ORIGINAL ARTICLE

Airborne cultivable microflora and microbial transfer in farm buildings and rural dwellings

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ABSTRACT

Objectives Exposure to environments rich in microorganisms such as farms has been shown to protect against the development of childhood asthma and allergies. However, it remains unclear where, and how, farm and other rural children are exposed to microbes. Furthermore, the composition of the microbial flora is poorly characterised. We tested the hypothesis that farm children are exposed indoors to substantial levels of viable microbes originating from animal sheds and barns. We also expected that environmental microbial flora on farms and in farm homes would be more complex than in the homes of rural control children.

Methods Dust samples were collected using passive samplers in the bedrooms of the following groups of children in rural Bavaria, Germany: (i) those living on farms (n=144), (ii) those regularly exposed to farm environments but not living on farms (n=149) and (iii) those never visiting farms (n=150). For farm children, additional samples were collected in animal sheds and barns. All samples were subjected to fungal and bacterial culturing.

Results Detectable levels of microorganisms were more often found in samples taken from farm dwellings than from other homes. Farm dwellings also showed higher microbial levels. Microbial counts of farm dwelling samples correlated with the counts in corresponding animal sheds and barns.

Conclusions Microorganisms are transported from animal sheds and barns into farm dwellings. Therefore, children living in these environments are exposed when indoors and when visiting animal sheds and barns. Indoor exposure may also contribute to the protective effect of the farm environment.

What this paper adds

- The farm environment has been shown to offer protection against asthma and allergies in children.
- The role of microorganisms has been recognised, but the microbial flora to which farm children are exposed is poorly characterised.
- This study shows that children living on farms are exposed to many microorganisms and that numerous fungal and bacterial taxa found in farm dwellings are also found in animal sheds and barns.
- Fewer microorganisms are found in the rural dwellings of non-farmers.

INTRODUCTION

Exposure to environments rich in microorganisms such as farms has previously been shown to protect against the development of childhood asthma and allergies.[1–7] In particular, frequent contact with farm animals during early childhood seems to be an important determining factor. In farm dwellings, environmental studies have shown increased levels of microbe associated molecular patterns such as endotoxin from Gram-negative bacteria, muramic acid from Gram-positive bacteria, and β(1-3)-D-glucans and extracellular polysaccharides from fungi, as compared to rural non-farm dwellings. However, little is known about the complex microbial flora which underlies exposure to these microbial markers. We hypothesised that children were exposed to substantial levels of viable microbes not only in animal sheds and barns, but also indoors in the farm dwelling. We also hypothesised that the indoor levels and spectra of microorganisms were determined by the microorganisms present in animal sheds and barns.

Air in animal sheds is highly contaminated with numerous species of moulds and bacteria, including high levels of actinomycetes.[8–11] Individuals working on a farm may transport microorganisms from the animal shed to the dwelling.[12 13] However, the microbial flora to which farm children may be exposed is poorly characterised.

The objectives of the present study were therefore to determine whether the microbes children were exposed to indoors in farm environments originated from animal sheds and barns, and to measure the levels of fungi and bacteria in farm and control rural dwellings. We therefore studied: (i) the microbial flora in the dwellings (bedrooms) of three groups of children living in a rural environment in the German Alpine region: one group living on farms, one group occasionally exposed to farm environments, and one group with no exposure to a farm environment; (ii) the microbial flora collected in the animal sheds and barns of farm dwellings; and (iii) the relationship between the

level of contamination in the farm dwelling and in associated animal sheds and barns.

This project is part of the GABRIEL Advanced Studies, which were designed to identify environmental microbial exposures in farm environments and determine the exposures protecting against the development of asthma and allergic diseases.

