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Correlation between Environmental Relative Moldiness Index (ERMI) values in French dwellings and other measures of fungal contamination

Delphine Méheust [a,b,*], Jean-Pierre Gangneux [a,c,d], Tiina Reponen [e], Larry Wymer [f], Stephen Vesper [f], Pierre Le Cann [a,b]

[a] Institut National de la Santé et de la Recherche Médicale (Inserm), U1085, Institut de Recherche Santé, Environnement & Travail (IRSET), F-35043 Rennes, France
[b] Ecole des Hautes Etudes en Santé Publique (EHESP), F-35043 Rennes, France
[c] Université de Rennes 1, Faculté de Médecine, F-35043 Rennes, France
[d] Centre Hospitalier Universitaire de Rennes (CHU), Service de Parasitologie-Mycologie, F-35033 Rennes, France
[e] University of Cincinnati, Department of Environmental Health, Cincinnati, OH 45267-0056, USA
[f] Environmental Protection Agency, Cincinnati, OH 45268, USA

HIGHLIGHTS

- The average ERMI was 15.7 for high versus 2.7 for the low ERMI dwellings in France.
- ERMI values were correlated with concentrations of fungi in air samples.
- ERMI values were correlated with the visible estimates of fungal contamination.
- Older dwellings were more likely to fall into the high ERMI category.
- ERMI values can possibly substitute for other estimates of fungal contamination.

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ABSTRACT

The Environmental Relative Moldiness Index (ERMI) is a DNA-based metric developed to describe the fungal contamination in US dwellings. Our goal was to determine if the ERMI values in dwellings in north western France were correlated with other measures of fungal contamination. Dust and air samples were obtained from 40 dwellings and analyzed by quantitative PCR and/or by culturing. These dwellings were also inspected and the amount of visible fungal growth estimated in m^2. The ERMI values in these dwellings ranged from −2.7 to 28.8 and the fungal contamination estimates ranged from 0 to 20 m^2. The 40 dwellings were divided into those with a low (<6) or high (>8) ERMI values (n = 20 in each). The average ERMI value was 15.70 for the high ERMI compared to 2.68 for the low ERMI dwellings. ERMI values were correlated (significant Kendall's tau values) with concentrations of fungi in air samples analyzed by QPCR or culturing. ERMI values were also correlated (significant Kendall's tau values) with the visible estimates of fungal contamination. Older dwellings were more likely to have higher ERMI values. These results suggest that the ERMI dust sample, which is quick and easy to collect, may be useful in making decisions about reducing fungal exposures in homes.

1. Introduction

Asthma prevalence in France is about 9 to 10% for children and 6 to 7% for adults (Delmas et al., 2009, 2010). A review of the scientific literature indicated that asthma is associated with observed water-damage and fungal growth (World Health Organization (WHO) Europe, 2009). As we spent 80% of our time in more and more insulated buildings, with sometimes poor ventilation and high dampness, we may be exposed to high concentrations of molds (Reboux et al., 2010). In addition, fungal infections are also on the rise (Bitar et al., 2009). Therefore quantitative metrics to describe the fungal contamination in dwellings are necessary to guide interventions and thereby to reduce fungal exposures (Vesper, 2011). In addition, any metric created should be simple to use and understand.

The Environmental Relative Moldiness Index (ERMI) is a metric that was created to quantify fungal contamination in US dwellings (Vesper et al., 2007). Standardized 5.0 mg dust samples were analyzed using a DNA-based technology called quantitative PCR (QPCR) for 36 indicator fungi; 26 Group 1 species associated with water-damaged dwellings and 10 Group 2 species found in dwellings independent of water damage (Vesper et al., 2007). The ERMI was calculated by taking the sum of the logs of the concentrations of the Group 1 fungi and subtracting the sum of the logs of the concentrations of Group 2 fungi. The resulting

* Corresponding author at: INSERM U1085, Ecole des Hautes Etudes en Santé Publique, Av. Pr Léon Bernard, CS 74312, 35043 Rennes Cedex, France. Tel.: +33 2 99 02 29 22; fax: +33 2 99 02 29 29.
E-mail address: Delphine.Meheust@ehesp.fr (D. Méheust).

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ERMI scale in the US ranges from about −10 to 20 (even higher in about 1% of dwellings). The ERMI scale was divided into quartiles. An ERMI of approximately five is the demarcation between third quartile and the fourth or the "highest fungal contamination" quartile in the US. When the QPCR analysis of the 36 species was applied to air samples and the same calculation principle was used, an "ERMI-like" metric was created (the ERMI itself is only based on a dust sample). Previously, we used the ERMI and ERMI-like estimates to help categorize fungal contaminations in French dwellings (Méheust et al., 2012). Other studies that have utilized the QPCR method for characterizing indoor fungi have included limited set of fungal species (Bellanger et al., 2009; Kaarakainen et al., 2009; Lignell et al., 2008).

In this study, 36 indicator fungi were quantified by QPCR in environmental samples obtained from 40 dwellings in north western France (Brittany). We assessed whether ERMI values in dust samples were correlated with air samples quantified using QPCR or using culturing methods. Also assessed was the correlation between ERMI-based estimates of fungal contamination and those estimates derived from visual inspection.

