



Storage mite concentrations are underestimated compared to house dust mite concentrations

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Abstract

Dwellings are increasingly well insulated to save energy and this leads to higher humidity and temperature, which improves conditions for mites. *Dermatophagoides* antigens are the main allergens involved and tested in atopic asthma. We developed three new species-specific quantitative PCR (qPCR) methods for house dust mites (*Dermatophagoides pteronyssinus* and *D. farinae*) and storage mites (*Acarus siro*, *Glycyphagus domesticus*, *Lepidoglyphus destructor*). We sampled dust with electrostatic dust collectors, in the bedrooms, under beds and in the kitchens of patients with allergies (n=24) and healthy controls (n=18). Mite quantification was carried out with the three new qPCRs and the qPCR previously described for the *Dermatophagoides* genus. The qPCRs were highly specific and efficient for house dust mite species and the storage mites. Storage mite concentrations were higher than house dust mite concentrations and were higher in dwellings of patients with allergies. Consequently, allergists should test more often patients against the storage mite antigens by prick tests or IgE serology. Dampness is a major factor in storage mite development and the presence of effective mechanical ventilation can reduce storage mite concentrations four-fold. In addition, to limit exposure to dust mites, treatments should be used throughout dwellings and not only in patients' bedrooms.

Keywords Allergy · Dwellings · Electrostatic dust collector · House dust mites · Storage mites · qPCR

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Introduction

Efforts to limit heat loss in domestic dwellings often induce a decrease in aeration and higher relative humidity and temperature rates, which encourages mite development. Over the last 3 decades, allergies linked to our domestic environment have become more common (Eder et al. 2006). The main allergenic particles involved are pollen grains, mites, pet animals, molds and insects (Bousquet et al. 2012). House dust mites (HDMs) (genus *Dermatophagoides*) are the most frequent mites in indoor habitats (beds, carpets, etc.) but storage mites (SMs) (*Acarus siro*, *Lepidoglyphus destructor*, *Glycyphagus domesticus*) have also been identified in stored food in dwellings (Thind and Clarke 2001; Morales et al. 2015). The mite's main allergen is found in *Dermatophagoides pteronyssinus* feces: Der p1 (Tovey et al. 1981). Other allergens are Der p2 from the same species, and Der f1 and Der f2 from *Dermatophagoides farinae*, Aca s13 from *A. siro*, as well as more than 100 others from 13 mites species (Colloff 2009). There are 33 groups of allergens.

There is sufficient evidence of a causal relationship between exposure to dust mite allergen and exacerbation of asthma in children sensitized to dust mites, but there is limited evidence of an association in children not sensitized and in adults, sensitized or not sensitized (Kanchongkittiphon et al. 2015).

The enzyme-linked immunosorbent assay (ELISA) is the most commonly used method for investigating allergens from mites in the environment (Dunn et al. 2008), followed by the Acarex test® (Dyn'R Medical Systems, Aix-en-Provence, France) based on the semi-quantitative estimation by colorimetry of the guanine contained in the excrement of mites. However, these tests cannot quantify mite species precisely, they only recognize four classes. The Acarex score shows a correlation with Der p1 levels, but cannot replace an antigen assay when accurate data are required (Ridout et al. 1993). It is therefore necessary to use a species-specific standard method to detect and quantify domestic mites.

Our study aims are to compare the concentration of HDMs (*D. pteronyssinus*, *D. farinae*) to the concentration of SMs (*A. siro*, *G. domesticus*, *L. destructor*) in the dwellings of patients with allergies and healthy controls by three new species-specific qPCR and establish the environmental factors which may influence the relationship among species of mites.

Materials and methods

Development of HDM and SM species-specific qPCR

Tested strains

To test specificity we used *D. pteronyssinus*, *D. farinae*, *L. destructor*, *G. domesticus* and *A. siro* (from the mite collection of the Laboratory of Parasitology, Faculty of Pharmacy, University of the Basque Country, Vitoria, Spain), *D. pteronyssinus* and *D. farinae* (from University of Paris 11, Chatenay-Malabry, France) and *A. siro* (from a cheese cave in Saint-Antoine, France). The strains were cultured on a 1:1 (wt:wt) autoclaved mixture of commercial mouse meal and dried yeast powder according to Cardona et al. (2004).

Individuals of the cockroach *Blattella germanica* were obtained from the 'Centre des Sciences du Goût et de l'Alimentation' (Faculty of Sciences, University of

Bourgogne-Franche-Comté, Dijon, France). The 38 filamentous fungi, four actinomycetes, four yeasts, two mycobacteria and one enterobacteria (Table S1) used were those currently isolated from indoor dust samples. Microorganisms were cultured onto Sabouraud (Becton–Dickinson, Franklin Lakes, NJ, USA), malt agar (Becton–Dickinson), dichloran glycerol 18% (DG18) (Oxoid, Basingstoke, UK), Actino Difco® (Becton–Dickinson, Le Pont-de-Claix, France) and R8 media (Amner et al. 1989) at 20, 30 or 52 °C for 7 days.

DNA from a cat (*Felis catus*) and a dog (*Canis familiaris*) was obtained from ear tip and testes tissue, respectively (Scherer et al. 2016).

DNA isolation

Two hundred microlitter of DNA-free water containing almost 400 mites, five young individuals of *B. germanica* or 10 mm² of fungi, actinomycetes, yeasts, mycobacteria and enterobacteria suspension was placed into 2-ml conical-bottom screw-cap tubes (MagNA Lyser Green Beads; Roche Diagnostics, Germany) containing 1.4-mm ceramic beads and 400 µl of lysis buffer from the NucleoSpin Plant II kit (Macherey–Nagel, Düren, Germany). The tubes were shaken in a MagNA Lyser Instrument (Roche Applied Science, Mannheim, Germany) 3× for 10 s at the maximum speed, with 1 min in the cooling block. The tubes were then heated in a boiling water bath for 10 min. The samples were placed on ice for 10 min, followed by centrifugation for 2 min at 6000×g. DNA was then purified with NucleoSpin Plant II kit according to the manufacturer's instructions. DNA from *F. catus* and *C. familiaris* was obtained with the High Pure PCR Template Preparation Kit as recommended by the manufacturer (Roche Applied Science). The DNA concentration of each extract was determined by a dosage program (160 nm) with a ND-1000 Nanodrop spectrophotometer (Thermo Fisher Scientific, USA).