MATERIALS AND METHODS

Study design

This environmental study is nested in phase III of the GABRIEL Advanced Studies (figure 1). The farms included in this study were mixed traditional family farms, with mainly cows and some small animals such as chickens; some farms had modest numbers of pigs. Crops produced were often grown as animal feed.[14] Three non-overlapping strata were defined: (i) farm children, that is children living on a farm run by their family at the time of assessment; (ii) exposed non-farm children, that is non-farm children regularly visiting animal sheds, barns or consuming cows milk produced on a farm; and (iii) non-exposed rural non-farm children. From the children participating in all phase II study modules, a stratified random sample of 895 subjects using nine dwelling strata (three for health status (those with asthma, those with atopy and controls) and three for the exposure (non-exposed children, exposed children and farm children)) was invited to participate in phase III in which we performed in-depth environmental sampling. For the nested study presented here, 444 subjects from phase III were selected (approximately 50 subjects per stratum) and data were obtained for 443. For farm children, sampling involved dwellings and associated barns and animal sheds.

Airborne dust samples

Airborne dust samples were collected by a passive collection method using electrostatic dust fall collectors (EDCs). Details about the use of EDCs in indoor and animal shed environments have been described elsewhere as well as the results of validation pilot studies.[15 16] Briefly, EDCs are plastic sample holders equipped with between two and four electrostatic cloths with a cloth exposure area of 0.0209 m^2 (Zeeman, Utrecht, The Netherlands). When exposed to air, EDCs capture settling dust due to their electrostatic properties. EDCs have been validated against active dust PM_{10} sampling methods such as the Harvard impactor.[16] Within each dwelling, one EDC was placed in the child's bedroom and also, if applicable, in whichever barn and/or animal shed in which the child spent most of his or her time, resulting in the collection of 150 EDCs from the non-exposed

