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The protective effect of rural life on mite sensitization disappears among urban migrants in the South of Vietnam

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ABSTRACT

Background: Rapid urbanization combined with rural migration to urban areas in southern Vietnam could be risk factors for allergen sensitization, contributing to chronic respiratory diseases (CRD). We aimed to evaluate the prevalence of mite sensitization and its relation to house dust characteristics among rural and urban native and migrating populations with CRD.

Methods: Rural ($n = 19$) and urban ($n = 46$) dwellings were defined on the basis of a home typology. Controls were western Belgian houses ($n = 14$). Besides the house characteristics, both endotoxin and mite allergens were measured in the settled dusts. The sensitization to mite allergens was defined by positive skin prick test (SPT) and concentration of specific IgE (sIgE)$\geq$ 0.7 U/mL. The prevalence of mite sensitization was evaluated among 610 patients with CRD and compared according to both their home types and places of birth and residences.

Results: The concentration of endotoxin (but not mite allergen) was higher in rural compared to urban dusts (440 (95%CI: 314–566) versus 170 (95%CI: 115–226) EU/mg; $p < 0.0001$). The prevalence of positive sIgE to Der p1 and Der p2 was significantly lower in rural (9% and 5%) compared to urban (15% and 9%) population, consistent with the positive SPT to mite (14% and 21%, respectively). Among the urban migrants, the risk of mite sensitization (SPT) was higher compared to the rural natives (OR: 1.79 (1.02–3.15), $p < 0.05$) and not different to the urban ones (OR: 1.35 (0.82–2.23) p NS).

Conclusion: In Vietnam, associated with higher endotoxin (but not allergen) dust concentrations, the risk of mite sensitization was lower in rural compared to the native urban population, but this protective effect could disappear among rural to urban migrants.

Keywords: Urban migration, Allergy, Home typology, Endotoxin, Allergens

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INTRODUCTION

The prevalence of allergic sensitization is rising in developing countries,[1] a phenomenon related to rapid urbanization.[2,3] In Vietnam, preliminary data suggest that an adult's migration from a rural to an urban area increases their risk of sensitization to mite allergens[4] that could contribute to a rapid increase of chronic respiratory diseases (CRD), which is one of the priorities of public health in Vietnam.[5] Compared to rural life, urban life is characterized by different outdoor and indoor pollution, disappearance of gut parasitosis, occupational factors, and food habits.

In western countries, simultaneous exposure to dust endotoxin and allergens plays a paradoxical role in allergic diseases.[6-8] On one side, exposure to indoor endotoxin from house dust has been reported as a protective factor for allergen sensitization early in the life,[9-11] while it is associated with more severe asthma in asthmatics adults.[12,13] On the other side, exposure to dust mite or cockroach allergens is a risk factor for clinical asthma in sensitized subjects.[14,15] In Vietnam, there are no published data on house dust characteristics from rural or urban dwellings. Since environmental control can prevent allergic diseases, there is a need to improve knowledge on the association between dust characteristics of both rural and urban Vietnamese houses with the risk of allergen sensitization.

Instead of selecting a number of random houses, we defined a "home typology" based on 4 typical and easily recognizable dwellings.[16] For each type of house, we compared their characteristics and the dust concentrations of mite allergens and endotoxin. We then compared the mite sensitization among a population with CRD, according to the home type, and migration from a rural to urban area. Fig. 1 summarizes the design of the study.

