HelmholtzZentrum münchen
German Research Center for Environmental Health

HITEA-ECRHS

Exposure to fungal and bacterial compounds in mattress dust in relation to concurrent respiratory symptoms and specific IgE. Updated results.

Christina Tischer
Helmholtz Zentrum München
Institute of Epidemiology I

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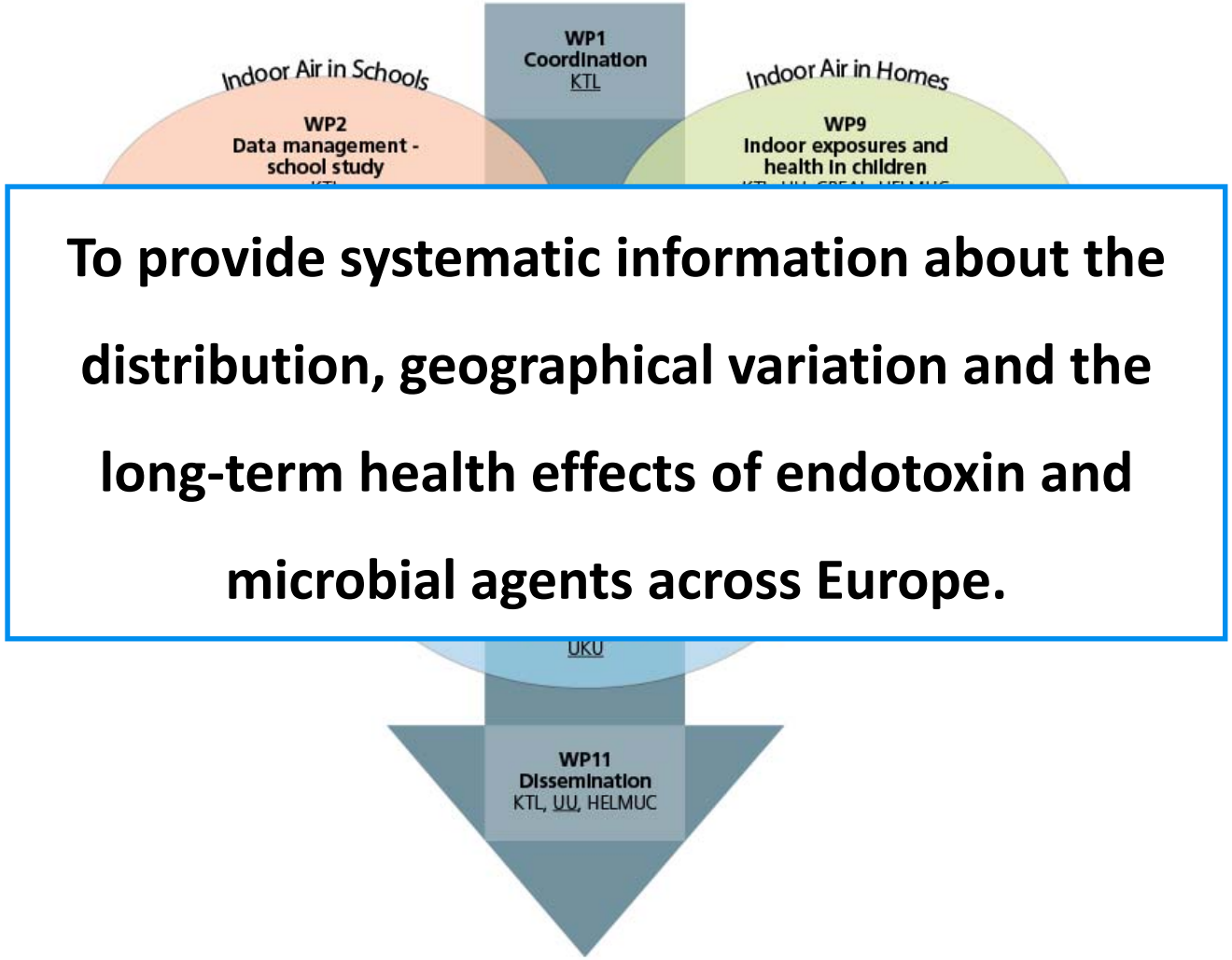
HELMHOLTZ ASSOCIATION

HITEA-ECRHS (WP 10): Background and Aim



To provide systematic information about the distribution, geographical variation and the long-term health effects of endotoxin and microbial agents across Europe.