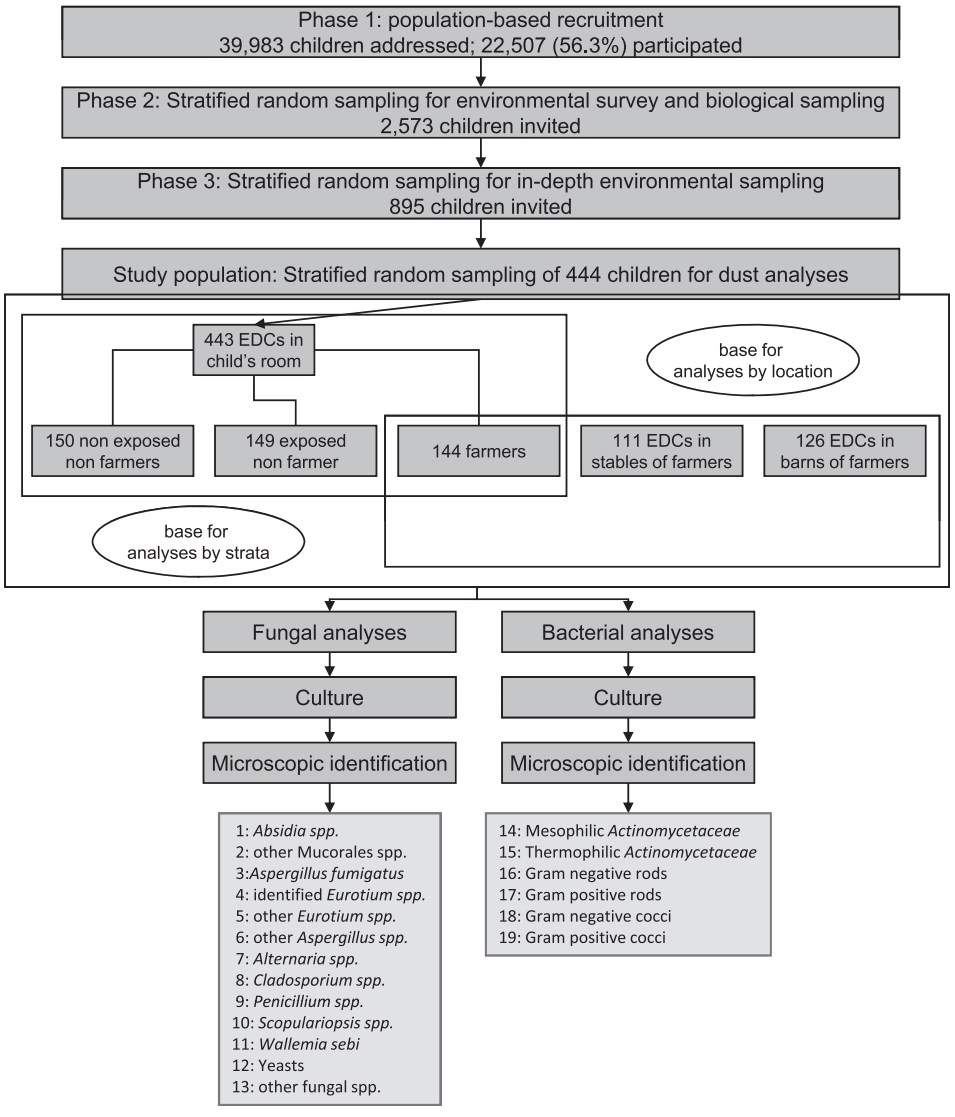

Figure 1 Flow chart of the GABRIEL Advanced Studies in Bavaria, Germany. Samples from 443 dwellings (bedrooms) were included in the current analyses from among the 895 children invited for in-depth exposure assessment within phase III of the GABRIEL Advanced Studies. For all participants, dust was collected in the child's room. For farm children, dust was additionally collected on the farm or the animal shed wherever possible. EDC, electrostatic dust fall collector.

children's bedrooms, 149 from exposed children's bedrooms, 144 from farm children's bedrooms, 111 from animal sheds and 126 from barns. EDCs were placed by a fieldwork team member. After exposure to air for 14 days, the families were asked to close the EDCs and returned them to the study centre in Munich by post, after which they were sent to Utrecht for further processing. The four electrostatic cloths were transferred individually into plastic bags and stored frozen: three cloths were stored at −80°C and one cloth was stored at −20°C. Samples stored at −20°C were used for the analyses described in this manuscript and were sent to the microbiology laboratory in Besançon, France, to be analysed for microbial composition within 4–8 months. Sampling was performed during the 2007–2008 winter, as high humidity levels were conducive to the development of fungi. A total of 680 EDCs were available for analyses.

Treatment of EDC cloths for microbiological analyses

Microbiological analyses were performed blindly, with the operators unaware of the characteristics of each sample. The cloths were put in a sterile bag with 20 ml of 0.1% Tween 80 and shaken for 10 min in a Stomacher (AES Laboratoire, Combourg, France). Samples of the washing solution were added to different growth media suitable for a range of microorganisms, and the analyses were performed as described below.

Culturing

Aliquots (100 µl) of the cloth washing solution were spread on five different agars: (i) Dichloran-Glycerol 18 (Oxoid, Basingstoke, England) with 0.5% chloramphenicol (Sigma-Aldrich, Steinheim, Germany) incubated at 30°C for mesophilic mould isolation, (ii) 3% malt-agar (AES, Bruz, France) with 10% salt and 0.5% chloramphenicol, incubated at room temperature for osmophilic fungal species, (iii) Difco actinomycetes isolation agar (Becton Dickinson, Le Pont de Claix, France) incubated at 30°C for mesophilic actinomycetes, (iv) R8 medium incubated at 52°C for thermophilic actinomycetes,[17] and (v) CHROMagar Orientation (CHROMagar, Paris, France) incubated at room temperature for bacterial growth and phenotypic differentiation. Because the different samples were blindly analysed and microbial contamination levels were unknown, four dilutions (pure, 10, 100 and 1000-fold dilutions) were used for the analysis of bacteria on the CHROMagar Orientation medium. For fungi and actinomycetes, three dilutions (pure, 10 and 100-fold dilutions) were systematically plated for counting and identification purposes. Microbial counts were then performed on those plates with the more concentrated inoculums that had sufficient colonies.