Design of probes and primers

Mitochondrial Cytochrome B genus (HDMs) and rRNA (SMs) sequences of the main mite species found in dwellings were retrieved from the GenBank database and were aligned using BioEdit Software (Tom Hall Ibis Biosciences, Carlsbad, CA, USA) to choose the most appropriate areas for primer design.

Primers and probes were designed using Primer Express® 3.0 for Real-Time PCR software (Applied Biosystems, Carlsbad, CA, USA). Parameters used to design primers and probes were chosen to ensure optimal specificity and efficiency for qPCR as described by Roussel et al. (2013).

qPCR, linear dynamic range, limit of detection and efficiency

PCR reactions contain 10 µl two-fold concentrated TaqMan® Brilliant III Ultra-Fast qPCR Master Mix (Agilent Technologies, USA), reverse and forward primers (Table 2) (final concentration 1 µM each) (Sigma-Aldrich, USA), a hydrolysis probe (Table 2) (final concentration 200 nM) (MWG Eurofins Operon, Germany), 5 µl of DNA sample and nuclease-free water to complete the 20 µl total volume of reaction mixture. Plates were centrifuged (1 min at 1000×g) and PCR reactions were run and monitored using Applied Biosystems 7500 FAST Real-Time PCR System and 7500 Software v.2.0.5. Sterile DNA-free water was extracted with samples and used as negative controls in each series of amplification.

Thermal cycles consisted of 3 min at 95 °C, followed by 45 cycles of 15 s at 95 °C and 1 min at 60 °C.

To generate calibration curves, DNA extracts were adjusted at 100 fg/μl with NucleoSpin Plant II kit elution buffer (Macherey–Nagel) and were then serially diluted (1:10), resulting in a five-point calibration curve range of 100–0.01 fg/μl. For each qPCR assay, the calibration curve allowed us to calculate the linear dynamic range (LDR), and PCR amplification efficiency. The LDR is the concentration range over which the quantification cycle (C_q) and concentration remain directly proportional to each other. The range interval and correlation coefficient (R² value) were then reported. Amplification was repeated 7× for each concentration and the limit of detection (LOD) was the lowest concentration at which DNA was detected in all seven samples. PCR amplification efficiency was established as the slope of the log-linear portion of the calibration curve: PCR efficiency = $10^{-1/\text{slope}} - 1$.

Analytical specificity

Analytical specificity, which refers to the qPCR assay detecting the appropriate organism and no others was tested against five mites, 38 filamentous fungi, four actinomycetes, four yeasts, two mycobacteria, enterobacteria, cockroach, cat and dog DNA extracts. DNA concentration was adapted to 10 fg/μl to test analytical specificity and each DNA extract was tested using each of the combinations of the three qPCR assays.

‘In silico’ specificity test

To verify the specificity of the qPCRs of storage mites (qPCRs of a group of three species) we used 84 mite sequences representing 56 species deposited in the GenBank to perform ‘in silico’ tests. The sequences were compared electronically with our probe/primer.

Applying novel qPCR on dust collected from dwellings

Recruitment and questionnaires

Twenty-four dwellings of patients with allergies (allergies confirmed by skin prick tests) and 18 dwellings of controls (without allergies) were recruited by the Medical Indoor Environment Counsellor (MIEC) of the ‘Mutualité de Bourgogne’ and the ‘Réseau d’Allergologie de Franche-Comté’ at the request of the allergist. For each dwelling, an environmental investigation was carried out and two questionnaires were distributed. The first was designed for the patients, the dwelling, the kitchen and the bedroom, the second involved clinical data.

Relative humidity and temperature measurement

Relative humidity and temperature was measured using a portable Testo 606-2 moisture meter (Testo, Aschaffenburg, Germany).

Table 1 Forward (Fwd) and reverse (Rev) primers and probe^a for *Dermatophagoides* spp. described by Roussel et al. (2013)

Fwd	DermaF1	5'-CCTCGTGGTGGAGTGCATT-3'
Rev	DermaR1	5'-TCAGCTGCAGTTATCCAGAGTCA-3'
Probe	DermaP1	5'-ATTAGACCAAGACCAAGGTGGCAACACC-3'

^aLabeled with FAM reporter and TAMRA quencher**Table 2** Forward (Fwd) and reverse (Rev) primers and probes^a for two dust mites and three storage mites

<i>Dermatophagoides pteronyssinus</i>		
Fwd	DpterCBF1	5'-CATCCAACCAGAGTGGTATTTCC-3'
Rev	DpterCBR1	5'-GCTATTGCGCATACTCCACCTA-3'
Probe	DpterCBP1	5'-TATGCAATCCTTCGGGCTATCCCATCA-3'
<i>Dermatophagoides farinae</i>		
Fwd	DfariCBF1	5'-CACACATTCAACCAGAGTGGTACTT-3'
Rev	DfariCBR1	5'-GGCTAACACTCCCCCTAGTTTAGA-3'
Probe	DfariCBP1	5'-CGCTTACGCGATCCTACGAGCCATT-3'
Storage mites (<i>Acarus siro</i> / <i>Glycyphagus domesticus</i> / <i>Lepidoglyphus destructor</i>)		
Fwd	SMF1	5'-CGCAAACCTGTGGTGGCGAGTA-3'
Rev	SMR1	5'-GCTCCTTGGTCCGTGTTTCA-3'
Probe	SMP1	5'-TCGGTCTCCACCCGACCCGTC-3'

^aLabeled with FAM reporter and TAMRA quencher

Electrostatic dust collection (EDC)

EDCs are electrostatic wipes (cloth surface: 20×17 cm) that are able to capture dust. This device has already been used for analysis with real time quantitative PCR (qPCR) (Scherer et al. 2014; Yamamoto et al. 2011). Three EDC were placed in each dwelling for 10 weeks. The first one was placed at a height of 1 m in the bedroom, the second on the floor under the bed and the third at a height of 1 m in the kitchen. EDC were returned by mail.

The EDC wipes were placed in a plastic bag with 20 ml of a solution of Tween 80 to 0.1% and washed in Stomacher for 10 min. Nine ml of rinsing liquid was then collected.