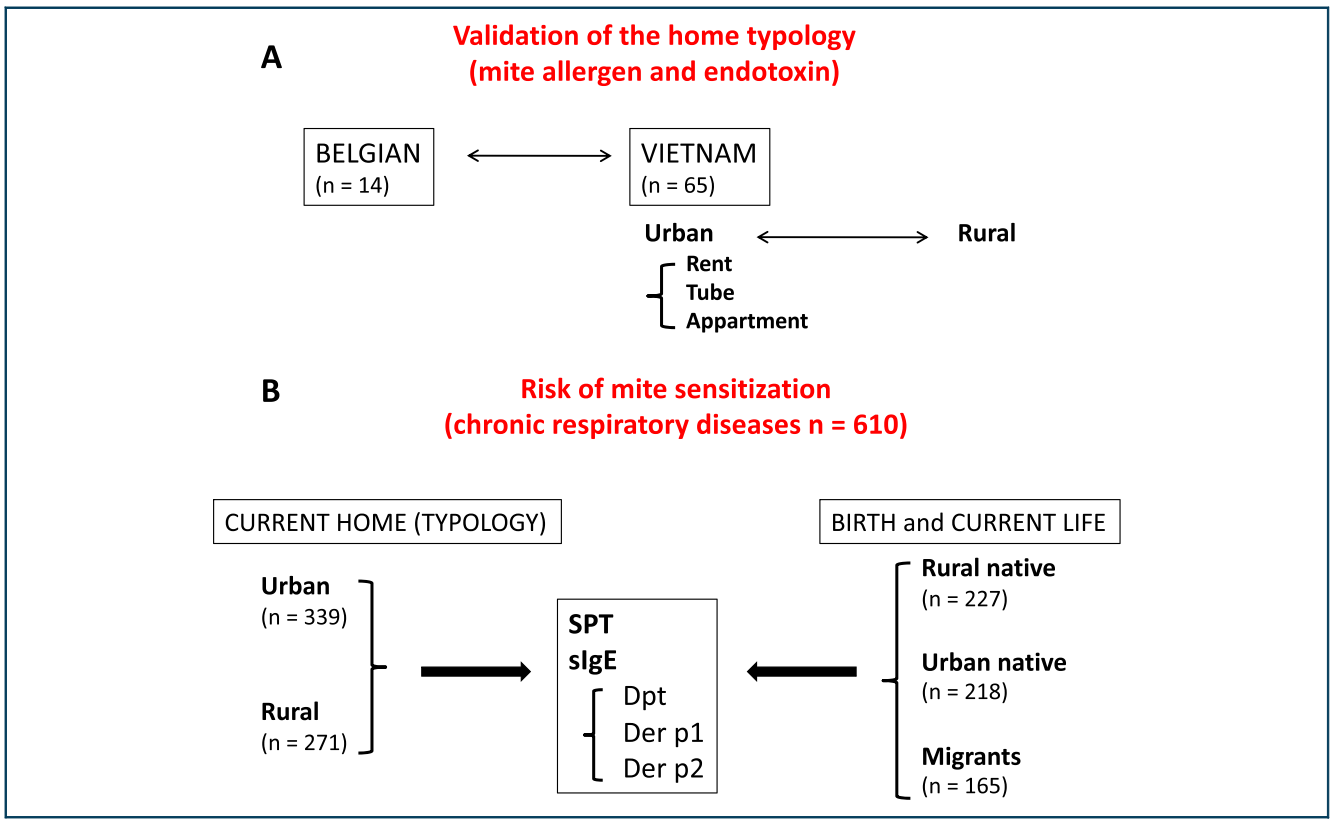

Fig. 1 General study design. A. Based on 4 typical and easily recognizable Vietnamese dwellings (tube, apartment, rent, traditional) a "home typology" was validated by significant differences of their characteristics and the concentrations of dust mite allergens and endotoxin. B. We then compared the risk and amplitude of mite sensitization (by skin prick tests [SPT] and measurements of specific IgE [sIgE]), among a population with Chronic Respiratory Diseases, according to the home type and migration from rural to urban area

Commercial extracts	Allergenic molecules	Stallergenes (ng/mL) Mean (±95%CI)	ALK (ng/mL) Mean (±95%CI)
Dermatophagoides pteronyssinus	Der p1	2075.8 (±136.8)	3928.4 (±108.6)
	Der group 2	887.4 (±108.6)	1527.9 (±247.2)
Dermatophagoides farinae	Der f1	8.4 (±2.3)	<Min
	Der group 2	23.7 (±3.7)	1.2 (±0.5)
Blomia tropicalis	Blo t5	0.3 (±0.01)	NA
Cockroach	Bla g1	12 (±4.8)	14.2 (±3.1)

Table 1. Allergen concentrations in commercial extracts. *CI: confidence interval.NA: not available*

METHODS

The home typology

Four types were defined: traditional house, rent, tube, and apartment. The "traditional house" (TRA) was characterized by a large garden with a main separated house, a small kitchen, and a frequently unflushable toilet (latrine); the "rent" (REN) by limited surface of the rooms, being in a row, and frequent unflushable toilet inside or outside; the "apartment" (APA) by a flushable toilet room inside and other rooms; and the "tube" (TUB) by at least 2 floors, a small width, and a great height, with flushable toilet inside.