Fungal identification

After 7 days of incubation, the colonies were counted and identified using macroscopic and microscopic criteria, following the keys in the *Atlas of Clinical Fungi*.[18] Slow-growing fungi were identified 14 days after incubation, using the same methods. Forty-four fungal species were identified, and taxa were built for use in further medical analyses. We particularly focused on genera and species commonly found in the farm environment and suspected of provoking allergic or pulmonary diseases among farmers, namely *Absidia* spp. (farmer's lung diseases), *Alternaria* spp. (allergy), *Aspergillus* spp. and more particularly *Aspergillus fumigatus* (asthma) and *Aspergillus glaucus* (farmer's lung diseases), *Cladosporium* spp. (allergy), *Penicillium* spp. (farmer's lung diseases and allergy), *Scopulariopsis* sp. (allergy) and *Wallemia sebi* (farmer's lung diseases).[11 19 20] Other fungi were classified into yeasts, other *Mucorales* species and unidentified moulds.

Table 1 Number and frequency of detected microorganisms, stratified according to exposure strata and location on the farm

	Non-exposed non-farm children dwellings		Exposed non-farm children dwellings		Farm children dwellings		Animal sheds		Barns		Farm vs non-exposed non-farm children dwellings	Farm vs exposed non-farm children dwellings
	N = 150		N = 149		N = 144		N = 111		N = 126			
	N	%	N	%	N	%	N	%	N	%	χ^2	χ^2
Absidia spp.	5	3.3	7	4.7	35	24.3	61	55.0	67	53.2	27.5***	22.9***
Other *Mucorales* spp.	12	8.0	11	7.4	31	21.5	48	43.2	45	35.7	10.8**	11.9**
Aspergillus fumigatus	32	21.3	40	26.9	55	38.2	74	66.7	88	69.8	10.0**	4.3*
Identified *Eurotium* spp.†	27	18.0	40	26.9	95	66.0	72	64.9	75	59.5	69.6***	45.1***
Other *Eurotium* spp.	21	14.0	22	14.8	51	35.4	51	46.0	53	42.1	18.2***	16.7***
Other *Aspergillus* spp.	57	38.0	66	44.3	95	66.0	75	67.6	87	69.1	23.0***	13.9***
Alternaria spp.	8	5.3	11	7.4	19	13.2	35	31.5	44	34.9	5.4*	2.7 (NS)
Cladosporium spp.	77	51.3	73	49.0	116	80.6	82	73.9	104	82.5	27.8***	31.9***
Penicillium spp.	113	75.3	113	75.8	127	88.2	80	72.1	73	57.9	8.1**	7.5**
Scopulariopsis sp.	5	3.3	10	6.7	41	28.5	66	59.5	69	54.8	35.2***	24.1***
Wallemia sebi	18	12.0	27	18.1	69	47.9	50	45.1	78	61.9	45.5***	29.5***
Yeast	12	8.0	19	12.8	44	30.6	72	64.9	48	38.1	24.2***	13.8***
Other fungi	44	29.3	46	30.9	66	45.8	72	64.9	78	61.9	8.5**	6.9**
Mesophilic *Actinomycetaceae*	38	25.3	45	30.2	111	77.1	109	98.2	117	92.9	78.7***	64.7***
Thermophilic *Actinomycetaceae*	8	5.3	27	18.1	45	31.3	79	71.2	71	56.4	33.4***	6.8**
Gram negative rods	79	52.7	87	58.4	121	84.0	95	85.6	112	88.9	33.2***	23.4***
Gram positive rods	89	59.3	91	61.1	132	91.7	90	81.1	111	88.1	41.2***	37.7***
Gram negative cocci	41	27.3	44	29.5	67	46.5	38	34.2	30	23.8	11.6**	9.0**
Gram positive cocci	106	70.7	95	63.8	133	92.4	94	84.7	109	86.5	22.7***	34.7***

χ^2 Test statistics representing differences in frequencies between exposure strata (farm vs exposed and farm vs non-exposed) are given.
*p<0.05; **p<0.001; ***p<0.0001.
†Identified *Eurotium* spp. and their teleomorphs: *Aspergillus hollandicus*, *Aspergillus glaucus*, *Eurotium amstelodami* and *Eurotium herbarorium*.
N, number of positive samples; NS, non-significant.