Sample analysis

DNA extraction was performed from the centrifuged washing liquid (8000×g, 10 min) using mechanical and thermal lysis as previously described by Scherer et al. (2014). In each extraction series, a sterile distilled water sample was included as a negative control, and negative and positive controls were added to each qPCR reaction.

Primers and hydrolysis probes were used in the qPCR analyses. Four specific qPCR targets were distributed as follows: one HDM genus (*Dermatophagoides*) using primers and probes developed in our laboratory (Roussel et al. 2013) (Table 1), and two HDM species (*D. pteronyssinus* and *D. farinae*) and one SM group (*A. siro*/*L. destructor*/*G. domesticus*) using the three new specific qPCRs described in Table 2.

Expression of qPCR results in the environment

qPCR results were expressed in fg/ μ l, using species-specific DNA standard calibration curves. A known number of *D. farinae* used with the DNA standard calibration curve allows us to express our results in 1000th of mite equivalent/ μ l. One, two, four, eight and 16 mites are the equivalent of 1102, 2032, 3887, 8008 and 15,832 fg/ μ l, respectively. Therefore, 1 fg/ μ l is the equivalent of 1000th of mite/ μ l. This calculation was made for *D. farinae* and extrapolated for the other groups (HDMs and SMs) and species (*D. pteronyssinus*).

Statistical analysis

Data were analyzed using the statistical program R (v.3.2.2) with packages lme4 (v.1.1.7), MASS (v.7.3.43) and pbkrtest (v.0.4.2) (R Development Core Team, Vienna, Austria). The dwelling characteristics of patients with allergies and controls were compared using the χ^2 test (qualitative variables) and Student's t test (quantitative variables). The comparisons of concentrations at three locations in the dwellings of the allergy and control groups were performed with a linear mixed-effect model. From this model, location effects were removed to obtain one concentration value by dwellings for each species. Species evaluations according to dwelling characteristics and concentrations of mites in dwellings were carried out using a linear model. The dwelling characteristics were automatically selected for each species using a stepwise model selection by Akaike's Information Criterion (AIC).

HDMs/SMs and *D. pteronyssinus*/*D. farinae* concentrations were compared with a paired Student's t test and the domestic mite relationship was determined with a Pearson correlation test.

Results

Development of HDM and SM species-specific qPCR

Design of primers/probes

The alignment of mite sequences enabled us to choose areas for primer and probe designs. Primer and probe sequences are listed in Table 2. DpterCBF1/R1/P1 was designed specifically to amplify *D. pteronyssinus*, DfariCBF1/R1/P1 specifically to amplify *D. farinae* and SMF1/R1/P1 specifically to amplify the three main SM species *A. siro*, *L. destructor* and *G. domesticus*.

Analytical specificity

Analytical specificity was first checked using BLAST searches, and then validated by qPCR assays against mites, fungi, actinomycetes, yeasts, mycobacteria, enterobacteria,

Table 3 Mean ($\pm$ SD) threshold cycles for the qPCR specificity study

Species extract (10 fg/ μ l)	DpterCBF1/R1/P1	DfariCBF1/R1/P1	SMF1/R1/P1
<i>Dermatophagoides pteronyssinus</i>	28.87 $\pm$ 0.05	Und.	Und.
<i>Dermatophagoides farinae</i>	Und.	26.21 $\pm$ 0.15	Und.
<i>Lepidoglyphus destructor</i>	Und.	Und.	28.26 $\pm$ 0.21
<i>Glycyphagus domesticus</i>	Und.	Und.	29.38 $\pm$ 0.22
<i>Acarus siro</i>	Und.	Und.	27.20 $\pm$ 0.16

Und. (undetermined): no amplification curve was obtained after 45 cycles of PCR

and cockroach, cat and dog extracts. No positive result was observed in qPCR assays using the three primers/probes designed (Table S1). Mite extracts were amplified as reported in Table 3, showing a good analytical specificity: only the target species were detected.

'In silico tests' on storage mites qPCR

Theoretically, four species would systematically 'match' with our 'probe/prime' storage mite systems (*Diadasiopus eickworti*, *Sancassania sphaerogaster*, *S. ochoai*, *S. salasi*) (Table S2).

Analytical characteristics of qPCR assays

The linear dynamic range (LDR) of qPCRs was calculated using calibration curves of targeted organisms, and found to be between 100 and 0.01 fg/ μ l (Table 4). The correlation coefficient was up to 0.96 for the three targets, showing a good linearity between logarithms for DNA concentration and Cq. The limit of detection (LOD) ranged from 10^{-4} to 10^{-5} mites per μ l of extract. Coefficients of variation for reproducibility were lower than 0.5 (Table 4).

Table 4 Performance of qPCR assays to detect specific mite strains extracted

Targets		Linear dynamic range		Limit of detection		PCR efficiency (%)	CV _{rep}
Primers/probes	DNA extract tested	Interval (fg/ μ l)	R ²	fg/ μ l	mites/ μ l		
DpterCBF1/R1/P1	<i>Dermatophagoides pteronyssinus</i>	100 to 0.01	0.985	0.1	10^{-4}	101	0.3
DfariCBF1/R1/P1	<i>D. farinae</i>	100 to 0.01	0.994	0.01	10^{-5}	94	0.4
SMF1/R1/P1	<i>Acarus siro</i>	100 to 0.01	0.968	0.1	10^{-4}	98	0.3

CV_{rep} coefficient of variation for reproducibility

Applying novel qPCR on dust from dwellings

Dwelling characteristics and clinical information

Dwellings were recruited from eastern France, which has a continental climate with maximum average temperatures of 5 °C in winter and an average relative humidity of 88%.

