Tal Lake water/ soil/ soil from Qinghai lake
	OTU13554 (g_*Pseudomonas/* f_*Pseudomonadaceae*)	0.94 (0.57-1.32)	$3.7 *10^{-6}$	biogas plant/ leaf cutter ant refuse dump/ cow manure Japan/ soil
	OTU2597 (g_unclassified/f_*Ruminococcaceae)*	0.98 (0.58-1.38)	$6.6 *10^{-6}$	Bovine reproductive tract/ manure/ cow manure/ descending colon ingesta from *Bos taurus*/ cow teat skin
	OTU7854 (g_ *Bacteroides/f_Bacteroidaceae*)	0.84 (0.48-1.19)	$1.6 *10^{-5}$	descending colon ingesta from *Bos taurus*/ sheep feces Ireland/ Okapi feces/ fecal sample *Procapra przewalskii*/ fecal sample *Bos taurus*/
	OTU1017	0.60 (0.34-0.86)	$2.1 *10^{-5}$	descending colon mucosa of *Bos*

	Taxa (further classification)	Effect	pValue	Sample site of BLAST hit[a]
	(g_*Alistipes*/f_*Rikenellaceae*)			*taurus*/ Argali sheep feces/ fecal sample from beef cattle/ fecal cattle sample/ Okapi feces
Presence/ absence		**OR (95%-C.I.)**		
	g_*Clostridium* (f_*Clostridiaceae*)	27.71 (6.03-127.45)	$5.3 *10^{-5}$	n/a
	g_*Facklamia* (f_*Aerococcaeceae*)	13.43 (3.77-47.84)	$1.4 *10^{-4}$	n/a
	OTU			
	OTU2597 (g_unclassified/ f-*Ruminococcaceae*)	50.81 (10.9-236.6)	$3.2 *10^{-6}$	Bovine reproductive tract/ manure/ cow manure/ descending colon ingesta from *Bos taurus*/ cow teat skin

[a] A BLAST search of the OTU sequences against NCBI database was performed (2016-03-09). Listed are up to five different sites where similar sequences with > 97% were found.

g_= genus; f_ = family; n/a = not applicable

Figure Legend

Figure 1: Taxonomic composition of the mattress dust and nasal microbiota on A) phyla and B) genera level

* Genus is unclassified, and only family is reported

Figure 2: Bland-Altman Plot for bacterial richness of mattress dust and nasal microbiota

Richness is shown as mean of 1000 rarefying steps to 1252 reads per subject.

Figure 3: Bacterial richness and Shannon-Index in the rarefied sample of mattress dust and nasal microbiota stratified for A) farming or B) regular exposure to cow/straw

Shown are the p-values of survey-weighted Wilcoxon tests.

Figure 4: Effects of A) farming and B) regular exposure to cow/straw on relative abundance of the main bacterial genera in mattress dust and nasal samples. In case of unclassified genus, family is reported, and this is indicated by (F).

Figure 5: Effects of richness and Shannon index of mattress dust and nasal microbiota, respectively, on asthma

Diversity measurements are shown unadjusted, adjusted for farming only and adjusted for farming + mutually adjusted. Mutually adjusted means additional adjustment for the respective diversity measurement in nasal swabs or mattress dust. Richness is shown on the z-transformed scale of the log10 (of richness), Shannon on the z-transformed scale of the original Shannon index.

A

B

Mattress Dust   **Nose**

A)

| Richness | Shannon-Index | Richness | Shannon-Index |

p-value=0.000008   p-value=0.000013   p-value=0.444014   p-value=0.913221

B)

p-value=0.0511   p-value=0.0678   p-value=0.0757   p-value=0.0199

Mattress Dust   **Nose**

A)

B)

Beta   Beta

Mattress Dust

Richness (crude)
+ adjusted for farming
+ mutually adjusted
Shannon (crude)
+ adjusted for farming
+ mutually adjusted

0.2 0.5 1.0 2.0
Odds Ratio (95%-C.I.)

Nose

0.2 0.5 1.0 2.0
Odds Ratio (95%-C.I.)