Bacterial identification

Bacterial colonies were selected from CHROMagar Orientation medium according to colony morphotype, subjected to Gram staining and classified. We classified and counted the actinomycetes cultured on Difco and R8 media according to growth temperature. We presented results of fungal identification according to bacterial taxa. We particularly focused on actinomycetes counts because these bacteria are known to be present in high amounts in the farm environment and to be responsible for immuno-allergic diseases such as farmer's lung disease.[11 19] Identification of each bacterial colony at the genus or species level will be continued in a further project on bacterial environmental characterisation as a specimen of every colony was re-isolated by sub-culturing and frozen at −80°C for analyses. Bacterial and fungal results were expressed in colony forming units (CFU) per plate and further converted to CFU per EDC and CFU/m^2 taking into account the dilution steps and the exposure surface area of the EDC (0.0209 m^2).

Statistical analyses

Due to the procedure used, levels below 200 CFU/EDC could not be detected. For calculation of the arithmetical mean, zero values were kept as such. For calculation of the geometric mean, non-detects were replaced by half of the limit of detection (1/2×200/0.0209 CFU/m^2=4784.69 CFU/m^2). The geometric means and 95% CIs of each microbial category stratified by exposure (non-exposed non-farm children, exposed non-farm children, farm children) and farm location (farm dwelling, animal shed, barn) were calculated. To test for differences in the frequencies of detectable microorganisms between exposure strata, a Pearson's χ^2 test was used. Spearman's r was used to calculate correlations between the corresponding viable microorganisms in dwellings and barns (n=125 matched pairs) and between dwellings and animal sheds (n=110 matched pairs). Statistical significance was set at the 5% level. STATA 10 SE was used for statistical analyses.

RESULTS

Numerous fungal and bacterial taxa were detected from the 680 analysed EDCs. However, only one fungal and three bacterial taxa, that is *Penicillium* spp., Gram negative rods, Gram positive rods and Gram positive cocci, were found in more than 50% of samples collected in the different environments (non-exposed dwelling, exposed dwelling, farm dwelling, animal shed and barn). There was a marked trend towards a higher proportion of samples with detectable levels of microorganisms by taxa collected from the dwellings of the farm children than from the dwellings of the non-exposed and exposed children (table 1).

In particular, the proportion of positive samples for each bacterial and fungal taxon was significantly higher in farm dwellings than in exposed or non-exposed dwellings. There was a trend for a higher proportion of detectable fungi and bacteria in exposed dwellings compared to non-exposed dwellings, but the difference was only significant for the thermophilic Actinomycetaceae group (data not shown).

The higher exposure in farm dwellings was evidenced by the proportion of detectable samples and by increased concentrations. As shown in table 2, the geometric mean concentration of detectable samples for every microbial parameter was higher in farm dwellings than in the two other types of dwellings, with the exception of *Scopulariopsis* sp. Moreover, for most taxa, the

Table 2 Geometric means and GSDs of microorganisms (in CFU/m^2) by exposure strata and by location on the farm, and Spearman correlation coefficients between microorganism levels in the farm dwelling and in the animal shed or barn