HITEA-ECRHS: Approach

Stage 1: ✓

- Sieved mattress dust samples (ECRHS II)
- Randomly selected from 14 centers
- **200 asthmatic and 200 matched controls**
- Sieving and aliquotting: IMPERIAL, UK
- Analysis: THL Kuopio, Finland
- **qPCR: Microbial agents that are associated with the health outcomes were selected:**
  - *Cladosporium Herbarum*
  - *Penicillium /Aspergillus / Paecilomyces variotii* group
  - Mycobacteria
  - Muramic acid
  - Gram positive bacteria
  - Gram negative bacteria

Stage 2: ✓

- 2008: Another 1000 sieved mattress dust samples from 22 centers („**random sample**“)
- Analyzed for endotoxin, (1,3)-ß-D-Glucan (Spanish fund) at IRAS (Utrecht) and Microbial comounds and species at THL (Finland)

HITEA-ECRHS: Statistical analysis

- Microbial compounds not normally distributed -> natural log transformation
- **Geographical variation** and distribution (by centre)
- **Correlation** between fungal and bacterial compounds (Spearman's Rho)
- **Determinants** of microbial compounds levels in mattress dust
  Significant determinants in multivariate models (linear mixed effect models)
- **Logistic regression models**:
  Interquartile range increase (IQR) exposure of microbial compounds and concurrent respiratory health outcomes as well as IgE to "aero-allergens":
  - Wheezing, chest tightness, shortness of breath (at rest/activity/woken up with shortness of breath), current allergic rhinitis (ever rhinitis AND current symptoms)
  - IgE "aero-allergens": > 0.35 kU/L to mite (Der p 1), cat, dog, timothy or Cladosporium h.
- **Linear regression models**:
  Interquartile range increase (IQR) exposure of microbial compounds and concurrent lung function parameters
  - FEV, FVC, FEV/FVC (Tiffeneau Index)

HITEA-ECRHS: Distribution

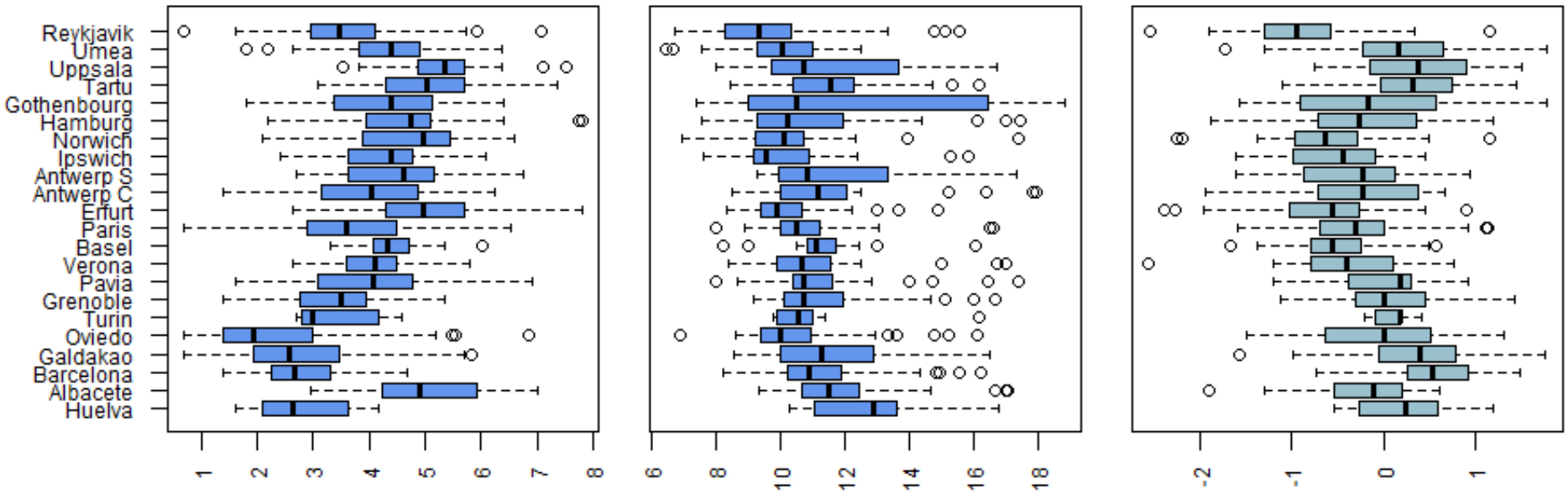
	N	1st Quartile	Median	Geom. Mean	3rd Quartile	IQR
Cladosporium H. (cells/mg)	944	24	63.5	61.11	154.2	130.25
Pen./Asp./Paec. (cells/mg)	954	14870	39100	59061.26	133900	119071.2
(1,3)-ß-D-Glucan (µg/mg)	962	0.52	0.87	0.87	1.48	0.96
Mycobacteria (cells/mg)	953	2699	5378	5918.155	12340	9641
Gram pos. bacteria (cells/mg)	929	278000	547200	540816.4	1109000	831193
Gram neg. bacteria (cells/mg)	951	30630	65890	70096.6	133500	102882
Muramic acid	944	9.80	14.85	14.64	22.70	12.9
Endotoxin (EU/mg)	963	1.07	2.49	2.42	4.83	3.76