2. Materials and methods

2.1. Dwelling selection and inspection

Forty dwellings in north western France (Brittany) were selected for the study. For 20 dwellings, investigation was requested following a complaint about problems of fungal growth and/or humidity, either registered at the Rennes city Department of Environmental Health, or at the Environment and Health Research Laboratory (LERES). The selection of control dwellings in the same geographic area was based upon ease of availability. Dwellings (22 apartments and 18 houses) with different ages (from the 17th century to 2011) and different sizes (from 17 to 300 m^2) were thus recruited. Most of them were located in urban settings (28/40). Inspections were carried out by a trained investigator during the winters of 2010–2011 and 2011–2012; most dwellings were heated at this time in the Brittany region. Visible fungal growth area was estimated in m^2 and a questionnaire was filled about the characteristics of the living space. At least one inhabitant of the home was present during inspection, but there was no human activity and no device that might influence airborne particles in the room during sampling.

2.2. Air and dust sampling

The Coriolis cyclone collector (Bertin Technologies, Saint-Quentin-en-Yvelines, France) was used at a flow rate of 300 L/min to collect two 10-min air samples: one in the living room and one in the bedroom of each of the 40 dwellings. The sampler was placed at a height of 1 m close to the sofa or bed, respectively. Airborne microorganisms were sampled in collection liquid (AES Chemunex, Bruz, France) with an initial volume of 15 mL. For the QPCR analysis, an aliquot of 1.5 mL of the sample was centrifuged at 5000 × *g* for 15 min and the supernatant fluid was removed to keep the pellet in 100 μL.

Dust samples were then collected from 40 dwellings by vacuuming 2 m^2 in the living room and 2 m^2 in a bedroom for 5 min each with a Mitest™ sampler-fitted vacuum, directly adjacent to the sofa or bed, respectively. One composite dust sample was collected from each home. Same protocol was used for hard surface floors (n = 35) and for carpeted floors (n = 5). This is the standard protocol developed by EPA. The dust was sieved through a 300 μM pore size mesh to remove large particles and other objects. The dust and air samples were stored at −20 °C until analyzed by QPCR.

2.3. Fungal identification and quantification using QPCR

Both an aliquot of 5.0 ± 0.1 mg of the dust and the centrifuged pellets of the two air samples were analyzed for each dwelling. The samples were spiked with 1×10^6 conidia of *Geotrichum candidum* as an external reference. Each extraction tube was shaken in the bead beater (Biospec Products, Bartlesville, OK) for 1 min and the DNA purified using the DNA-EZ extraction kit (GeneRite, Cherry Hill, NJ).

Methods and assays have been reported previously for performing QPCR analyses (Haugland et al., 2002, 2004). Briefly, the standard reaction assays contained 12.5 μL of "Universal Master Mix" (Applied Biosystems Inc., Foster City, CA), 1 μL of a mixture of forward and reverse primers at 25 μM each, 2.5 μL of a 400 nM TaqMan probe (Applied Biosystems Inc.), 2.5 μL of 2 mg/ml fraction V bovine serum albumin (Sigma Chemical, St. Louis, MO) and 2.5 μL of DNA free water (Cepheid, Sunnyvale, CA). To this mix was added 5 μL of the DNA extract from the sample. All primer and probe sequences used in the assays as well as known species comprising the assay groups are at the website: http://www.epa.gov/nerlcwww/moldtech.htm. Primers and probes were synthesized commercially (Applied Biosystems, Inc.).

Reactions were performed with thermal cycling conditions consisting of 2 min at 50 °C, 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C for template denaturation and 1 min at 60 °C for probe and primer annealing and primer extension. The Cycle threshold determinations were automatically performed by the Roche 480 software (Roche, Penzberg, Germany) using the second derivative maximum method. Assays for each target species and the internal reference (*G. candidum*) were performed in separate tubes of the 96-well plate format.

2.4. Cultivation analysis of air samples

Coriolis samples were also plated on malt extract agar (MEA, Merck, Germany). The numbers of fungal colony forming units (CFU) per plate were determined on days 3 and 7 after incubation at 25 °C. The total fungal concentrations were adjusted with the total liquid volume after Coriolis sampling. Results for air samples were thus expressed as CFU per cubic meter of air sampled.

2.5. Statistical analyses

Associations between dust ERMI values and other measurements (ERMI-like values and concentrations of culturable fungi in living room and bedroom air samples, age of dwelling, size of the contaminated area, and size of the dwelling) were evaluated by means of the Kendall coefficient ("Kendall's tau"). Values for Kendall's tau were calculated by the ranking procedure described in Hollander and Wolfe (1973) and using Microsoft Excel 2007 to perform the data manipulation. Corresponding p-values were obtained from Table A.21 in Hollander and Wolfe (1973).

3. Results

The ERMI values in these dwellings ranged from −2.74 to 28.79 (Fig. 1). There was an obvious break in the assembled ERMI values (no six or seven value ERMIs) and so the dwellings were divided into low (<6) or high (>8) ERMI dwellings. The mean ERMI values were 2.68 for low vs. 15.70 for high ERMI dwellings and these means were significantly different (Table 1). The differences between Sum of the Logs of the Group 1 and Group 2 fungi were also significantly different in low vs. high ERMI dwellings (Table 1). Furthermore, significant differences were observed for most of the mean concentrations of individual species in low vs. high ERMI dwellings (data not shown).

The ERMI values and "ERMI-like" values based on air samples in the living room or bedroom were significantly correlated (concordance 71.9% with average living room plus bedroom) (Table 2). The ERMI-like values in these dwellings ranged from −2.58 to 17.06 in