	Non-exposed non-farm dwelling		Exposed non-farm dwelling		Farm dwelling		Animal shed		Barn		Farm dwelling/animal shed (n=110)		Farm dwelling/barn (n=125)	
	GM/1000	GSD	GM/1000	GSD	GM/1000	GSD	GM/1000	GSD	GM/1000	GSD	Spearman's r	p Value	Spearman's r	p Value
Absidia spp.	5	0.1	5	0.1	9	1.6	48	5.7	38	6.5	0	***	0	***
Other *Mucorales* spp.	5	0.1	5	0.3	7	0.9	19	3.4	15	3.6	0	NS	0	*
Aspergillus fumigatus	6	0.6	8	1.2	10	1.9	55	4.1	34	6.0	0	***	0	***
Identified *Eurotium* spp.†	6	0.6	7	0.8	23	2.6	187	8.7	110	9.8	0	***	0	***
Other *Eurotium* spp.	6	0.4	6	0.5	11	1.7	69	7.3	39	9.5	0	***	0	NS
Other *Aspergillus* spp.	9	1.2	10	1.4	20	2.1	131	5.7	78	7.6	0	***	0	***
Alternaria spp.	5	0.1	5	0.1	6	0.3	11	2.2	12	2.5	0	*	0	NS
Cladosporium spp.	10	1.1	9	0.8	23	1.6	189	5.7	316	6.4	0	*	0	NS
Penicillium spp.	22	1.8	28	2.1	48	2.0	122	5.6	57	5.3	0	*	0	*
Scopulariopsis sp.	5	0.0	6	0.4	7	0.8	59	4.3	30	6.6	0.26	**	0.28	**
Wallemia sebi	6	0.3	7	0.8	14	2.0	51	7.6	108	8.1	0	**	0	**
Yeast	5	0.1	6	0.4	10	1.7	126	7.2	34	7.5	0	*	0	*
Other fungi	8	0.8	8	0.9	11	1.6	77	5.9	67	5.8	0	*	0	NS
Mesophilic *Actinomycetaceae*	6	0.4	7	0.8	41	3.4	1682	5.3	477	4.1	0	***	0	***
Thermophilic *Actinomycetaceae*	5	0.1	6	0.5	9	1.7	71	4.8	30	5.7	0	***	0	*
Gram negative rods	15	2.0	17	2.7	122	5.2	7022	8.6	2258	12.3	0	**	0	***
Gram positive rods	13	1.4	17	2.9	246	5.3	5604	9.3	2792	15.6	0	**	0	***
Gram negative cocci	9	1.5	9	1.8	21	4.9	68	4.6	15	14.5	0	NS	0	NS
Gram positive cocci	27	3.7	29	4.7	454	6.0	25 368	10.0	1841	16.6	0	***	0	***

*p<0.05; **p<0.001; ***p<0.0001.
†Identified *Eurotium* spp. and their teleomorphs: *Aspergillus hollandicus*, *Aspergillus glaucus*, *Eurotium amstelodami* and *Eurotium herbarorium*.
Mean values are expressed in colony forming unit per square metre (CFU/m^2). Non-detects have been replaced by half of the limit of detection for GM calculations.
Spearman's r is shown for farm children for whom electrostatic dust fall collectors from either farm source were returned: child's room and animal shed (N=110) or child's room and barn (N=125).
GM, geometric mean; GSD, geometric SD; NS, non-significant.

Table 3 Number of samples with or without specified microorganisms identified in farm dwellings and/or other indoor farm source (animal shed or barn)

	Present in farm dwelling/animal shed or barn				Proportion of +/+ of all farm dwelling positives	Proportion of −/+ of all farm dwelling negatives
	−/−	+/−	−/+	+/+		
Absidia spp.	30	0	39	29	100%	57%
Other *Mucorales* spp.	36	8	39	15	65%	52%
Aspergillus fumigatus	16	3	46	33	92%	74%
Identified *Eurotium* spp.*	8	9	26	55	86%	76%
Other *Eurotium* spp.	25	8	40	25	76%	62%
Other *Aspergillus* spp.	8	7	26	57	89%	76%
Alternaria spp.	42	3	45	8	73%	52%
Cladosporium spp.	0	4	19	75	95%	100%
Penicillium spp.	1	12	10	75	86%	91%
Scopulariopsis sp.	19	5	49	25	83%	72%
Wallemia sebi	16	8	37	37	82%	70%
Yeast	24	0	42	32	100%	64%
Other fungi	11	5	40	42	89%	78%
Mesophilic *Actinomycetaceae*	0	0	17	81	100%	100%
Thermophilic *Actinomycetaceae*	11	2	52	33	94%	83%
Gram negative rods	0	1	17	80	99%	100%
Gram positive rods	2	3	8	85	97%	80%
Gram negative cocci	29	19	24	26	58%	45%
Gram positive cocci	2	3	6	87	97%	75%