Parameters	Belgium (n = 14)	All houses Vietnam (n = 65)	Rural (Vietnam) (n = 19)	Urban (Vietnam)		
				Rent (n = 12)	Apartment (n = 18)	Tube (n = 16)
Area (m^2)[a]	160 (125–198)	87 (74–102)	115 (96–133)	21 (17–25)	64 (54–75)	132 (104–166)
Occupants >4	2 (15)	14 (22)	7 (36.8)	1 (8.3)	3 (16.7)	3 (18.8)
Density (person/ $100m^2$)[a]	2.2 (1.5–2.9)	7.0 (5.7–8.7)	3.81 (3.02–4.66)	16.43 (12.06–22.07)	6.16 (4.74–7.77)	4.68 (2.90–6.86)
Smoking (Yes)	2 (15)	17 (27)	3 (15.8)	6 (50.0)	5 (29.4)	3 (18.8)
Air conditioner[b] (bed/liv)	0	19/2	3/0	0/0	9/2	6/0
Louver (Yes)[b]	NA	30 (48.0)	13 (72.2)	7 (58.3)	5 (29.4)	5 (31.3)
Toilet (Unflushed)[b]	0	26 (40)	13 (68.4)	11 (91.7)	1 (5.6)	1 (6.3)
Pet (Yes)[b,c]	7 (53.8)	25 (40)	15 (83.3)	3 (25.0)	2 (11.8)	5 (31.3)
Animals (Yes)[b]	0	12 (19)	10 (55.6)	1 (8.3)	0	1 (6.3)
Wood-cooking (Yes)	0	3 (5)	1 (5.6)	1 (8.3)	0	1 (6.3)
Season (Dry/ Rain)	NA	35/30	10/9	6/6	13/5	6/10

Table 2. Characteristics of houses. *Data were expressed as n (%), except for Area and Density (Mean [95% confident interval]); NA: not available. a. Belgium – Vietnam, t-test, $p < 0.05$. b. Houses in Vietnam, ANOVA, $p < 0.05$. c. Multivariate analysis, $p < 0.05$*

Based on their distribution in rural villages and urban cities (and districts in Ho Chi Minh City), TRA houses were defined as "rural", and REN, APA, and TUB as "urban" dwellings.

Controls were urban or suburban Belgian homes, all having western-style facilities.

Sampling of dusts

Dusts were collected by battery-operated handle vacuum cleaner, 5 minutes separately from the bedroom (mattress and floor), living room, and kitchen (floors) in 65 Vietnamese houses. As controls, one sample was obtained from the mattresses in 14 Belgian houses. Each sample was manually sieved and divided into 2 aliquots. Dust samples were kept at −20 °C until extraction.

Measurement of allergens

The dust samples from bedroom (Vietnam and Belgium) were extracted to measure allergens. The fine dust samples were extracted (at a concentration of 10 mg/ml) in phosphate buffered saline with Tween (PBS-T, pH 7.4), followed by rotation mixing for 2 hours, at room temperature. After 10 minutes of centrifugation at 1000×*g*, supernatants were stored at −20 °C until analysis. Der p1, Der 2 (group 2 allergen), Blo t5 and Bla g1 allergens were quantified by enzyme-linked immunosorbent assay (ELISA) kits (Indoor Biotechnologies, UK) according to manufacturer's instructions, except that tetramethylbenzidine was used instead of 2, 2′–Azinobis [3–ethylbenzothiazoline–6–sulfonic acid] diammonium salt. Allergen concentrations were expressed as ng/g of dust. The lower limit of detection was 0.2–0.8 ng/ml corresponding to 2–8 ng/g dust for Der Group 2 and Der p1, respectively. A sample under the limit of detection was defined as "measurable". Only data with measurable levels (>0 ng/g) were used for statistical analysis. Each determination was done in duplicate to assume repeatability.