HITEA-ECRHS: Geographical distribution

Cladosporium H. (cells/mg)

Pen./Asp./Paec. (cells/mg)

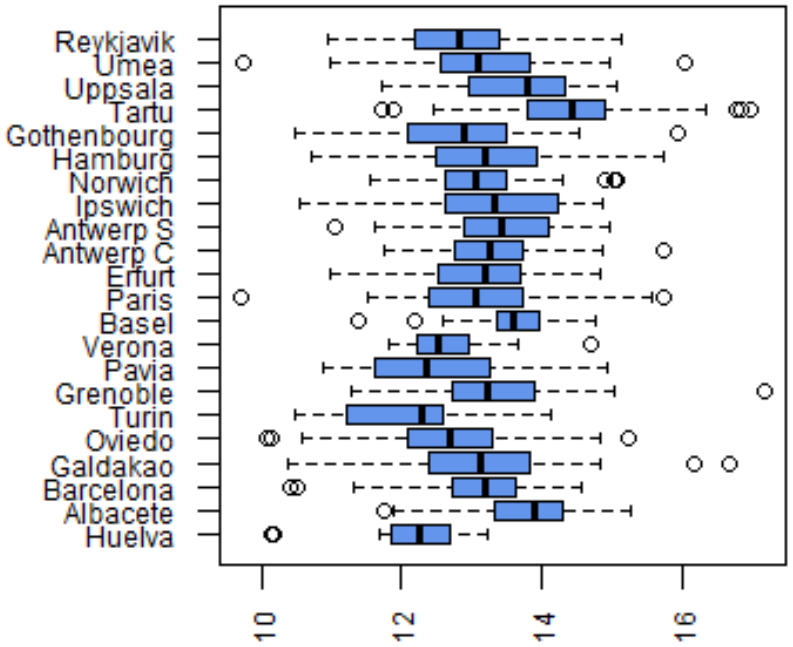
(1,3)-ß-D-Glucan (µg/mg)



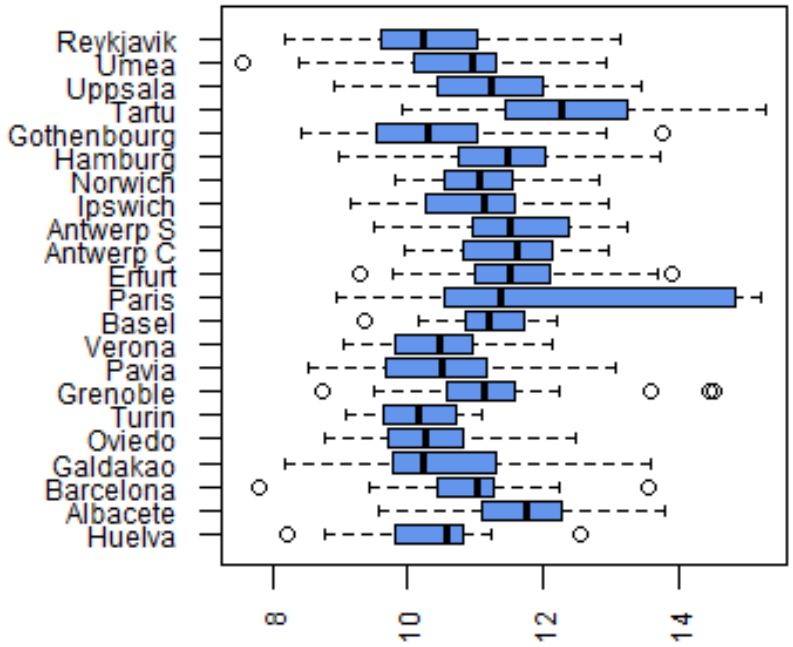
Natural log-transformed

HITEA-ECRHS: Geographical distribution

Gram positive bacteria (cells/mg)