N=98 farm children with samples from the child's room, animal shed and barn included.
*Identified *Eurotium* spp. and their teleomorphs: *Aspergillus hollandicus*, *Aspergillus glaucus*, *Eurotium amstelodami* and *Eurotium herbarorium*.

microbial concentrations in the farm dwellings were significantly and positively correlated with the concentrations in the corresponding animal sheds and barns.

The fungal patterns of non-exposed and exposed dwellings were very similar and characterised by the dominant presence of *Penicillium* spp., whereas the microflora from the farm dwellings were more similar to the animal shed microflora with *Eurotium* spp., other *Aspergillus* spp., *Penicillium* spp., *Cladosporium* spp. and Gram positive cocci identified as the dominant groups of microorganisms. Similarities were also found between the bacterial patterns of farm dwellings, animal sheds and barns, and bacterial contamination of non-exposed dwellings was very low compared to that of farm dwellings.

The similarity of the results between animal sheds, barns and farm dwellings is also corroborated by the findings presented in table 3 which show that in a majority of the farm dwellings (58% to 100% depending on the microorganism), a positive sample for a given microorganism in the dwelling corresponded to a positive sample in the animal shed or barn.

As shown in table 4, the concentrations of different microorganisms were lower in farm dwellings compared to animal sheds and barns, with ratios for the arithmetic mean varying from 1:6 (*Penicillium* spp.) to 1:64 (unidentified *Eurotium* spp.) for fungi, and from 1:11 (mesophilic actinomycetes) to 1:171 (Gram negative rods) for bacteria.

DISCUSSION

Relationships between the presence of fungi in indoor and outdoor air have been studied previously, and the authors found that outdoor factors may influence the presence of viable mould in indoor environments.[21] We sought to describe the association between the patterns and levels of viable microorganisms on the farm and in the indoor environment of children by performing a large and extensive environmental study. The similarities in species found in farm dwellings and barns strongly suggest that microorganisms are transported from animal sheds and barns into farm dwellings. Indeed, there is a marked tendency towards an increased number of microbial species and higher levels in farm dwellings compared to rural dwellings. Moreover, this strong indication of transport or flow from barns and animal sheds into dwellings clearly modifies the overall composition of airborne microflora in children's bedrooms. While the bedroom indoor environment of non-exposed children is characterised by a predominance of *Penicillium* spp., as previously reported,[22 23] other fungi such as *Aspergillus glaucus* and its teleomorph

Table 4 Arithmetic means (in 10^3 CFU/m^2) and ratio of arithmetic mean of level of microorganisms found in animal sheds and farm dwellings

	Arithmetic means (10^3 CFU/m^2)		Arithmetic mean ratio (animal shed/farm dwelling)
	Animal shed (N=111)	Farm dwelling (N=144)	
Absidia spp.	835	32	26
Other *Mucorales* spp.	145	14	10
Aspergillus fumigatus	1055	59	18
Identified *Eurotium* spp.*	4012	177	23
Other *Eurotium* spp.	3005	47	64
Other *Aspergillus* spp.	1724	75	23
Alternaria spp.	75	4	19
Cladosporium spp.	2183	51	43
Penicillium spp.	639	109	6
Scopulariopsis sp.	817	13	63
Wallemia sebi	1411	54	26
Yeast	1964	31	63
Other fungi	715	31	23
Mesophilic *Actinomycetaceae*	6994	624	11
Thermophilic *Actinomycetaceae*	1073	66	16
Gram negative rods	173 551	1013	171
Gram positive rods	279 564	3270	85
Gram negative cocci	15 733	431†	37
Gram positive cocci	388 932	6334	61

Arithmetic mean ratio is the animal shed mean/farm dwelling mean.
*Identified *Eurotium* spp. and their teleomorphs: *Aspergillus hollandicus*, *Aspergillus glaucus*, *Eurotium amstelodami* and *Eurotium herbarorium*.
†One missing value.