The dwelling profiles, observed in this study, were mainly houses equipped with controlled mechanical ventilators (CMV) (a quarter of the kitchens and a 10th of the bedrooms). Single-flow CMVs are pipes equipped with a motor to extract indoor air and release it outside the building. Double-flow CMVs include a second circuit allowing the recovery of part of the calories (1 dwelling per 10 equipped with CMV). There are no air conditioning installations in the dwellings. They had gas heating and were aired every day. The kitchen walls were painted and the floors tiled, whereas the bedroom walls were decorated with wallpaper and had parquet floors. The mean relative humidity was 48.3% (37.6–66.9%) and the mean temperature was 20.8 °C (17.4–23.6 °C). Evidence of damp (visible molds, mildew smell, blistered paint, and unstuck wallpaper)

Table 5 Dwelling characteristics and comparison between dwellings of patients with allergies and those of controls using the χ^2 test (qualitative variables) and Student's t test (quantitative variables)

Questions	Patients with positive skin prick tests (n = 24)		Healthy controls (n = 18)		P
Dwelling					
Area	Rural (10)	Urban (14)	Rural (4)	Urban (14)	0.18
Location	Apartment (5)	House (19)	Apartment (5)	House (13)	0.78
Potted plants	Yes (20)	No (4)	Yes (16)	No (2)	0.77
Pets	Yes (13)	No (11)	Yes (7)	No (11)	0.30
Use of plant-health products	Yes (14)	No (10)	Yes (8)	No (10)	0.27
Type of heating	Gas (12)	Other ^a (12)	Gas (5)	Other ^a (13)	0.18
Damp evident	Yes (4)	No (20)	Yes (6)	No (12)	1.16
m ² /inhabitants	Median (30)		Median (47)		0.04
Kitchen					
Ventilation	Yes (18)	No (6)	Yes (14)	No (4)	0.48
Floor covering	Tile (18)	Other ^b (6)	Tile (15)	Other ^b (3)	0.77
Wall covering	Paint (9)	Other ^c (15)	Paint (10)	Other ^c (8)	0.57
Indoor compost	Yes (11)	No (13)	Yes (7)	No (11)	0.77
Aeration frequency	Median (every day)		Median (every day)		0.79
Bedroom					
Anti-HDM treatments	Yes (7)	No (17)	Yes (1)	No (17)	0.04
Floor covering	Parquet (13)	Other ^b (11)	Parquet (13)	Other ^b (13)	0.77
Wall covering	WallPaper (11)	Other ^c (13)	Paper (10)	Other ^c (8)	0.39
Aeration frequency	Median (every day)		Median (every day)		0.79
Inhabitants					
Smokers	Yes (9)	No (15)	Yes (3)	No (15)	0.35

^aElectricity, fuel, wood

^bLinoleum, parquet

^cPaper, paint, fiber

was observed in 25% of the dwellings. The inhabitants were mainly non-smokers. The number of m² per inhabitant was significantly higher in the dwellings of controls (t test: $P=0.04$). The patients with allergies used significantly more anti-HDM treatment than controls (χ^2 test: $P=0.04$). Comparative characteristics of dwellings of allergy patients and controls are presented in Table 5.

The 24 patients selected for this study were chronic allergy sufferers (seasonal allergies excluded) with a hypersensitive response to domestic mites (skin prick test positive for *D. pteronyssinus* or *D. farinae*) but also to pollen (7), cats (3), molds (3), dogs (2) and cockroaches (1). Nineteen had rhinitis, 15 asthma, 12 conjunctivitis, two chronic nasal dysfunction and one atopic dermatitis.

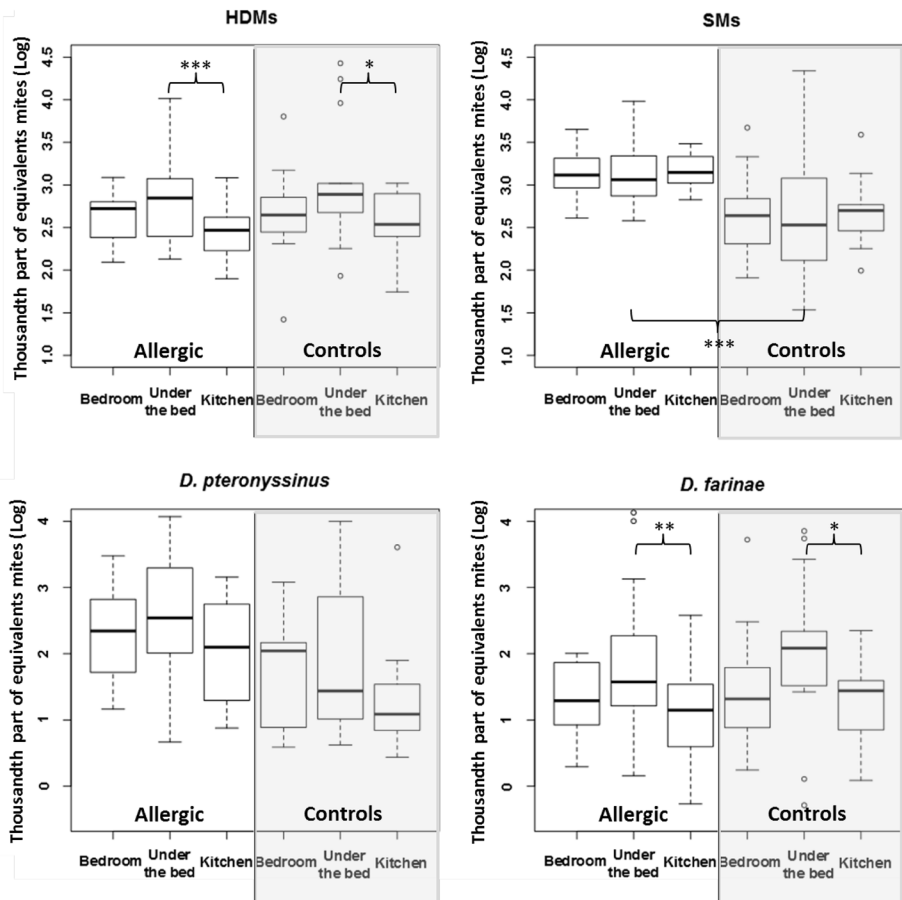


Fig. 1 Contamination of house dust mites (HDMs), storage mites (SMs), *Dermatophagoides pteronyssinus* and *D. farinae* (denominated in log 1000th of mite equivalents) at the three sites (bedroom, under the bed, kitchen) in the dwellings of patients with allergies and controls. *** $P < 0.005$, ** $0.005 < P < 0.01$, * $0.01 < P < 0.05$

Contamination profiles of dwellings

With EDC, HDMs and SMs were detected by qPCR in all dwellings. The abundance of targets ranged from 0 to 21,000 fg/ μ l (i.e., 0–21,000 one-thousandth of mite equivalent per μ l) and contamination in the dwellings of patients with allergies and controls are presented in Fig. 1.