Controls were commercial extracts (*Dermatophagoides pteronyssinus, Dermatophagoides farinae, Blomia tropicalis,* and cockroach) from ALK and Stallergenes, the last associated with a prevalence of 15% positive skin prick test (SPT) among Vietnamese patients with CRD.[4] Since very low concentrations of Bla g1 and Blo t5 allergens were detected in extracts from ALK and Stallergenes (Table 1) and house settled dusts (data not shown), only Der p1 and Group 2 are presented in the results. This low sensitivity for measurement of Blot 5 has been previously reported by Yi et al..[17]

Measurement of endotoxin

The second fine dust samples were extracted by constant rotating mixing for 2 hours, at room temperature, to obtain a final concentration of 1mg/5 ml PBS. The suspensions were rocked vigorously for 1 minutes and then placed in an ultrasonic bath at 75 °C for 30 minutes. After centrifugation, supernatants were collected and assayed immediately. The β-1,3-glucan blocker (Lonza, USA) was added in the extracts to eliminate the contamination of β-1,3-glucan. Endotoxin was analyzed by using Limulus Amebocyte Lysate Kinetic-QCL™ assay (Lonza, US license No. 1775, USA) and expressed as Endotoxin Units (EU)/mg dust. The lower limit of detection was 0.005 EU/mL corresponding to 0.001 EU/mg dust. Repeatability was calculated by duplicate dosages.

Population characteristics and mite-specific IgE measurements

Mite sensitization was evaluated among 610 patients with chronic respiratory diseases. Based on a picture of 9 representative homes in Ho Chi Minh City,[4] each patient identified their own type of dwelling as TRA (n = 271), REN (n = 56), APA (n = 25), and TUB (n = 258). The types APA, REN, TUB were combined as "urban" and TRA defined as "rural".

We also defined the patients as rural natives, urban, or migrants (from rural to urban areas), based on the question, "Where was your place of birth: rural or city?", followed by, "Do you still live there? If no, when did you move?" A migrant was a person who moved from a rural setting and lived at least for 1 year in the urban environment.

The concentrations of IgE specific to *Dermatophagoides pteronyssinus* (Dpt) extracts and to the recombinant proteins Der p1, Der p2, and Der p23 were measured by ImmunoCAP (references d1, d202, d203, d209 from Phadia IDM 1000, Uppsala, Sweden). Skin Prick Test (SPT) to Dpt was performed according to a standard method.[4]

