

Wheeze in infancy: protection associated with yeasts in house dust contrasts with increased risk associated with yeasts in indoor air and other fungal taxa

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Abstract

Background: While fungal exposures are assumed to provoke wheeze through irritant or allergenic mechanisms, little is known about the differential effects of indoor and outdoor fungi on early-life wheeze.

Methods: In a Boston prospective birth cohort of 499 at-risk infants, culturable fungi in bedroom air and dust and outdoor air were measured at the age of 2–3 months. Wheeze was determined using bimonthly telephone questionnaires. Odds ratios were estimated for an interquartile increase in fungal natural log-transformed concentrations, adjusting for predictors of wheeze and potential confounders.

Results: Increased odds of 'any wheeze' (≥ 1 vs 0 episodes) by age one were positively associated with indoor dust *Alternaria* [odds ratio (OR) = 1.83; 95% confidence interval (CI), 1.07–3.14], *Penicillium* [OR = 1.18; (0.98–1.43)], and *Cladosporium* [OR = 1.47; (1.16–1.85)]; indoor air *Penicillium* [OR = 1.26; (0.92–1.74)]; and outdoor air *Cladosporium* [OR = 1.68; (1.04–2.72)]. In contrast, indoor dust yeasts were protective [OR = 0.78; (0.66–0.93)]. 'Frequent wheeze' (≥ 2 vs < 2 episodes) by age one was borderline associated with dust yeasts [OR = 0.86; (0.70–1.04)] and indoor air yeasts [OR = 1.53; (0.93–2.53)]. *Alternaria* concentration was associated with any wheeze for children with maternal mold sensitization [OR = 9.16; (1.37–61.22)], but not for those without maternal mold sensitization [OR = 1.32; (0.79–2.20)].

Conclusions: While wheeze rates were higher with exposures to fungal taxa considered to be irritant or allergenic in sensitive subjects, yeasts in the home had a strong protective association with wheeze in infancy. Molecular microbiologic studies may elucidate specific components of innate microbiologic stimulants that lead to contrasting effects on wheeze development.

Abbreviations

a_w , water activity (or equilibrium relative humidity, %ERH) is the vapor pressure generated by moisture present in a hygroscopic product. It represents the moisture content on a surface that is available for fungal growth; Bla g 1 or 2, cockroach allergen from *Blattella germanica*; bn, billion; CFU, colony-forming unit; CI, confidence interval; D_{50} , aerodynamic diameter cut point where 50% of the particles are captured. Above this cut point, a greater percentage is captured; DG18, dichloran glycerol culture media; EPS-Pen/Asp, extracellular polysaccharides from *Penicillium* or *Aspergillus*; EU, endotoxin unit; IgE, immunoglobulin E; Inc, incorporated company; IQR, interquartile range; lpm, liters per minute; LRI, lower respiratory illness; Ltd, limited company; r, correlation coefficient; SD, standard deviation; Th1, type 1 T helper cell; Th2, type 2 T helper cell.

Fungi have indoor and outdoor sources (1–3). While fungi are a known risk of irritant or allergy-induced wheeze (4–7), little is known about the differential responses of children to infant exposure to individual indoor and outdoor airborne and dust-borne fungal taxa. Moreover, a cross-sectional study suggested that certain indoor fungi (*Penicillium* and *Eurotium*) may be protective (8).

Infancy is a vulnerable period when exposures may influence immune development, allergy, and wheeze (9). We hypothesized that transient elevations in early-life (age, 2–3 months) fungal exposures are associated with wheeze in children by age one. We assessed whether associations were independent of reports of dampness or other known predictors of wheeze (10, 11). Finally, we investigated sources of vulnerability, focusing on maternal asthma or sensitization.

Methods

Study protocol

In a prospective Boston birth cohort study, 499 infants (including six sets of twins) born to women with history of asthma and allergies were recruited between 1994 and 1996 (10). The study was approved by the Institutional Review Board of Boston's Brigham and Women's Hospital.