The quantity of HDMs was similar in the dwellings of patients with allergies and those of controls. By contrast, SM concentrations from the dwellings of patients with allergies were higher than those of controls (t test: $P=0.00004$).

SM concentrations were similar in bedrooms, under beds and in kitchens. The highest concentrations of HDMs were found under beds, regardless of whether the dwellings belonged to patients with allergies (linear mixed-effect model: $P=0.0006$) or controls ($P=0.03$). There were significant differences in HDM concentrations between the EDC locations for *D. farinae*—under beds vs. in kitchens; linear mixed-effect model, $P=0.003$ (allergy patients) and $P=0.01$ (controls)—but not for *D. pteronyssinus* (Fig. 1).

Factors influencing HDM and SM contamination

SM concentration increased when damp was observed (linear model with stepwise selection by AIC: $P=0.03$) and decreased four-fold with the presence of CMV ($P=0.0004$). These factors did not seem to influence the HDM concentrations (*Dermatophagoides* spp., *D. pteronyssinus* or *D. farinae*). However, the anti-HDM treatment (dust mite-proof, chemical treatment of bedding part) effects were limited to a slight and non-significant reduction

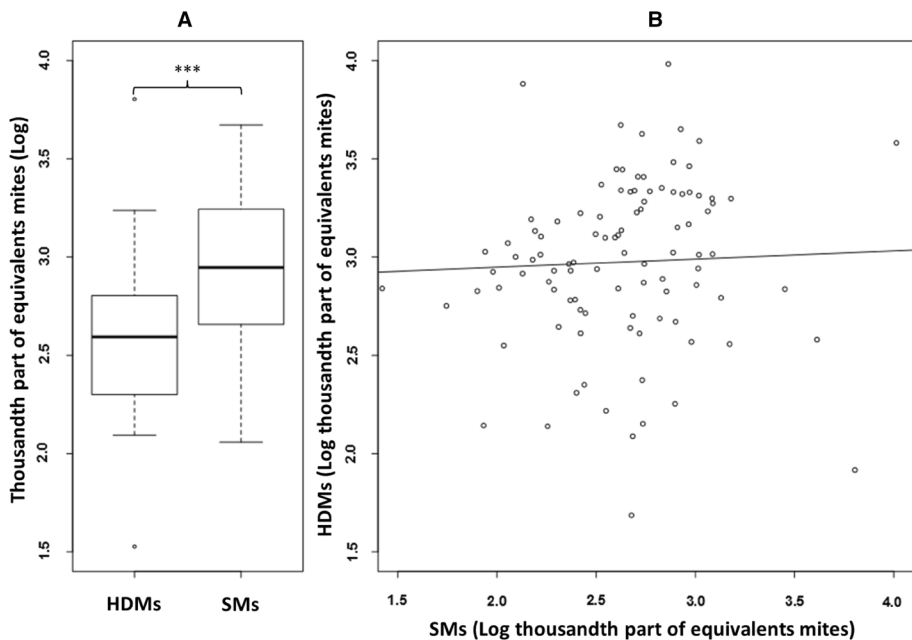


Fig. 2 Relationship between house dust mites (HDMs) and storage mites (SMs) concentrations (denominated in log 1000th of mite equivalents) (a) and their correlation (b). *** $P<0.005$

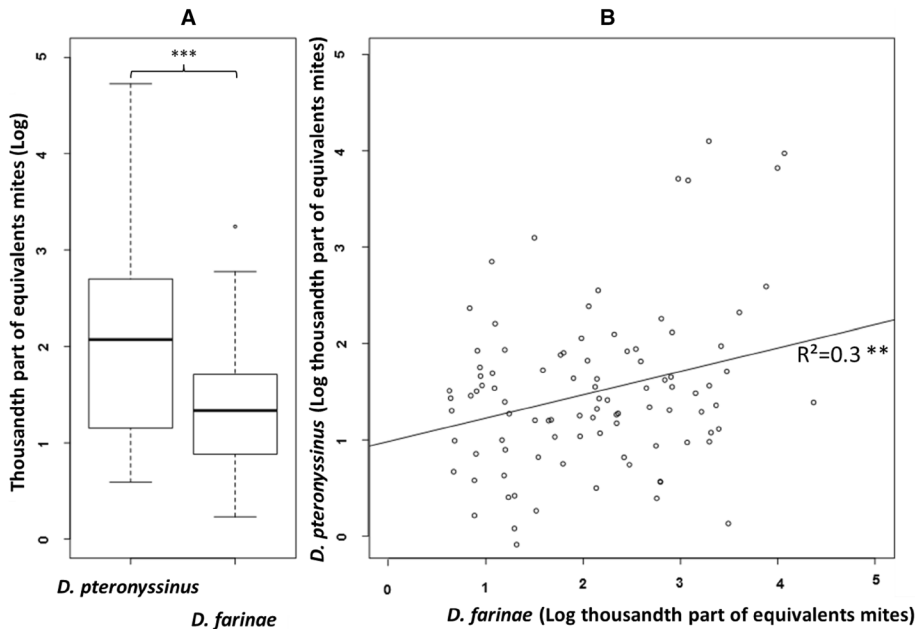


Fig. 3 Relationship between *Dermatophagoides pteronyssinus* and *D. farinae* concentrations (denominated in log 1000th of mite equivalents) (a) and their correlation (b). *** $P < 0.005$, ** $0.005 < P < 0.01$

in HDM (*Dermatophagoides* spp., *D. pteronyssinus* or *D. farinae*) and SM concentrations throughout the dwellings.

Relationship between contamination and mites

Comparing the HDM and SM concentrations, we found that the SM concentrations were on average higher than the HDM concentrations (paired t test: $P = 0.0001$) (Fig. 2a). SM and HDM concentrations were not significantly correlated (Fig. 2b). Apparently, high HDM concentrations did not lead to high SM concentrations or vice versa.