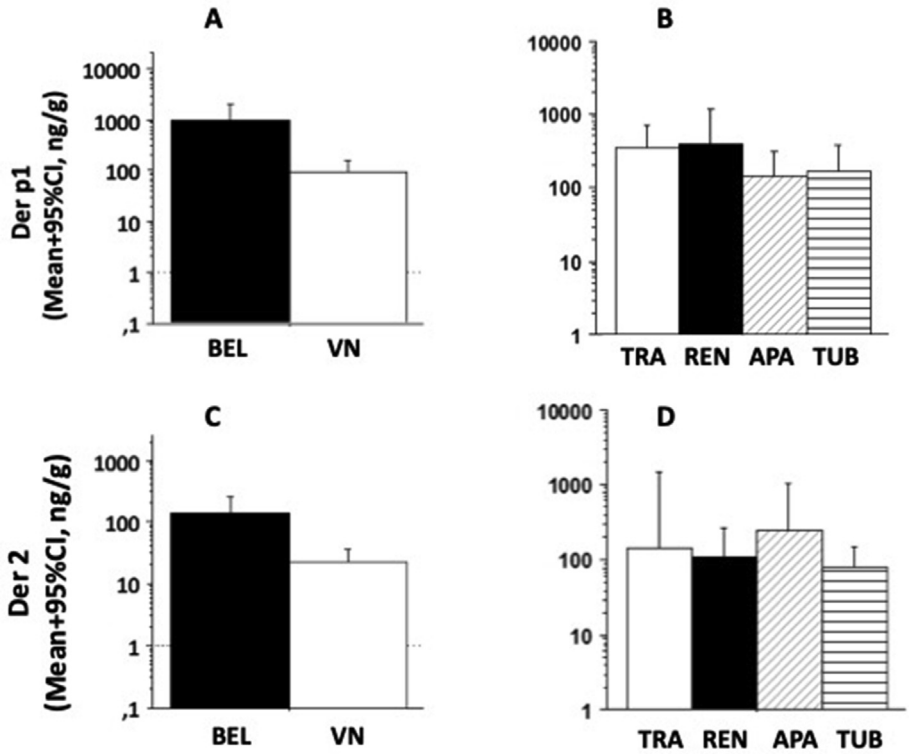

Fig. 2 Dust mite allergen concentrations. Mean of Der p1 (A) and Der Group 2 (Der 2) (C) concentrations (+95% CI) in Vietnam (VN) compared to Belgium (BEL); mean of Der p1 (B) and Der Group 2 (Der 2) (D) concentrations (+95% CI) according to the Vietnamese home types (traditional (TRA), rent (REN), apartment (APA) and tube (TUB)). Statistics: Mann-Whitney U (2 groups) or Kruskal-Wallis (3 groups) tests

Demographics and specific diagnosis of CRD (asthma, COPD, others), based on spirometry (Master Screen PFT Carefusion Ltd, Germany) before and after bronchodilator were obtained in each patient.[4]

The study was approved by the Ethics Committee of Pham Ngoc Thach hospital (CS/PT/13/12) and was enrolled in the clinicalTrials.gov database (NCT02517983).

Statistics analysis

The concentrations of allergens and endotoxin in dusts were expressed as mean ± 95% Confident Interval (CI). Due to the small number of data (less than 30 cases by group) the differences of allergens or endotoxin concentrations between groups were compared using non-parametric Mann-Whitney U (2 groups) or Kruskal-Wallis test (≥3 groups), and paired Wilcoxon test or Friedman test (≥3 groups) was used for comparisons within groups.

ANOVA and unpaired *t*-test were applied to compare the concentrations of sIgE. Chi square or ANOVA was used to test the nominal data characterizing the houses or the prevalence of sIgE sensitization. A multivariate model was used to evaluate the possible confounders (i.e. the statistically significant parameters from the bivariate analysis). Chi square tests were used to test the differences between frequencies (house types, measurable allergens). The predictors of allergen sensitization among rural, migrants and urban were tested by regression analysis and calculation of odd ratio (OR, 95% CI).

Analyses were performed with StatView statistical package version 5.0 (SAS Institute Inc., USA) and SPSS v23.0 (IBM, Armonk, NY, USA). Values of $p < 0.05$ were considered statistically significant.

RESULTS

Characteristics of the different types of Vietnamese houses

The characteristics of Vietnamese and western-style houses are compared in Table 2. The Vietnamese rural house had more frequent

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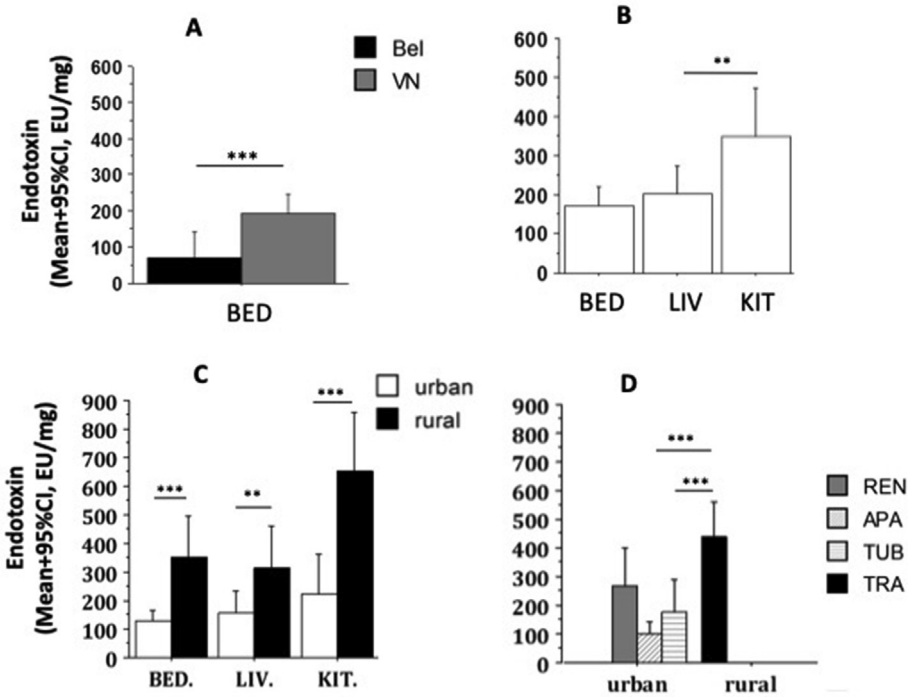