Early-life fungal exposure assessment when infants were aged 2–3 months has been described previously (12). Air samples were collected at 45 lpm using a Burkard culture plate sampler with dichloran glycerol (DG18) culture media. Indoor air was sampled 1–1.5 m above the bedroom floor demarcated for dust collection. Outdoor air was collected 3 m from the main entrance, but could not be collected if temperatures were $<2.2^{\circ}\text{C}$ because culture plates would freeze. Positive-hole corrections scaled counts to an estimate of colony-forming units (CFUs) that would have been observed without co-impaction events (13). After air sampling, dust was collected from the bedroom floor, using a canister vacuum cleaner (The Eureka Co., Bloomington, IN, USA). Dust suspensions were spread-plated on DG18 media. Fungal colonies were identified to genus level using morphological methods (14) and reported as CFUs per gram of dust. In the subset with winter visits, fungi were measured again when infants were 8–9 months old. Mean annual fungal concentrations were calculated as the average of the levels measured at the 2–3 and 8–9 month home visits.

Definition of fungal predictors

Fungal distributions were highly skewed and were represented as natural logarithm (ln)-transformed, continuous variables (adding 1 CFU per unit for values below the limit of detection). We classified exposures as follows: (i) individual genera, (ii) 'total fungi' [sum of all detectable fungi from each medium (outdoor/indoor air and dust)], (iii) 'high fungal exposure' (binary variable, $>75^{\text{th}}$ percentile of any detectable genera), and (iv) an ordinal count of genera that were $>75^{\text{th}}$ percentile.

Definition of other predictors

We adjusted for other predictors of wheeze, details of their ascertainment being reported elsewhere (10, 11). These included birthweight, self-reported physician-diagnosed maternal asthma, winter birth, and maternal smoking during pregnancy. We did not adjust for environmental tobacco smoke, as it was rarely ($<1\%$) reported. We also assessed whether results were independent of early-life exposures to cockroach allergen (≥ 2 U/g maximum Bla g 1 or 2 in living room dust), dust mite allergen (≥ 10 $\mu\text{g/g}$ Der p or f 1 baby's bed), and endotoxin (\geq median of measured samples = 81.3 EU/mg, in living room dust).

Previously, we found early-life fungi associated with increased risk of lower respiratory illness (LRI; physician-diagnosed croup, bronchitis, bronchiolitis, or pneumonia) by age one (12). LRI is associated with wheeze and may be on the pathway between fungal exposures and wheeze. We evaluated the timing between the first episode of wheeze and LRI.

Maternal sensitization ascertainment by serum IgE has been described previously (15). Mothers were classified with 'mold atopy' if sensitized to *Alternaria* or *Aspergillus* species and 'any atopy' if sensitized to ≥ 1 allergen (mold, cat, dog, ragweed, ryegrass, cockroach, or dust mite) or total IgE ≥ 200 IU/ml (15).

Outcome definition

Outcome ascertainment has been described elsewhere (10). Bimonthly telephone questionnaires were administered to the child's primary caregiver, asking 'Since we last spoke with you on (date given), has your child had symptoms of wheeze?'. We defined 'any wheeze' as ≥ 1 report by age one versus no report. 'Frequent wheeze' was defined as ≥ 2 reports compared with <2 .

Statistical analysis

We compared outdoor and indoor air fungi and calculated Spearman correlations due to residual skewed distributions after ln transformation. We compared unadjusted and adjusted logistic models, beginning with indoor air and dust using the full data set. Odds ratios were scaled to an interquartile increase in ln-transformed concentration of the specific taxon. With fewer outdoor winter measures, we performed subset analyses on infants with measurable outdoor fungi, thereby maintaining constant sample size across models: (i) indoor air and dust jointly in the same model, (ii) outdoor air alone, (iii) outdoor air and indoor dust jointly, and (iv) outdoor and indoor air and indoor dust jointly. Sensitivity analyses excluding potential outliers (the highest two/three fungal concentrations) and also with generalized estimating equations to account for correlation in the twins were performed.

Fungi were included together in final models, checking correlations ($r \geq 0.8$) to avoid collinearity. Models were validated using the Lasso approach for model shrinkage to reduce the

probability of chance findings (16). We also fit models for the binary 'high exposure' and the ordinal 'count' of fungal genera >75th percentile.

We used Wald and likelihood ratio tests to examine whether maternal sensitization or asthma modified the association between fungi and wheeze.

We used SAS 9.1 (SAS Institute Inc, Cary, NC, USA), except R 2.6.1 for the Lasso (17).

Results

Cohort characteristics

Of the 499 children, 211 (42%) and 96 (19%) experienced 'any wheeze' and 'frequent wheeze', respectively. Most (86%) wheeze episodes occurred after the early-life fungal assessments. Although 27% experienced ≥ 1 episode of LRI, 97% of this 27% experienced their first wheeze prior to their first LRI. Population characteristics have been described elsewhere (11–13). Children included 47% girls, 75% Caucasians, and 9% low-income families (<\$30k/year). Forty-four percent of children reported visible dampness in the year preceding their age 2–3 month home visit.