As far as the two main HDM species are concerned, the concentrations of *D. pteronyssinus* were significantly higher than those of *D. farinae* (paired t test: $P = 0.0003$) (Fig. 3a) and these concentrations are significantly correlated (Pearson correlation test: $R^2 = 0.3$; $P = 0.006$): higher concentrations of *D. pteronyssinus* associate with higher concentrations of *D. farinae* (Fig. 3b).

Discussion

Three novel species-specific qPCR methods can be used to quantify *D. pteronyssinus*, *D. farinae* and *A. siro/G. domesticus/L. destructor*. Comparatively, SMs concentrations are higher than HDMs concentrations and are higher in dwellings of patients with allergies than in dwellings of controls. SM and HDM concentrations are independent and damp is a

factor encouraging SM development, but the presence of controlled mechanical ventilators reduces SM concentrations.

The new primers and probes obtained for the HDM targets *D. pteronyssinus* and *D. farinae* can only detect the targeted species and reduce the LOD to 10^{-4} and 10^{-5} vs. 10^{-3} and 10^{-4} mite equivalents per μl , respectively, in our previous study (Roussel et al. 2013). The new qPCR target can detect the three main SM species in the indoor environment: *A. siro*, *G. domesticus* and *L. destructor*, with a low LOD (10^{-4} mite equivalents per μl).

Three methods have been used in environmental studies to estimate exposure to mites: mite counts, assays for specific mite allergens by ELISA, and guanine measurements (Platts-Mills et al. 1992). Mite counts by microscopic analysis make it possible to specify the founding species (Platts-Mills et al. 1992). However, determining species requires expert knowledge, and this method is too time-consuming to be applied to a large number of samples. Quantifying the guanine production of mites is limited by the amount of guanine that is not produced by HDMs (Hallas et al. 1993). ELISA is the most commonly used method for investigating the human–environment with species-specific monoclonal antibodies to bind group I (Der p I and Der f I) allergens (Indoor Biotechnologies, Charlottesville, VA, USA; Phadia, Uppsala, Sweden). Measurements are usually performed on dust from floors and mattresses (Brunekreef et al. 2002; Gent et al. 2012).

The results of detection and quantification of *Dermatophagoides* spp. in dust by qPCR correlated with ELISA assays for Der p I quantification, matching the previously reported correlation between mite counts and Der p I quantification (Lind 1986). Quantities of dust necessary for assays are different for qPCR and ELISA tests: 100 mg of dust were needed to perform the ELISA assays whereas qPCR experiments were performed with 15 mg of dust (Roussel et al. 2013). The detection limit for the qPCR experiments mainly ranged from 1 to 0.1 pg/ μl , making it possible to detect 1 to 13 mites per g of dust (Roussel et al. 2013). This limit of detection is equivalent to the LOD of the ELISA Der p I method (Lind 1986) and is higher than that of ELISA, allowing the detection of *A. siro* in grain (Krizkova-Kudlikova et al. 2007). The Acarex test is simple to identify sources of mites in houses (Haouichat et al. 2000) but in qPCR experiments, a single dust extract of 15 mg (vs. 100 mg for ELISA or Acarex test) allows us to quantify HDMs, SMs, fungi and bacteria.

Domestic mites were detected in all dwellings in this study. The strong presence of HDMs and their effects on health are recognized by the medical community (Boquete et al. 2006). SMs were present in higher concentrations in dwellings of patients with allergies but their implications for health have not been thoroughly explored. SM concentrations were similar in bedrooms and kitchens, whereas HDM concentrations were higher in bedrooms, particularly under beds. SMs feed on food waste and colonize foodstuffs such as flour and cheese. Mattress conditions are ideal for HDMs due to the damp and heat (caused by human sweat) and food (skin, keratinized fragments) (Boor et al. 2014). HDMs and SMs are detected in bedrooms and kitchens because air circulates from the main rooms (bedrooms, living rooms) towards kitchens and bathrooms. CMV extracts the air polluted by kitchens and bathrooms and new air enters the dwelling through air vents above windows in living rooms and bedrooms. Furthermore, inhabitant movements allow air to flow within the dwelling. Mites are able to move quickly (7 mm/min) (Naegelé et al. 2013), but this movement plays a limited role in dwellings, and ‘invasion’ does not exceed 1 m from the origin (Naegelé et al. 2016).

Our results demonstrate a significant decrease in SM concentrations with the presence of an efficient CMV. However, the observation of damp is linked to an increase in SM concentrations, as damp is one of the main factors in mite development. Depending on the relative humidity, a variety of mite species can be found in dwellings. Relative humidities

of 50–60% (*D. farinae*) and 70–80% (*D. pteronyssinus*) will encourage the presence of HDMs (Crowther et al. 2006) whereas a high upper rate of 85% will promote SM development (Aspaly et al. 2007). Other factors such as temperature and food availability will modify the potential of each species to develop in dwellings. HDMs thrive at a temperature between 26 and 32 °C but can survive at 15 °C (Arlian et al. 1990). However, SMs can withstand lower temperatures. SMs exposed to gradually decreasing temperatures during climatic changes of approaching winter, in grain under warehouse conditions, survived at – 15 °C for 96 days (Ždárková and Vladimír 1993). When the conditions are not optimal, SMs are able to block or prolong their stage of development (Wharton 1976). These behavioral adaptations could be the cause of the proliferation of SMs in dwellings in our study.

Included in the MIEC recommendations are removing carpets, lowering of temperature, airing more regularly and for longer, and anti-HDM treatments (mattress covers, miticide spray). These physical and chemical treatments led to a slight decrease in HDM and SM concentrations in this study. Indeed, anti-HDM treatments are only effective locally, whereas allergens are aerosolized throughout dwellings.

Exposure to allergens can lead to conjunctivitis, rhinitis and asthma (Lin et al. 2012). Dust allergens were among the first pollutants to be associated with respiratory effects (Blanc et al. 2005). Mites' feces are a major allergen in domestic dust. *Dermatophagoides pteronyssinus* produced, on average, 20 excrements a day containing 100 pg of Der p1 each (Tovey et al. 1981). Thresholds of sensitization fixed at 2 µg/g of dust were found to lead to antibody production (Calderón et al. 2015).