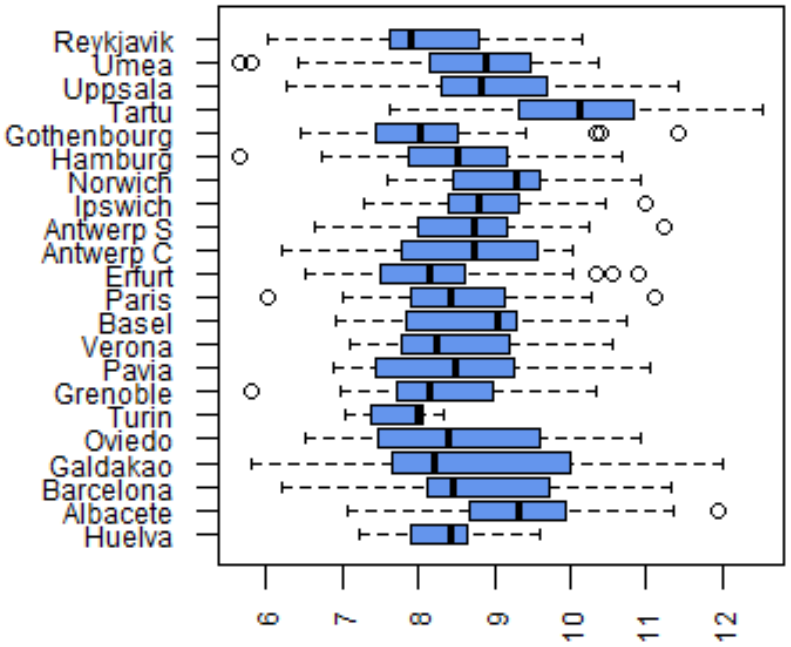
Gram positive bacteria (cells/mg)



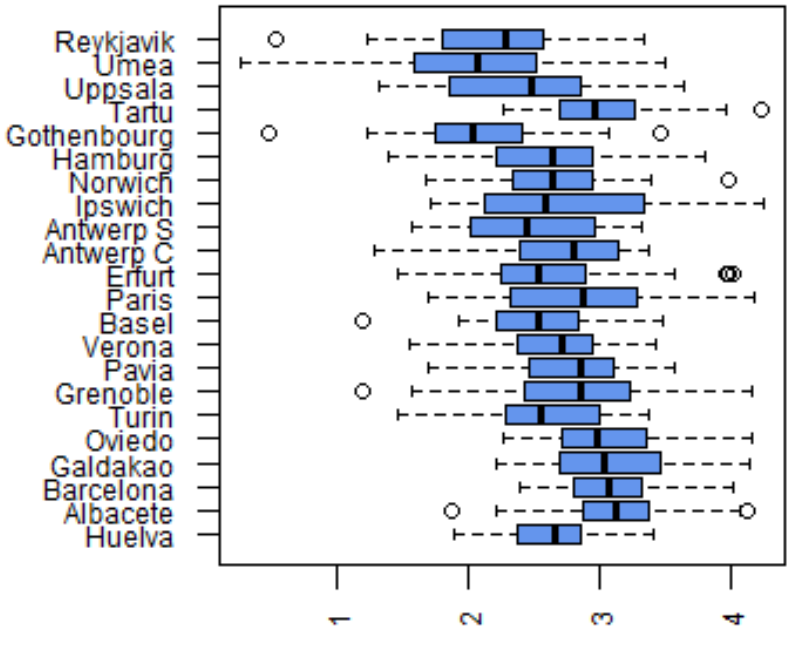
Natural log-transformed

HITEA-ECRHS: Geographical distribution

Mycobacteria (cells/mg)



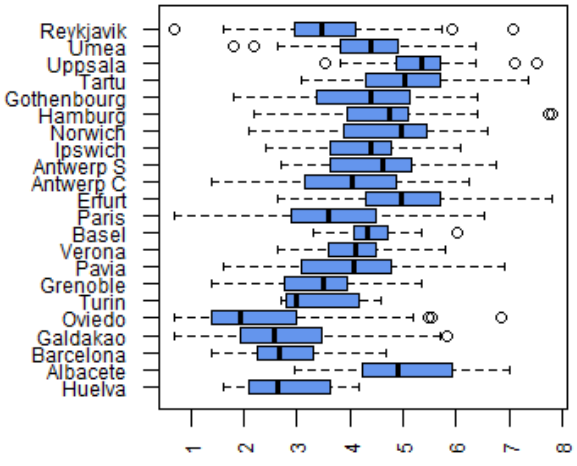
Muramic acid (cells/mg)



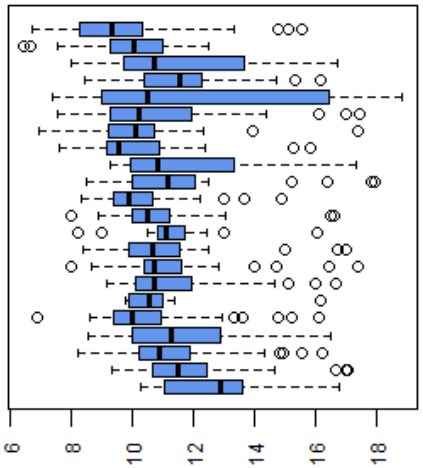
Natural log-transformed

HITEA-ECRHS: Geographical distribution

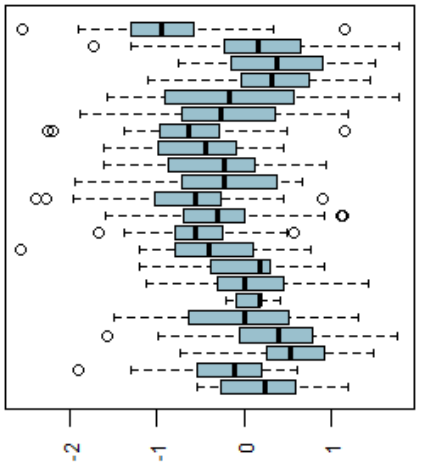
Cladosporium H. (cells/mg)