Characteristics of indoor versus outdoor air and dust fungi

Among the subset with two visits, total outdoor air ($r = 0.82$, $P < 0.01$, $n = 111$), indoor air ($r = 0.78$, $P < 0.01$, $n = 195$), and dust fungi ($r = 0.87$, $P < 0.01$, $n = 81$) measured at visit 1 were highly correlated with respective mean annual concentrations. The following results focus on exposures at visit 1.

Alternaria, *Aureobasidium*, *Cladosporium*, nonsporulating, and total fungal concentrations were greater in outdoor air, while *Aspergillus*, *Penicillium*, and yeasts were greater in indoor air (Table 1). All distributions were positively skewed. Correlation coefficients (Supplemental Material, Table S1 a, b) ranged from -0.30 to 0.51 , with the exception of outdoor–indoor air *Cladosporium* (0.74) and nonsporulating fungi (0.66). For total fungi, levels in air and dust were highest in summer/fall and lowest in winter/spring. However, individual taxa in dust did not vary significantly by season (Fig. 1). Outdoor air *Alternaria*, *Cladosporium*, and *Penicillium* were highest in the summer/fall, whereas outdoor air yeasts were lowest; this was generally reflected in the indoor air patterns of the specific taxa.

Fungal exposure predicts wheeze

Elevated odds of 'any wheeze' occurred for increased dust-borne *Alternaria* concentrations (OR = 1.83; 95% CI, 1.07–3.14) and for both dust-borne (OR = 1.47; 95% CI, 1.16–1.85) and outdoor airborne (OR = 1.68; 95% CI, 1.04–2.72) *Cladosporium* in models with other fungi included (Table 3). There was a trend toward an increased risk with elevated indoor airborne (OR = 1.26; 95% CI, 0.92–1.74) and dust-borne (OR = 1.18; 95% CI, 0.98–1.43) *Penicillium*. Yet, dust-borne yeasts had protective associations

(OR = 0.78; 95% CI, 0.66–0.93). Findings were consistent considering genera separately (Table 2) or jointly (Table 3).

Estimates for 'frequent wheeze' risk were less precise than those for 'any wheeze' (Supplemental Material, Tables S2 and S3). Adjusting for other genera, indoor airborne yeasts were borderline associated with increased odds (OR = 1.53; 95% CI, 0.93–2.53), whereas dust-borne yeasts appeared borderline protective (OR = 0.86; 95% CI, 0.70–1.04). There were suggestions of associations with dust-borne *Alternaria*, *Cladosporium*, and *Aspergillus*.

Associations were independent of winter birth, low birth-weight, maternal smoking during pregnancy, and maternal asthma. Models that adjusted for either birth or sampling season categorically yielded consistent results. Relationships were not confounded by gender, ethnicity, income, day care attendance or LRI by age one, maternal atopy, building type, endotoxin, indoor allergens (cockroach and dust mite), and self-reported mold/dampness.

We obtained consistent results in sensitivity analyses that (i) excluded extremely high fungal exposures, (ii) accounted for the correlation among the twins, and (iii) with the Lasso approach. Models treating fungi continuously were more precise than with binary fungi (>75th percentile, results not shown). Reported mold/dampness was not significantly associated with any wheeze (OR = 1.27; 95% CI, 0.88–1.83) or frequent wheeze (OR = 1.06; 95% CI, 0.67–1.69). Wheeze was not associated with binary 'high fungal exposure' (any fungi >75th percentile), ordinal 'count', or total fungal exposure variables.

Effect modification by maternal mold sensitization

Dust-borne *Alternaria* was associated with higher odds of 'any wheeze' for children with mold-sensitized mothers [(OR = 9.16; 95% CI, 1.37–61.22), $n = 46$] than those with nonsensitized mothers [(OR = 1.32; 95% CI, 0.79–2.20), $n = 315$] (interaction $P = 0.05$). However, associations were not modified by maternal 'any atopy' or asthma. Effect modification of 'frequent wheeze' was not observed.

Discussion

While we found increased odds of wheeze in the first year of life with elevated infant fungal exposures in indoor dust (*Alternaria*, *Penicillium*, and *Cladosporium*), indoor air (*Penicillium*), and outdoor air (*Cladosporium*), the odds of wheeze were reduced with elevated indoor dust-borne yeast levels, even after adjusting for visible home dampness, endotoxin, or other known wheeze risk factors (10, 11).