The Global Allergy and Asthma European Network suggests a panel of allergens (pollen, animals, molds, insects and HDM) to be tested but the SM allergens are not listed (Bousquet et al. 2012). *Dermatophagoides pteronyssinus* and *D. farinae* are the only HDM allergen sources suggested by this network. According to Høst et al. (2003), HDM allergens should also be tested and SM allergens are not mentioned despite the fact that tests for SM allergens (*A. siro*, *L. destructor*, *G. domesticus*) are available (Phadia, Uppsala, Sweden). However, the clinical effects of *A. siro* (allergen Aca s13) are well known (van Hage-Hamsten and Johansson 1998) and this species has been associated with indoor respiratory allergy, mainly in rural areas (Iraola et al. 2014). SMs of the *Acarus* genus are a major cause of deterioration in stored food and are found all over the world.

In conclusion, it is essential to use a quantitative tool (qPCR) that is sensitive and highly specific in order to quantify allergen exposure. SMs are particularly common in dwellings of patients with allergies but quantities of them are underestimated and their effects on health are probably not sufficiently acknowledged by allergists. To limit our exposure to HDMs and SMs, an effective CMV should be set up as well as anti-HDM treatment used throughout dwellings, and not only in patients' bedrooms.

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Table S1 Mean of threshold cycles for the qPCR specificity study

Tested strains	DpterCBF1/R1/P1	DfariCBF1/R1/P1	SMF1/R1/P1
Molds			
<i>Acremonium strictum</i>	Und.	Und.	Und.
<i>Alternaria alternata</i>	Und.	Und.	Und.
<i>Aspergillus flavus</i>	Und.	Und.	Und.
<i>Aspergillus fumigatus</i>	Und.	Und.	Und.
<i>Aspergillus niger</i>	Und.	Und.	Und.
<i>Aspergillus ochraceus</i>	Und.	Und.	Und.
<i>Aspergillus penicillioides</i>	Und.	Und.	Und.
<i>Aspergillus sydowii</i>	Und.	Und.	Und.
<i>Aspergillus versicolor</i>	Und.	Und.	Und.
<i>Aureobasidium pullulans</i>	Und.	Und.	Und.
<i>Chaetomium globosum</i>	Und.	Und.	Und.
<i>Cladosporium cladosporoides</i>	Und.	Und.	Und.
<i>Cladosporium herbarum</i>	Und.	Und.	Und.
<i>Cladosporium sphaerospermum</i>	Und.	Und.	Und.
<i>Epicoccum nigrum</i>	Und.	Und.	Und.
<i>Eurotium amstelodami</i>	Und.	Und.	Und.
<i>Lichteimia corymbifera</i>	Und.	Und.	Und.
<i>Mucor</i>		Und.	Und.
<i>Paecilomyces variotii</i>	Und.	Und.	Und.
<i>Cladosporium cladosporoides</i>	Und.	Und.	Und.
<i>Pencillium brevicompactum</i>	Und.	Und.	Und.
<i>Pencillium chrysogenum</i>	Und.	Und.	Und.
<i>Penicillium citrinum</i>	Und.	Und.	Und.
<i>Penicillium corylophilum</i>	Und.	Und.	Und.
<i>Penicillium crustosum</i>	Und.	Und.	Und.
<i>Penicillium italicum</i>	Und.	Und.	Und.
<i>Penicillium olsonii</i>	Und.	Und.	Und.
<i>Penicillium piceum</i>	Und.	Und.	Und.
<i>Penicillium purpurogenum</i>	Und.	Und.	Und.
<i>Penicillium variable</i>	Und.	Und.	Und.
<i>Rhizomucor</i>		Und.	Und.
<i>Rhizopus oryzae</i>	Und.	Und.	Und.
<i>Rhizopus stolonifer</i>	Und.	Und.	Und.
<i>Scedosporium apiospermum</i>	Und.	Und.	Und.
<i>Scedosporium prolificans</i>	Und.	Und.	Und.
<i>Scopulariopsis brevicaulis</i>	Und.	Und.	Und.
<i>Stachybotrys chartarum</i>	Und.	Und.	Und.
<i>Trichoderma viride</i>	Und.	Und.	Und.
<i>Wallemia sebi</i>	Und.	Und.	Und.
Actinomycetes			
<i>Laceyella sacchari</i>	Und.	Und.	Und.
<i>Nocardiopsis composita</i>	Und.	Und.	Und.
<i>Saccharopolyspora rectivirgula</i>	Und.	Und.	Und.
<i>Streptomyces albus</i>	Und.	Und.	Und.
Yeasts			
<i>Candida albicans</i>	Und.	Und.	Und.
<i>Candida diffluens</i>	Und.	Und.	Und.
<i>Candida neoformans</i>	Und.	Und.	Und.
<i>Cryptococcus uniguttulatus</i>	Und.	Und.	Und.
Mycobacteria			
<i>Mycobacterium immunogenum</i>	Und.	Und.	Und.
<i>Mycobacterium chelonae</i>	Und.	Und.	Und.
Enterobacteria			
<i>Klebsiella oxytoca</i>	Und.	Und.	Und.
Cockroach			
<i>Blattella germanica</i>	Und.	Und.	Und.
Cat			
<i>Felis catus</i>	Und.	Und.	Und.
Dog			
<i>Canis familiaris</i>	Und.	Und.	Und.

Und. (undetermined): no amplification curve was obtained after 45 cycles of PCR

Table S2 ‘In silico’ tests: alignment of our storage mites group qPCR system

(forward/reverse primers and probe) with mite sequences (DNA 28S region) in GenBank

Identification	Species	Error primer forward	Error probe	Error primer reverse
JQ000408.1	Acaridae gen. n. sp. n. AD469	2	0	0
JQ000412.1	<i>Acarus immobilis</i>	0	0	0
EU152621.1	<i>Acarus siro</i>	0	0	0
JQ000411.1	<i>Acarus siro</i>	0	0	0