Eurotium amstelodami are also common in farm dwellings. *A glaucus* is commonly present on farms, especially when hay is distributed to cattle.[24–26] Other fungal species observed in the farm children's bedroom, such as *Absidia* spp. and *Wallemia sebi*, and bacteria such as actinomycetes species, have also been frequently detected in the farm environment[11 27] and are suspected of being involved in farmer's lung diseases. Moreover, our study showed that for numerous taxa, the concentrations of microorganisms in animal sheds and farm dwellings are positively and significantly correlated, corroborating the hypothesis of a noticeable transfer from the animal shed to the farm dwelling. For actinomycetes and most of the fungal groups, the ratio between arithmetic mean exposures in the dwelling compared to the animal shed is between 1:10 and 1:40. For these microorganisms, it can be extrapolated that when a child has spent 10–40 h in his or her bedroom, he or she has been in contact with the same number of microorganisms as if he or she had stayed 1 h in the animal shed. Similarly, bacterial contamination of the bedroom seems influenced by the environment in the animal sheds and barns. This may be due to the propensity of fungal and actinomycetes spores to be easily disseminated in the atmosphere[12] and carried into dwellings on the hands, clothes or shoes of farmers and their children.[13 28 29]

The findings for *Penicillium* spp. were somewhat different. On the one hand, the low arithmetic mean ratio between the air in farm dwellings and the air in animal sheds, and the positive correlation between the numbers of colonies found in these two locations, suggest that the indoor air in a farm dwelling is contaminated by *Penicillium* spp. spores originating from the farm. On the other hand, *Penicillium* species were also the predominant fungal flora in non-farming dwellings, which is concordant with previous studies showing that *Penicillium* species are among the most typical fungi found in the indoor air of homes.[12 30 31] Apart from the farm environment, there are likely to be other sources of *Penicillium* species, as highlighted by studies on mould contamination in indoor air.[22 32–34] More detailed identification of *Penicillium* species would have been of interest to determine if the patterns of individual species found in farm dwellings and non-exposed dwellings are different. However, the identification of *Penicillium* species is particularly difficult even in highly specialised laboratories, as further studies combining molecular, biochemical and physiological approaches are required.

In this study we focused on bacteria and fungi that were easily obtained using standard culture media. Furthermore, we targeted mesophilic and thermophilic fungi and actinomycetes because of their propensity to replicate in indoor environments.[35] We acknowledge that these microorganisms represent only a small fraction of all airborne microorganisms because numerous bacteria and fungi are difficult or even impossible to culture.[35] Other approaches such as microscopy or DNA cloning,[36] that bypass the culturing step, could also be used to assess the microbial diversity in farm dwellings. However, such non-culture based methods, especially the biomolecular approaches, are expensive and difficult to apply to large environmental studies with large series of samples. Both approaches are however complementary and one is not necessarily superior to the other. Nonetheless, the microbial analyses in this study appear to be relevant, since in a separate analysis we have shown that increased diversity of viable microorganisms enhances the protective effect conferred by animal shed fungal and bacterial flora on childhood asthma and atopy.[14]

In conclusion, this study is an important step in the understanding of microbial exposure in farm dwellings because it provides data suggesting transport of microbial species between animal sheds and farm dwellings. This transport clearly determines children's exposure to microorganisms, as it modifies the composition of airborne microflora in the children's bedrooms.

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