Fig. 3 Dust endotoxin concentrations and home types. The mean (+95% CI) concentrations of dust endotoxin: **A**: in Vietnam (VN) compared to Belgium (BEL) dust from bedroom (BED); **B**: in each room in Vietnamese houses: bedroom (BED), living room (LIV), kitchen (KIT); **C**: for each room, in regard with the rural or urban dwellings; **D**: rural (TRA) compared to other home types (REN, APA, TUB). Statistics: Mann-Whitney U (2 groups) (A, C and D) and Friedman tests (B). **$p < 0.01$; ***$p < 0.001$

unflushable toilets (latrine), farm animals and pets, compared to Vietnamese urban and western-style homes. After running multivariate analysis, only having pets remained significant, the others characteristics being confounders with rural life.

Mite allergens (Der p1 and Der 2) were more frequently measurable in Belgian dusts (100% and 93%, respectively) compared to Vietnamese dusts (45% and 23%, respectively) (Chi-square; $p < 0.001$). The Belgian concentrations were higher (963 (±652) and 126 (±64) ng/g, respectively) compared to the Vietnamese concentrations (257 (±153) and 117 (±58) ng/g, respectively) ($p > 0.05$) (Fig. 2 A and C). Within the Vietnamese home types, the mite concentrations were similar in the settled dusts (Fig. 2B and D).

The endotoxin dust concentration was lower in Belgian compared to Vietnamese bedrooms, (71 (±65) versus 192 (±54) EU/mg, respectively; $p < 0.001$) (Fig. 3A). Among the Vietnamese homes, the endotoxin concentrations were significantly ($p < 0.05$) higher in kitchen compared to livingroom and to bedroom (Fig. 3B). The endotoxin concentration was higher in rural compared to urban bedrooms (352 (±129) versus 126 (±42) EU/mg; $p < 0.001$), and it was significant for each room ($p < 0.001$) (Fig. 3C). The endotoxin concentrations from Vietnamese rural dwellings were significantly higher compared to each urban type (Fig. 3D).

In regard to rural and urban areas, the endotoxin concentrations were not associated with flushable or unflushable toilets, the presence of animals, or presence of smokers (Fig. 4A-B-C).

Prevalence of mite sensitization according to home type

Allergen sensitization was evaluated among 610 patients with CRD and then associated with rural (n = 271) or urban (n = 339) type of homes (Fig. 5). The prevalence of positive SPT to mite and positive IgE to Der p1, Der p2 and Der p23 was higher among urban compared to rural patients (Fig. 5C).