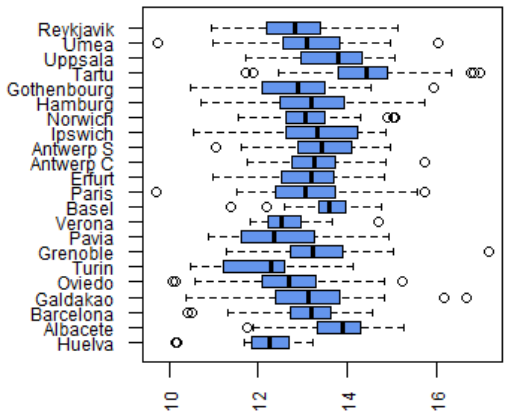
Pen./Asp./Paec. (cells/mg)



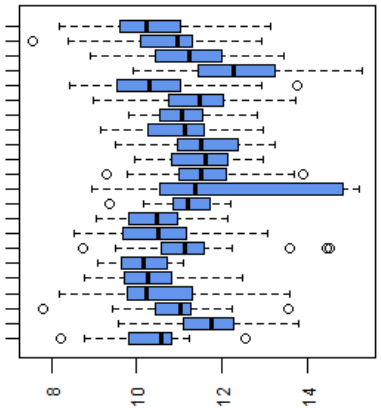
(1,3)-ß-D-Glucan (µg/mg)



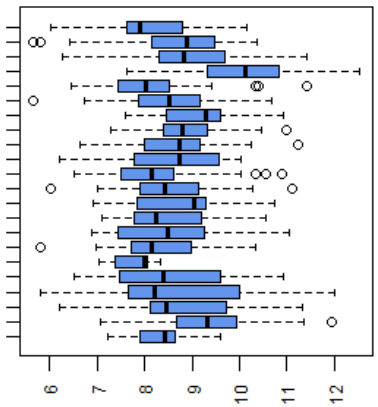
Gram + bacteria (cells/mg)



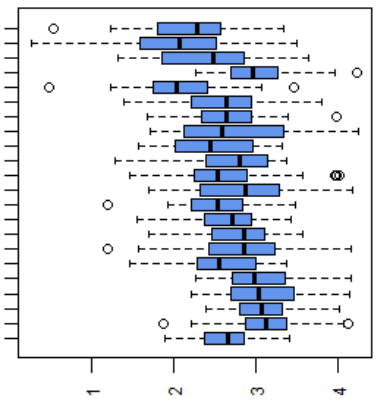
Gram - bacteria (cells/mg)