How do fungi modify infant wheeze risk?

Fungi may be irritants, leading directly to airway inflammation, or may increase infection risk, leading to inflammation and bronchoconstriction (5). Early-life wheeze is usually induced by viral illnesses in vulnerable infants (18). While mold predicted increased risk of LRI (12), 97% children experienced their first wheeze prior to their first LRI, making

Table 1 Distributions of fungal exposures (Statistics calculated for all measured samples, including values below the limit of detection)

Fungi	# Samples	# Detectable	Geometric mean	Median	75th percentile (ln*)	Maximum	IQR (ln-transformed*)
<i>Alternaria</i>							
Outdoor air (CFU/m ³)	384	139	3.0	0.0	11.1 (2.5)	1977.8	2.5
Indoor air (CFU/m ³)	494	124	2.0	0.0	11.1 (2.5)	122.2	2.5
Bedroom floor dust (CFU/g)	412	236	90.0	454.5	3846.2 (8.3)	363636.4	8.3
<i>Aureobasidium</i>							
Outdoor air (CFU/m ³)	384	113	2.5	0.0	11.1 (2.5)	266.7	2.5
Indoor air (CFU/m ³)	494	88	1.6	0.0	0.0 (0.0)	266.7	0.0
Bedroom floor dust (CFU/g)	412	329	812.4	2857.1	10172.4 (9.2)	295000.0	3.1
<i>Cladosporium</i>							
Outdoor air (CFU/m ³)	384	344	99.5	161.1	466.7 (6.1)	10088.9	2.6
Indoor air (CFU/m ³)	494	384	33.1	44.4	211.1 (5.4)	9877.8	2.9
Bedroom floor dust (CFU/g)	412	322	812.4	3779.8	8485.7 (9.0)	3240000.0	2.9
<i>Aspergillus</i>							
Outdoor air (CFU/m ³)	384	131	2.7	0.0	11.1 (2.5)	1100.0	2.5
Indoor air (CFU/m ³)	494	311	9.0	11.1	33.3 (3.5)	866.7	3.5
Bedroom floor dust (CFU/g)	412	374	2697.3	4791.3	14264.2 (9.6)	11175000.0	2.4
<i>Penicillium</i>							
Outdoor air (CFU/m ³)	384	287	18.2	22.2	66.7 (4.2)	1033.3	4.2
Indoor air (CFU/m ³)	494	423	30.0	33.3	88.9 (4.5)	10400.0	2.0
Bedroom floor dust (CFU/g)	412	368	1998.2	3846.2	9523.8 (9.2)	1619047.6	2.1
Yeasts							
Outdoor air (CFU/m ³)	384	120	2.7	0.0	11.1 (2.5)	300.0	2.5
Indoor air (CFU/m ³)	494	172	3.0	0.0	11.1 (2.5)	700.0	2.5
Bedroom floor dust (CFU/g)	412	367	4023.9	8496.2	25000.0 (10.1)	2630434.8	2.2
<i>Zygomycetes</i>							
Bedroom floor dust (CFU/g)	412	57	3.0	0.0	0.0 (0.0)	342105.3	0.0
Total							
Outdoor air (CFU/m ³)	384	368	445.9	533.3	972.2 (6.9)	10411.1	1.5
Indoor air (CFU/m ³)	494	368	244.7	300.0	588.9 (6.4)	10400.0	1.8
Bedroom floor dust (CFU/g)	412	412	59874.1	71633.3	137416.7 (11.8)	11225000.0	1.7

IQR, interquartile range.

*ln-transformed=ln (untransformed concentration+1); addition of 1 CFU for values below the limit of detection.

LRI less likely to be on the pathway between early-life fungal exposures and wheeze expression.

Inhalant infant allergy is often undetectable. Nevertheless, the increased wheeze response to *Alternaria* of children with mold-sensitized mothers may be an early manifestation of allergic symptoms (19). We could not evaluate modification of infant wheeze risk by maternal sensitivity to other genera, as we only tested maternal IgE for *Alternaria* or *Aspergillus*.

Increased ambient basidiospore and ascospore concentrations during infancy were associated with increased wheeze odds at age 2 in a birth cohort of 514 low-income, predominantly Mexican children in an agricultural region of California (20). However, in a subgroup of this population, there was no *in vitro* evidence for a relative increase in Th2 expression.

