QUANTITATIVE PCR DETERMINATION OF MICROBES IN RELATION TO OBSERVED MEASURES OF MOULD IN HOMES

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ABSTRACT

The objective of this study was to evaluate a simple passive sampling approach in combination with quantitative PCR as a tool to objectively and reliably assess moisture damage and mould contamination in homes. In the HOME study carried out in New Zealand, airborne dust that had settled on electrostatic wipes over a period of four weeks was collected from 93 residential homes. The samples were analysed for fungal and bacterial content using quantitative PCR (qPCR). The study found that specifically measurements of total fungal DNA and *Penicillium/Aspergillus* group were strongly associated with observations of visible mould, mould odour and moisture damage in the home.

INTRODUCTION

Moisture damage and visible mould in buildings are consistently linked with ill health, and microbes are suggested to be a key factor in the adverse exposure /1,2/. Practical tools to reliably assess moisture related microbial contamination in buildings and to potentially predict health hazardous situations are lacking. The objective of this study was to evaluate a simple passive sampling approach in combination with quantitative PCR as a tool to assess mould contamination in homes. Our hypothesis was that a long-term sample of airborne settled dust analysed for DNA of specific fungal groups can be used to objectively predict conditions of moisture damage and visible mould indoors.

METHODOLOGY

The HOME study was launched to investigate whether housing characteristics, particularly mould levels, in New Zealand homes are related to new onset wheezing in children. In total 450 children were enrolled in the wider Wellington area into this case-control study, with one wheezing child compared to two non-wheezing children (controls). Researchers trained in mycological observation assessed each child's bedroom for presence and extent of visible mould, condensation, leaks and mould odour, as part of a home visit. The mycological assessment of the child's bedroom involved an examination of seven locations (e.g. walls, ceilings, floors) for signs of visible mould, with all these observations being rated on a mould severity scale from 0 – 3, categorizing the visible mould based on extent. Homes were categorized into low (mould score=0), medium (mould score=1-3) and high (4-21) mouldiness. Moreover, parents were asked about observations of leaks, condensation, visible mould and mould odour they had observed in the house in the past 12 months.

KIITOKSET

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Airborne settled dust was passively collected over four weeks on electrostatic wipes /3/ in the study children's bedrooms. Wipes were extracted in sterilized water with 0.05% Tween 20, and the extract subjected to beat milling and subsequent DNA purification. Quantitative PCR assays targeting total fungal DNA, *Penicillium* spp./*Aspergillus* spp./*Paecilomyces variotii*, *Cladosporium cladosporioides*, *Aspergillus versicolor*, Gram-positive and Gram-negative bacteria have been developed and described previously /4,5,6/.

The log transformed microbial measurement data were analysed against the home mould score using Pearson's correlation test and home characteristics using Student's T-Test.

RESULTS AND DISCUSSION

For this analysis, levels of fungal and bacterial groups were determined from airborne settled dust collected in 93 children's bedrooms: *Cladosporium cladosporioides* (median $4.0x10^2$ cells/wipe), *Aspergillus versicolor* (86% of samples <LOD), *Penicillium* spp./*Aspergillus* spp./*Paecilomyces variotii* group (Pen/Asp; median $4.0x10^4$ cells/wipe), total fungi (median $4.9x10^4$ cells/wipe), Gram-positive (median $2.0x10^6$ cells/wipe) and Gram-negative bacteria (median $8.7x10^5$ cells/wipe).

Levels of total fungi and Pen/Asp groups were significantly elevated in homes in which mould odour, visible dampness and leaks or moisture damage were reported. Moreover, these mould groups were correlated highly significantly with a mould score calculated based on the extent of visible mould observed in these homes. Levels of fungi and bacteria in low, medium and high mould score homes are presented in Table 1. Both Gram-positive and Gram-negative bacterial levels were significantly higher in homes where leaks or moisture damage and mould odour were observed, and in homes where more than one child slept in the bedroom. Levels of *C. cladosporioides* were significantly elevated in homes with reported leaks or moisture damage.

Table 1. Levels of fungal and bacterial groups measured via quantitative PCR in airborne settled dust from homes with low, medium and high researcher observed mould score. Median (25^{th} percentile – 75^{th} percentile) levels are shown; results are expressed as cell equivalents per wipe.

qPCR assays	Low mould (N=36)	Medium mould (N=27)	High mould (N=30)
Total fungi*	$2.7x10^4$ ($1.6x10^4$ - $6.2x10^4$)	$5.1x10^4$ ($1.9x10^4$ - $1.1x10^5$)	$1.1x10^5$ ($4.5x10^4$ - $1.7x10^5$)
Penicillium/Aspergillus group*	$2.6x10^4$ ($1.2x10^4$ - $4.7x10^4$)	$4.3x10^4$ ($1.3x10^4$ - $1.1 x10^5$)	$1.2x10^5$ ($2.7x10^4$ - $3.6 x10^5$)
Cladosporium cladosporioides	$2.7x10^2$ ($1.9x10^2$ - $6.2x10^2$)	$4.5x10^2$ ($2.3x10^2$ - $8.7 x10^2$)	$6.8x10^2$ ($2.8x10^2$ - $1.8x10^3$)
Gram-positive bacteria	$1.6x10^6$ ($1.1x10^6$ - $3.6x10^6$)	$2.2x10^6$ ($1.3x10^6$ - $3.6x10^6$)	$3.2x10^6$ ($1.0x10^6$ - $5.1x10^6$)
Gram-negative bacteria	$6.3x10^5$ ($2.9x10^5$ - $1.2x10^6$)	$8.7x10^5$ ($3.6x10^5$ - $2.0x10^6$)	$1.6x10^6$ ($6.4x10^5$ - $2.7x10^6$)

*significant ($p<0.05$) in Pearsons's correlation test when analysed against the home mould score

CONCLUSIONS

Quantitative PCR analyses in combination with a standardized passive sampling approach for settled dust is a promising tool to objectively measure mould contamination in residential homes, both in epidemiological study settings and to support building inspections for moisture and mould damage in practical situations. Specifically, measurements of total fungal DNA and *Penicillium/Aspergillus* group were strongly associated with observations of visible mould, mould odour and moisture damage in this study.

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