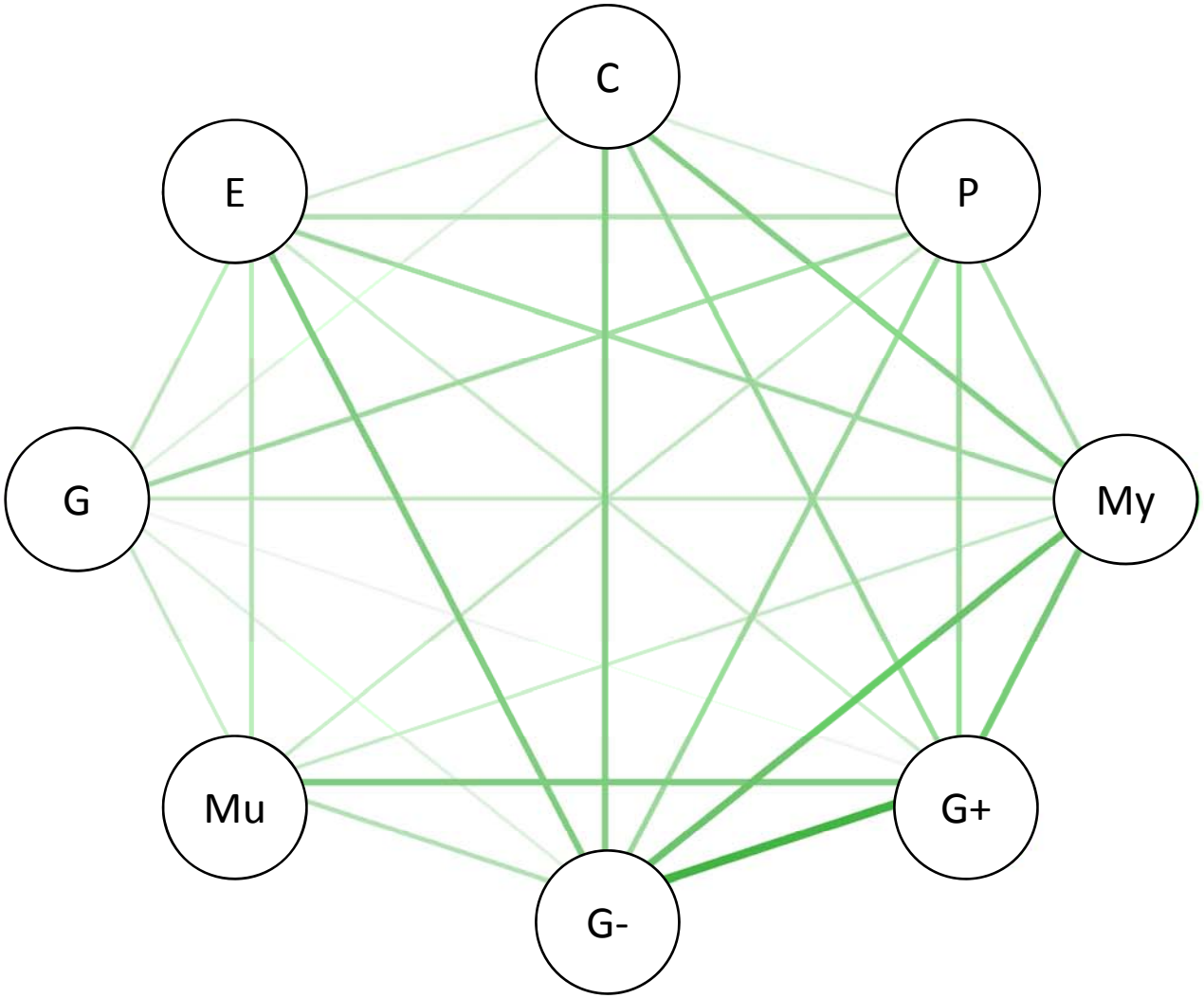
Mycobacteria (cells/mg)



Muramic acid (cells/mg)



HITEA-ECRHS: Correlation microbial compounds



C = Cladosporium H.
P = Pen./Asp./Paec.
My = Mycobacteria
G+ = Gram positive bacteria
G- = Gram negative bacteria
Mu = Muramic acid
G = (1,3)-ß-D-Glucan
E = Endotoxin

HITEA-ECRHS: Correlation microbial compounds

	Cladosporium herbarum	Pen./Asp./Paec.	Myco-bacteria	Gram-pos. bacteria	Gram-neg. bacteria	Muramic acid	(1,3)-ß-D-Glucan	Endotoxin
Cladosporium herbarum								
Pen./Asp./Paec. variotii group	0.16***							
Myco-bacteria	0.46***	0.34***						
Gram-positive bacteria	0.40***	0.38***	0.53***					
Gram-negative bacteria	0.4***	0.38***	0.58***	0.73***				
Muramic acid	0	0.23***	0.22***	0.50***	0.29***			
(1,3)-ß-D-Glucan	0.13***	0.36***	0.24***	0.08*	0.13***	0.23***		
Endotoxin	0.20***	0.30***	0.36***	0.21***	0.49***	0.29***	0.25***	

p<0.05/**p<0.01 / ***p<0.001 ***

HITEA-ECRHS: Determinants – Linear mixed effect models

Cladosporium H. (cells/mg)	Geometric Mean Ratio (95%)
Cat at home:	1.46 (1.06-2.01)***
Cat in bedroom:	1.50 (1.22-1.85)***
Dog at home:	1.50 (1.15-1.94)***
Dog in bedroom:	2.38 (1.83-3.08)***
Longitude (per 10°E):	1.52 (1.15-2.01)**
Season – summer:	1.84 (1.45-2.34)***
Season – autumn:	1.78 (1.47-2.15)***

Pen./Asp./Paec. (cells/mg)	Geometric Mean Ratio (95%)
Observed condensation:	1.82 (1.30-2.56)**
Damp spots at home p12m:	1.57 (1.08-2.73)**
Dog at home:	1.81 (1.23-2.67)**
Dog in bedroom:	2.38 (1.83-3.08)***
Summer temperature (unit = 10°C)	4.03 (1.59-10.24)***

(1,3)-ß-D-Glucan (µg/mg)	Geometric Mean Ratio (95%)
Dog at home:	1.41 (1.01-1.97)
Carpet/rug completely:	0.58 (0.44-0.77)***
Summer temperature (unit = 10°C)	1.78 (1.09-2.90)*
Season – autumn:	1.35 (1.03-1.77)*