The protective role of dust-borne yeasts adds to the small, but growing evidence that not all fungi or fungal products need be risk factors for sensitization or a Th2 bias (21). While unmeasured confounders may explain the protective role of yeasts, we adjusted for several known potential confounders and predictors of early-life wheeze. Yeasts are unicellular fungi that are phylogenetically diverse, whereas the

species we found to be harmful in our study are multicellular fungi. Variability in pathogen-associated molecular patterns (PAMPs) within yeast species as well as between unicellular and multicellular fungi may differentially stimulate innate responses through variable pattern recognition receptors (PRRs). Toll-like receptor 2 (TLR2) recognizes PAMPs from fungi, thereby initiating signal transduction pathways that induce expression of genes that regulate innate and subsequent adaptive immune responses (22). Molecular microbiologic studies may elucidate specific components of innate microbiologic stimulants that lead to contrasting effects on wheeze development.

In a birth cohort of 574 at-risk infants, high dust (1–3)- β -D-glucan concentrations (>60 μ g/g) appeared protective of recurrent wheezing by age one (23). Differential glucan levels between genera (24) and types of yeasts (25) may explain the variable associations with respiratory and allergic disease (26). Taxonomically resolved exposure measures may clarify this inconsistency (27). The direction and magnitude of wheeze response may be related to the route, dose, and timing of exposure and the child's vulnerability factors (28). As gut immune responses are a key to the development of