EU152624.1	<i>Acotyledon paradoxa</i>	1	0	0
JQ000416.1	<i>Acotyledon paradoxa</i>	1	0	0
JQ000395.1	aff. <i>Alabidopus</i> sp. AD591	2	0	0
JQ000391.1	<i>Afrocanestrinia</i> sp. n. AD456	3	0	0
JQ000431.1	<i>Alcedinectes alcyon</i>	2	0	0
JQ000394.1	<i>Arrunsithiana</i> nr. <i>spicantis</i> AD764	3	0	0
EU152627.1	<i>Askinasia antillarum</i>	2	0	0
JQ000424.1	<i>Askinasia antillarum</i>	2	0	0
JQ000423.1	<i>Askinasia</i> sp. AD304	2	0	0
JQ000419.1	<i>Boletoglyphus extremiorientalis</i>	2	0	0
JQ000420.1	<i>Boletoglyphus ornatus</i>	2	0	0
JQ000417.1	<i>Aleuroglyphus ovatus</i>	2	0	0
EU152612.1	<i>Canestrinia pentodontis</i>	3	0	0
JQ000392.1	<i>Canestrinia pentodontis</i>	3	0	0
EU152613.1	<i>Canestrinia</i> sp. n. AD775	3	0	0
JQ000393.1	<i>Canestrinia</i> sp. n. AD775	3	0	0
JQ000418.1	<i>Capillaroglyphus polypori</i>	2	0	0
EU152631.1	<i>Cerophagus nearcticus</i>	2	1	0
JQ000428.1	<i>Cerophagus nearcticus</i>	2	1	0
JQ000390.1	<i>Chaetodactylus lithurgi</i>	3	0	0
EU152614.1	<i>Chortoglyphidae</i> sp. AD591	2	0	0
JQ000397.1	<i>Chortoglyphus arcuatus</i>	1	0	0
EU152615.1	<i>Chortoglyphus sciuricola</i>	1	0	0
JQ000396.1	<i>Chortoglyphus sciuricola</i>	1	0	0
JQ000398.1	<i>Chortoglyphus</i> sp. AD792	1	0	0
JQ000413.1	<i>Cosmoglyphus</i> sp. AD702	1	0	0
EU152690.1	<i>Dermoglyphus</i> sp. n. AD780	3	3	0
JQ000506.1	<i>Dermoglyphus</i> sp. n. AD780	3	3	0
JQ000410.1	<i>Diadasiopus eickworti</i>	0	0	0
EU152620.1	<i>Eburneopus eclecticus</i>	2	0	0
EU152628.1	<i>Ewingia coenobitae</i>	2	0	0
JQ000425.1	<i>Ewingia coenobitae</i>	2	0	0
JQ000404.1	<i>Glycyphagus hypudaei</i> c. PBK-2011	2	1	0
EU152619.1	<i>Glycyphagus hypudaei</i> s.l. AD614	2	1	0
JQ000429.1	Heterocoptidae gen. n. sp. AD552	2	2	0
EU152632.1	Heterocoptidae sp. n. AD552	2	2	0
EU152629.1	<i>Histiogaster arborsignis</i>	2	0	0
JQ000426.1	<i>Histiogaster</i> sp. AD356	2	0	0
JQ000383.1	<i>Hyadesia</i> aff. <i>curassaviensis</i> AD605	1	2	0
EU152608.1	<i>Hyadesia curassaviensis</i>	1	3	0
JQ000382.1	<i>Hyadesia curassaviensis</i>	1	3	0
JQ000432.1	<i>Hypodectes</i> sp. AD946	3	0	0
JQ000433.1	Hypoderatidae gen. sp. AD887	2	0	0
EU152603.1	<i>Kanoetus</i> sp. n. AD763	2	2	0
JQ000357.1	<i>Kanoetus</i> sp. n. AD763	2	2	0
EU152611.1	<i>Chaetodactylus krombeini</i>	3	0	0
JQ000389.1	<i>Chaetodactylus krombeini</i>	3	0	0
JQ000409.1	<i>Lardoglyphus</i> sp. AD983	2	0	0
EU152712.1	<i>Listrophoroides aethiopicus</i>	2	3	0
EU152711.1	<i>Listrophoroides cricetomys</i>	3	2	0

EU152618.1	<i>Marsupialichus brasiliensis</i>	2	0	0
JQ000403.1	<i>Marsupialichus brasiliensis</i>	2	0	0
JQ000421.1	<i>Naiacus</i> sp. AD305	1	0	0
EU152626.1	<i>Naiadacarus arboricola</i>	2	0	0
JQ000422.1	<i>Naiadacarus arboricola</i>	2	0	0
EU152633.1	<i>Neottialges vitzthumi</i>	1	0	0
JQ000430.1	<i>Neottialges vitzthumi</i>	1	0	0
EU152617.1	<i>Nycteriglyphites pennsylvanicus</i>	2	0	0
JQ000400.1	<i>Nycteriglyphites pennsylvanicus</i>	1	0	0
EU152634.1	<i>Picidectes chapini</i>	2	0	0
JQ000434.1	<i>Picidectes chapini</i>	2	0	0
JQ000499.1	<i>Ptyssalgidae</i> gen. n. sp. AD1153	3	3	0
JQ000386.1	<i>Roubikia</i> sp. AD863	1	1	0
EU152623.1	<i>Sancassania</i> aff. <i>sphaerogaster</i> AD361	0	0	0
JQ000415.1	<i>Sancassania</i> aff. <i>sphaerogaster</i> AD361	0	0	0
EU152622.1	<i>Sancassania nesbitti</i>	1	0	0
JQ000414.1	<i>Sancassania nesbitti</i>	1	0	0
AY191833.1	<i>Sancassania ochoai</i>	0	0	0
AY191834.1	<i>Sancassania salasi</i>	0	0	0
KY922092.1	<i>Sancassania</i> sp. AD513	0	0	0
EU152625.1	<i>Schwiebea pseudotsugae</i>	2	0	0
EU152610.1	<i>Sennertia zhelochovtsevi</i>	3	0	0
JQ000388.1	<i>Sennertia zhelochovtsevi</i>	3	0	0
EU152630.1	<i>Sennertonix manicati</i>	1	1	0
JQ000427.1	<i>Sennertonix manicati</i>	1	1	0
JQ000371.1	<i>Tortonia</i> sp. AD560	2	0	0
KM277805.1	<i>Tyrophagus</i> sp. DL-2014	2	0	0
KM277806.1	<i>Tyrophagus</i> sp. DL-2014	2	0	0
EU152616.1	<i>Aeroglyphus robustus</i>	2	0	0
JQ000399.1	<i>Aeroglyphus robustus</i>	2	0	0