***p<0.05, **p<0.01, ***p<0.001**

HITEA-ECRHS: Determinants – Linear mixed effect models

Gram positive bacteria (cells/mg)	Geometric Mean Ratio (95%)
Dog allowed in bedroom:	1.53 (1.15-2.05)**
Age of mattress (ref. since ECRHS I):	
> 1 year but not in ECRHS I	1.17 (1.00-1.38)***
< 1 year	1.96 (1.38-2.79)***
Current smoking:	1.52 (1.15-2.01)**
Season – autumn:	0.79 (0.64-0.97)

Gram negative bacteria (cells/mg)	Geometric Mean Ratio (95%)
Observed -	
Damp patches bedroom:	1.54 (1.17-2.02)***
Cat allowed in bedroom:	1.24 (1.01-1.53)
Dog at home:	1.60 (1.23-2.08)***
Dog allowed in bedroom:	1.80 (1.38-2.35)***
Age of mattress (ref. since ECRHS I):	
> 1 year but not in ECRHS I	1.22 (1.05-1.41)***
< 1 year	1.90 (1.37-2.64)***
Owing a bird:	1.30 (1.00-1.68)*

Mycobacteria (cells/mg)	Geometric Mean Ratio (95%)
Cat allowed in bedroom:	1.59 (1.31-1.92)***
Dog at home:	1.88 (1.48-2.39)***
Dog allowed in bedroom:	2.10 (1.66-2.66)***
Bed in living room:	1.39 (1.03-1.88)*

Muramic acid (cells/mg)	Geometric Mean Ratio (95%)
Water damage ever:	1.12 (1.03-1.22)*
Dog at home:	1.27 (1.08-1.49)***
Dog allowed in bedroom:	1.22 (1.05-1.41)***
Current smoking:	1.13 (1.04-1.24)**
Carpet/rug completely:	0.84 (0.74-0.96)*

***p<0.05, **p<0.01, ***p<0.001**

HITEA-ECRHS: Study population

Total study population	n / N (%)
Female	486 / 956 (**51%**)
Age (in years, median)	**44 years**
Complete full time education (in years, median)	**at 20 years**
Currently employed	815 / 955 (**85%**)
Asthma score, past 12 months	
0 (no symptoms)	628 / 950 (**66%**)
1	212 / 950 (**22%**)
2	63 / 950 (**7%**)
3	23 / 950 (**2%**)
4	14 / 950 (**2%**)
5	10 / 950 (**1%**)
Wheezing past 12m	185 / 954 (**19%**)
Woken up with chest tightness p12m	99 / 954 (**10%**)
Shortness of breath at rest p12m	51 / 953 (**5%**)
Shortness of breath following activity p12m	170 / 953 (**18%**)
Woken up by an attack of shortness of breath 12m	59 / 954 (**6%**)
Current allergic rhinitis p12m	356 / 890 (**40%**)
IgE to "aero-allergens" (> 0.35 kU/L)*	211 / 862 (**24%**)

***Definition specific IgE to "aero-allergens":** either to mite (Der p 1), cat, dog, timothy or *Cladosporium herbarum*

HITEA-ECRHS: Risk factors

		n / N (%)
Smoking status	Ever smoker	553 / 955 (**58%**)
	Current smoker	299 / 954 (**31%**)
	Never smoked	402 / 955 (**42%**)
Mould/Dampness	**Observed** – damp patches in bedroom	71 / 904 (**8%**)
	Observed – mould/mildew in bedroom	47 / 904 (**5%**)
	Observed – condensation on bedroom windows	208 / 899 (**23%**)
	Damp spots at home p12m	197 / 950 (**21%**)
	Ever mould/mildew at home	334 / 952 (**24%**)
	Current mould/mildew p12m	150 / 222 (**68%**)
	Ever water damage	290 / 937 (**31%**)
	Current water damage	94 / 283 (**33%**)
Pets indoor	Cat at home	182 / 955 (**19%**)
	Dog at home	156 / 955 (**16%**)
	Owing bird	77 / 955 (**8%**)
	Bed in living room	54 / 955 (**6%**)
	Age of mattress < than 1 year	50 / 950 (**5%**)
Season dust sampling	Winter	227 / 907 (**25%**)
	Spring	256 / 907 (**28%**)
	Summer	129 / 907 (**14%**)
	Autumn	295 / 907 (**33%**)

HITEA-ECRHS: Results fungal compounds (aORs)