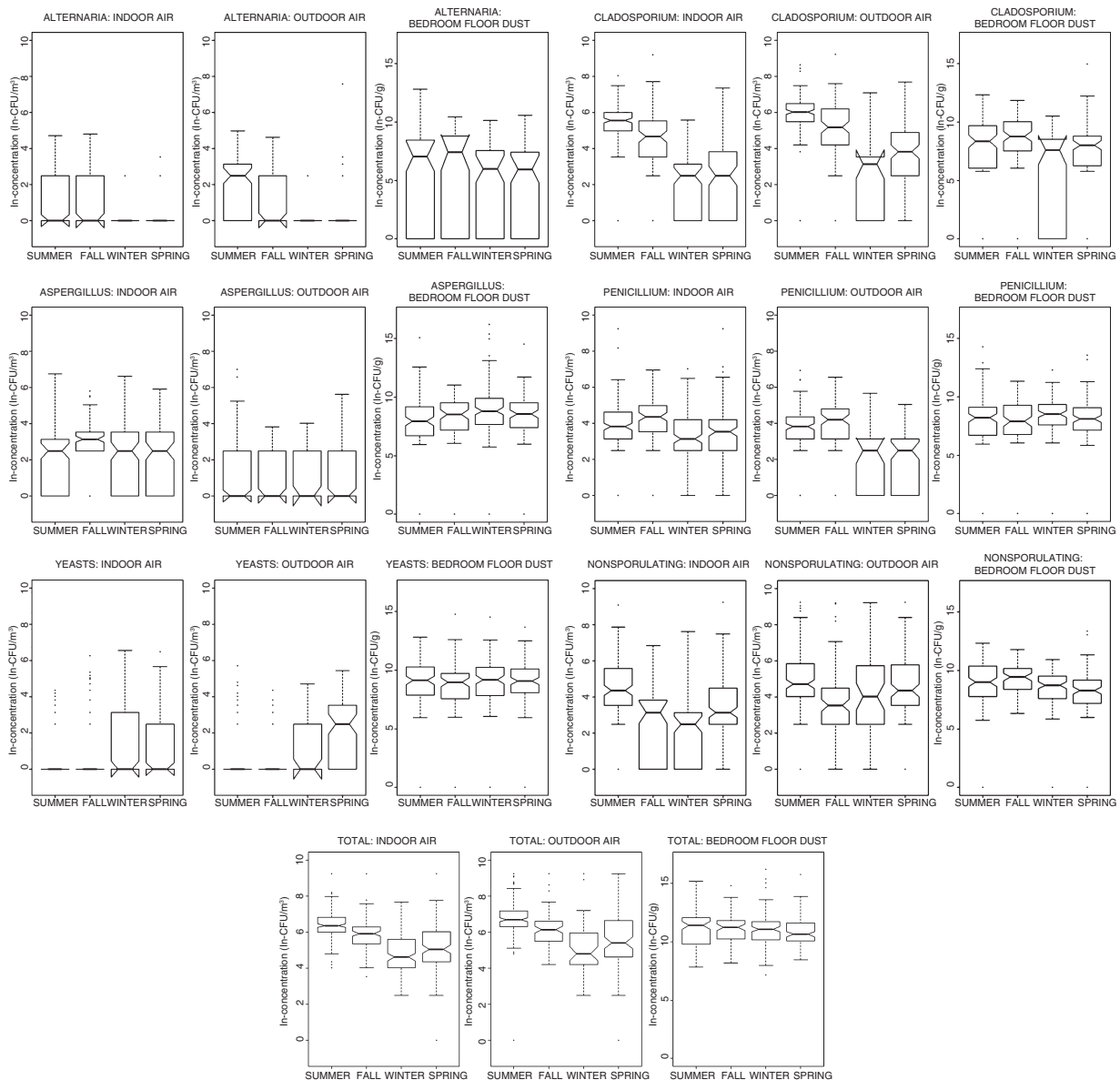


Figure 1 The distribution of airborne (indoor/outdoor) and dust-borne (bedroom floor dust) fungal concentrations by sampling

season, at the homes of children (aged 2–3 months) in the Epidemiology of Home Allergens and Asthma Study.

immune tolerance (29), ingestion of floor dust by infants and toddlers may result in protection, whereas aerosolized yeasts may be irritants. Moreover, the yeasts that aerosolize may differ from yeasts in floor dust.

Method for ascertainment of mold exposure may influence findings: no gold standard

Our study is one of the most comprehensive evaluations of early-life fungal effects on infant wheeze risk. Few studies have simultaneously measured fungi in outdoor air and indoor dust and air. Despite the potential health significance of exposures, cost considerations lead most studies to rely on

visible mold. A Connecticut study of high-risk children measured indoor airborne, but not outdoor or dust culturable fungi (30). They reported increased age one wheeze risk for those 36 children with high *Penicillium* levels (30). Exposure to >75th percentile of indoor airborne *Penicillium* (but no other fungi) at the age of 3 months predicted increased age one wheeze risk in a smaller study of 103 at-risk infants from the low-income urban population of Syracuse, NY (31).

Indoor and outdoor airborne, but not dust-borne measurements were available for the Inner-City Asthma Study of mold-sensitive children (aged 5–11 years) with asthma (6). Outdoor fungi predicted more asthma symptoms, whereas indoor fungi predicted more unscheduled emergency room or

Table 2 Associations of early-life fungi with any wheeze by age one (Analyses evaluate associations of each fungal genus separately)

Fungal genera	Any wheeze (≥ 1 vs 0 episodes)					
	Full data set ($n = 407$)		Subset with outdoor air measures ($n = 316$)			
	OR†	95% CI	Indoor air and dust		Outdoor and indoor air and dust	
			OR†	95% CI	OR†	95% CI
<i>Alternaria</i>						
Outdoor air (ln-CFU/m ³)	–	–	–	–	1.07	(0.71–1.61)
Indoor air (ln-CFU/m ³)	1.01	(0.67–1.50)	1.01	(0.66–1.55)	0.98	(0.61–1.56)
Bedroom floor dust (ln-CFU/g)	1.47*	(0.95–2.28)	1.84*	(1.12–3.02)	1.84*	(1.12–3.02)
<i>Cladosporium</i>						
Outdoor air (ln-CFU/m ³)	–	–	–	–	1.46*	(0.97–2.21)
Indoor air (ln-CFU/m ³)	0.80	(0.59–1.08)	1.04	(0.71–1.52)	0.80	(0.50–1.29)
Bedroom floor dust (ln-CFU/g)	1.28*	(1.08–1.52)	1.40*	(1.14–1.73)	1.43*	(1.16–1.77)
<i>Aspergillus</i>						
Outdoor air (ln-CFU/m ³)	–	–	–	–	1.07	(0.73–1.58)
Indoor air (ln-CFU/m ³)	1.03	(0.69–1.54)	0.97	(0.62–1.53)	0.96	(0.60–1.52)
Bedroom floor dust (ln-CFU/g)	1.03	(0.87–1.23)	1.04	(0.86–1.26)	1.04	(0.86–1.26)
<i>Penicillium</i>						
Outdoor air (ln-CFU/m ³)	–	–	–	–	0.91	(0.50–1.65)
Indoor air (ln-CFU/m ³)	1.05	(0.82–1.33)	1.15	(0.87–1.52)	1.17	(0.87–1.56)
Bedroom floor dust (ln-CFU/g)	1.19*	(1.02–1.39)	1.21*	(1.02–1.44)	1.21*	(1.01–1.43)
Yeasts						
Outdoor air (ln-CFU/m ³)	–	–	–	–	0.99	(0.66–1.49)
Indoor air (ln-CFU/m ³)	1.16	(0.85–1.57)	1.02	(0.71–1.45)	1.02	(0.70–1.49)
Bedroom floor dust (ln-CFU/g)	0.89*	(0.78–1.03)	0.85*	(0.72–0.99)	0.85*	(0.72–0.99)
Total						
Outdoor air (ln-CFU/m ³)	–	–	–	–	1.15	(0.84–1.56)
Indoor air (ln-CFU/m ³)	1.06	(0.77–1.45)	1.25	(0.84–1.86)	1.13	(0.72–1.79)
Bedroom floor dust (ln-CFU/g)	1.07	(0.82–1.39)	1.04	(0.77–1.41)	1.05	(0.77–1.42)

OR, odds ratio; CI, confidence interval.

†Odds ratio for an interquartile increase in the ln-transformed fungal concentration: Exceptions; interquartile range for indoor airborne *Aureobasidium* and dust-borne *Zygomycetes* = 0.0 CFU/m³; Neither *Aureobasidium* nor *Zygomycetes* were predictors of any wheeze by age one.

All models presented adjust for winter birth (yes/no), low birthweight quartile (yes/no), maternal smoking during pregnancy (yes/no), and maternal history of physician-diagnosed asthma (yes/no).

* $P \leq 0.1$.

clinic visits in the past 2 weeks. As the investigators suggest, the short duration of air sampling and lack of dust measures could have led to an underestimate of the role of indoor mold on asthma symptoms.

Compared with airborne fungi, bedroom dust fungi were most consistently linked to wheeze, likely because dust cultures represent a more time-integrated exposure than 1-min air samples (1). There is no gold standard method to estimate mold exposure (5), and ideal studies use a combination of approaches. While concentrations vary temporally within homes (32), fungi in early life were highly correlated with average annual concentrations. Moreover, our early-life measures represent a vulnerable period when exposures may influence immune and respiratory development (9).

Despite fungal seasonal variability, fungal–wheeze associations were independent of season; children born in winter had

reduced wheeze risk (OR = 0.58; 95% CI, 0.38–0.90), relative to other birth seasons. Other seasonally variable *in utero* or postnatal exposures (e.g., aeroallergens, vitamin D) may explain the role of birth season in early-life wheeze (33).

Study limitations

In addition to sampling time and taxonomic resolution limitations, our analytical technique required spores to be culturable, but nondetectable culturable fungi may include nonviable spores and fungal fragments that carry allergens or irritants. Strongly hydrophilic (water activity, $a_w > 0.9$) fungi may be underrepresented due to the high solute concentration in the culture media used, DG18 (34), or because very few homes were severely water-damaged. In fact, hydrophilic fungi (e.g., *Fusarium*, *Stachybotrys*) were rarely recovered as their growth and survival rely on continuously wet materials (4).

Table 3 Associations of early-life fungi with any wheeze by age one (Analyses evaluate associations of all listed fungal genera jointly)†