IQR increase in exposure:	Cladosporium H.	Pen./Asp./Paec.	(1,3)-ß-D-Glucan
Wheeze 12m	1.02 (0.77-1.36)	1.02 (0.81-1.29)	1.10 (0.85-1.43)
Chest tightness 12m	0.90 (0.63-1.29)	0.97 (0.72-1.30)	0.93 (0.66-1.31)
Shortness of breath at rest p12m	1.24 (0.77-2.00)	0.90 (0.60-1.35)	0.82 (0.54-1.26)
Shortness of breath following activity p12m	1.15 (0.87-1.53)	1.04 (0.82-1.31)	0.93 (0.70-1.22)
Woken up with shortness of breath p12m	0.90 (0.58-1.43)	0.90 (0.62-1.33)	0.97 (0.63-1.49)
Current allergic rhinitis p12m	0.80 (0.63-1.01)	1.07 (0.88-1.29)	1.18 (0.94-1.47)
Specific IgE to "aero-allergens" (> 0.35 kU/L)	0.86 (0.66-1.12)	1.12 (0.91-1.38)	1.02 (0.79-1.31)

***Adjusted for:** gender, age, education (completed, in years), damp spots in the home, cat at home, dog at home, current smoking, season of dust sampling

HITEA-ECRHS: Results bacterial compounds (aORs)

IQR increase in exposure:	Gram + bacteria	Gram - bacteria	Mycobacteria	Muramic acid
Wheeze 12m	1.20 (0.96-1.51)	0.85 (0.68-1.07)	1.17 (0.92-1.49)	1.18 (0.90-1.54)
Chest tightness 12m	1.22 (0.92-1.62)	1.04 (0.79-1.38)	0.93 (0.68-1.26)	1.24 (0.88-1.75)
Shortness of breath at rest p12m	1.32 (0.92-1.88)	1.08 (0.75-1.54)	1.35 (0.91-2.01)	1.22 (0.78-1.92)
Shortness of breath following activity p12m	**1.45 (1.15-1.84)**	**1.26 (1.02-1.57)**	1.24 (0.97-1.58)	**1.49 (1.13-1.97)**
Woken up with shortness of breath p12m	1.11 (0.78-1.57)	1.06 (0.75-1.50)	0.92 (0.63-1.36)	**1.59 (1.02-2.48)**
Current allergic rhinitis p12m	1.12 (0.93-1.34)	1.00 (0.83-1.99)	1.08 (0.89-1.32)	1.02 (0.82-1.27)
Specific IgE to "aero-allergens" (> 0.35 kU/L)	0.89 (0.72-1.10)	0.94 (0.76-1.15)	**0.79 (0.62-1.00)**	0.99 (0.77-1.27)

***Adjusted for:** gender, age, education (completed, in years), damp spots in the home, cat at home, dog at home, current smoking, season of dust sampling

HITEA-ECRHS: Lung function parameters

Linear regression model (ß, p-value)*:

IQR increase in exposure:	Cladosporium H.	Pen./Asp./Paec.	(1,3)-ß-D-Glucan
FEV 1	0.02, p=0.32	-0.007, p=0.72	0.02, p=0.45
FVC	0.03, p=0.38	0.02, p=0.43	0.02, p=0.53
FEV 1/FVC – Tiffeneau Index	0.001, p=0.77	-0.03, p=0.22	-0.002, p=0.65

IQR increase in exposure:	Gram + bacteria	Gram - bacteria	Mycobacteria	Muramic acid
FEV 1	-0.005, p=0.81	-0.01, p=0.61	0.002,p=0.95	0.02, p=0.52
FVC	0.0008, p=	1.04 (0.79-1.38)	0.93 (0.68-1.26)	1.24 (0.88-1.75)
FEV 1/FVC – Tiffeneau Index	1.32 (0.92-1.88)	1.08 (0.75-1.54)	1.35 (0.91-2.01)	1.22 (0.78-1.92)

***Adjusted for:** gender, age, education (completed, in years), damp spots in the home, cat at home, dog at home, current smoking, season of dust sampling, weight, height.

HITEA-ECRHS: Summary

- Geographical variation of microbial compounds and species
- Microbial compounds moderate to highly correlated, especially bacterial compounds
- Significant determinants of microbial compounds in mattress dust:
    - Having a cat or a dog
    - Damp patches in the home / Bedroom
    - Age of mattress
    - Season of dust sampling
    - Current smoking
- **Higher levels of gram positive / negative bacteria** as well as **muramic acid** in mattress dust were associated with a higher risk for **shortness of breath after strenuous activity** in the past 12 months.
- Higher levels of **muramic acid** were additionally associated with **woken up with shortness of breath** in the past 12 months.
- There was no evidence of an association of either FEV 1 , FVC or FEV 1/FVC with microbial compounds and species in mattress dust
- There was no association with exposure to higher levels of microbial compounds and species in mattress dust in relation to Bronchial Hyper responsiveness (data not shown)

HITEA-ECRHS: Future steps

- **Health outcomes**:
  - Respiratory symptoms
  - Lung function
  - BHR?

- Include **Endotoxin**?

- **Additional paper**? Geographical variation / determinants …

- **…**