Fungal genera	Any wheeze (≥1 vs 0 episodes)					
	Full data set (n = 407)		Subset with outdoor air measures (n = 316)			
	OR‡	95% CI	Indoor air and dust		Outdoor and indoor air and dust	
	OR‡	95% CI	OR‡	95% CI	OR‡	95% CI
<i>Alternaria</i>						
Outdoor air (ln-CFU/m ³)	–	–	–	–	0.90	(0.56–1.43)
Indoor air (ln-CFU/m ³)	1.09	(0.69–1.71)	0.91	(0.56–1.47)	0.87	(0.52–1.47)
Bedroom floor dust (ln-CFU/g)	1.45	(0.92–2.29)	1.84*	(1.08–3.12)	1.83*	(1.07–3.14)
<i>Cladosporium</i>						
Outdoor air (ln-CFU/m ³)	–	–	–	–	1.68*	(1.04–2.72)
Indoor air (ln-CFU/m ³)	0.79	(0.56–1.11)	1.09	(0.72–1.67)	0.85	(0.51–1.41)
Bedroom floor dust (ln-CFU/g)	1.28*	(1.07–1.54)	1.41*	(1.13–1.76)	1.47*	(1.16–1.85)
<i>Aspergillus</i>						
Outdoor air (ln-CFU/m ³)	–	–	–	–	1.17	(0.77–1.79)
Indoor air (ln-CFU/m ³)	1.04	(0.68–1.59)	0.98	(0.60–1.60)	1.01	(0.60–1.68)
Bedroom floor dust (ln-CFU/g)	0.97	(0.81–1.17)	0.96	(0.78–1.18)	0.98	(0.80–1.21)
<i>Penicillium</i>						
Outdoor air (ln-CFU/m ³)	–	–	–	–	0.68	(0.34–1.37)
Indoor air (ln-CFU/m ³)	1.10	(0.85–1.43)	1.21	(0.89–1.64)	1.26	(0.92–1.74)
Bedroom floor dust (ln-CFU/g)	1.18*	(1.00–1.39)	1.19	(0.99–1.44)	1.18	(0.98–1.43)
Yeasts						
Outdoor air (ln-CFU/m ³)	–	–	–	–	1.03	(0.66–1.61)
Indoor air (ln-CFU/m ³)	1.19	(0.86–1.64)	1.13	(0.77–1.66)	1.12	(0.74–1.69)
Bedroom floor dust (ln-CFU/g)	0.84*	(0.73–0.97)	0.78*	(0.66–0.92)	0.78*	(0.66–0.93)

OR, odds ratio; CI, confidence interval.

†Models adjust for winter birth (yes/no), low birthweight quartile (yes/no), maternal smoking during pregnancy (yes/no), and maternal history of physician-diagnosed asthma (yes/no).

NOTE – associations are also independent of gender; black ethnicity; low family income; day care attendance in the first year of life; maternal atopy; building type; living room dust endotoxin (≥median = 81.3 EU/mg) and cockroach allergen (Bla g 1 or 2 ≥ 0.05 U/g [=2 ng/g]); dust mite allergen (≥10 µg/g Der p or f 1 baby's bed); lower respiratory illness by age one (croup, bronchitis, bronchiolitis, or pneumonia); and visible reports of mold, water damage, or dampness in the home, at any time over the year before birth (results not shown).

‡Odds ratio for an interquartile increase in the ln-transformed fungal concentration.

*P ≤ 0.05.

While we had sufficient power to assess ‘any wheeze’, we lacked power to fully evaluate the risk of ‘frequent wheeze’, which is more predictive of future asthma (35). We recognize that the number of fungi evaluated may lead to chance findings. However, we found consistent results with the Lasso method (16).

Conclusions

In one of the most comprehensive evaluations of early-life fungal health effects, our prospective birth cohort has shown that while wheeze rates were higher with exposures to fungi usually considered to be irritant or allergenic in sensitive subjects, yeasts in the home had a strong protective association with wheeze in infancy. The influences of fungi on the risk of wheeze may vary by form, functional differences in genera; by timing, dose, or mode of exposure; or by inherited susceptibility factors. Molecular microbiologic studies may elucidate specific components of innate microbiologic stimulants that lead to contrasting effects on wheeze development. Such

studies may result in groundbreaking findings as translational research that could contribute to the development of new targeted pharmacologic or biotic primary or secondary asthma and allergy therapies.

Author contributions

Behrooz Behbod analyzed and interpreted the data and was responsible for writing this manuscript as first author. Joanne Sordillo and Elaine Hoffman assisted with the analysis and interpretation of data and critically revising for important intellectual content. Soma Datta assisted with acquisition of data and critically revising for important intellectual content. Michael Muilenberg and Ginger Chew assisted with the acquisition and interpretation of data and critically revising for important intellectual content. James Scott, Thomas Platts-Mills, and Joel Schwartz assisted with the interpretation of data and critically revising for important intellectual content. Harriet Burge assisted with the design and acquisition of data and critically revising for important intellectual content. Diane

Gold was responsible for the conception and design, acquisition, analysis, and interpretation of data and critically revising for important intellectual content. All authors have given final approval of the version to be published.

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Conflict of interest

All authors of this manuscript declare that they do not have a conflict of interest including relevant financial interests, activities, relationships, and affiliation.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Spearman correlations between fungal exposures.

Table S2. Associations between early-life fungi and frequent wheeze by age one (analyses evaluate associations of all listed fungal genera separately).

Table S3. Associations between early-life fungi and frequent wheeze by age one (analyses evaluate associations of all listed fungal genera jointly).

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