Among the 383 patients living in urban areas, 165 subjects were "migrants", i.e. being native to rural areas before moving to the city. The prevalence of asthma and COPD, and the anomalies of spirometry were comparable for the 3 groups of patients regardless of their residences (Table 3). The majority

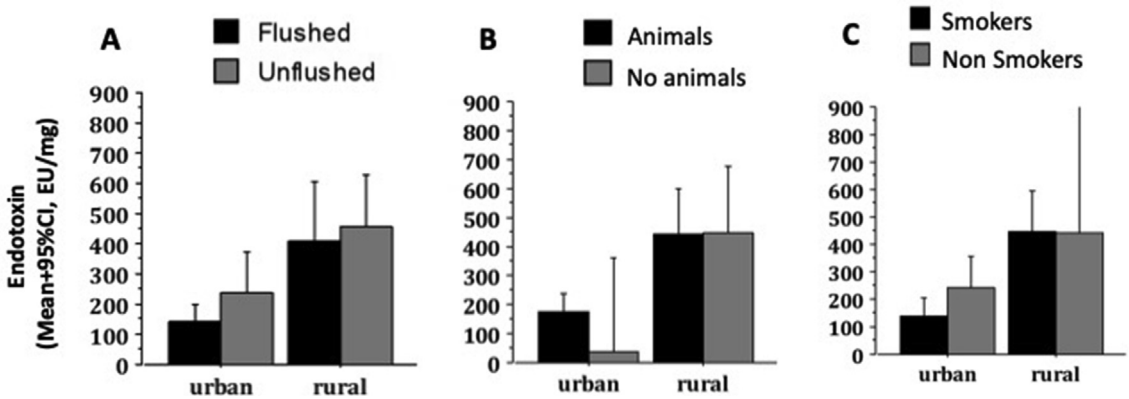
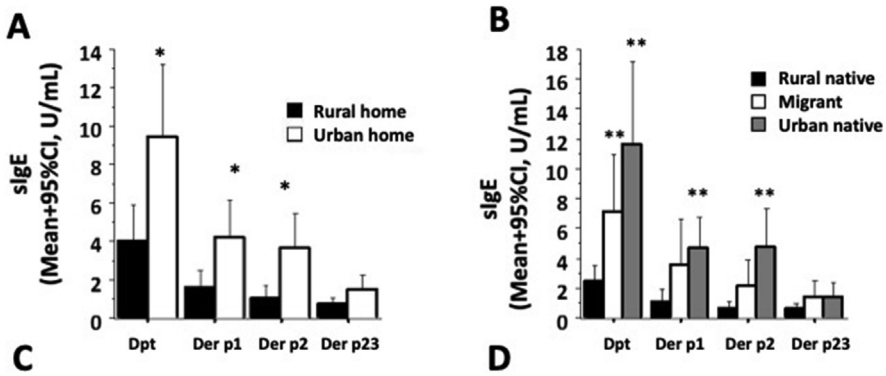


Fig. 4 Dust endotoxin concentrations in regard with the home characteristics. The mean (+95% CI) concentrations of dust's endotoxin among rural (i.e. traditional) or urban (i.e. apartment, rent and tube) houses according to some environmental characteristics: **A**: presence of unflushable (latrine) compared to flushable toilets; **B**: presence of animals or not; **C**: presence of smokers or not. Statistics: Mann-Whitney U (2 groups) test

of the migrants were exposed to both rural and urban environments for at least 10 years. The rural population was characterized by fewer females, a higher cumulative smoking, and older age.

The prevalence of positive SPT to dust mite extract was lower among rural compared to migrant and urban patients as well as the prevalence of IgE sensitization to Der p1 and Der p2, the migrants reaching the prevalence of sensitization of urban people (Fig. 5D). The OR of sensitization among rural, migrant and urban were respectively: 1.0, 1.79 (1.02–3.15) ($p < 0.05$), 2.42 (1.45–4.05) ($p < 0.001$) for positive SPT to Dpt; 1.0, 1.71 (0.85–3.45) (p NS), 2.99 (1.62–5.53) ($p < 0.001$) for IgE to Der p1 and 1.0, 2.03 (0.76–5.44) (p NS), 4.68 (1.99–10.96) ($p < 0.001$) for Der p2. The OR of the sensitization to Dpt (SPT) of the urban native compared to the migrants was not significant (OR: 1.35 (0.82–2.23) p NS).



	Rural home	Urban home	P
n	271	339	
SPT Dpt +	37 (14%)	72 (21%)	< 0.02
sIgE Dpt +	80 (30%)	124 (37%)	0.07
IgE Der p1 +	24 (9%)	51 (15%)	< 0.02
IgE Der p2 +	13 (5%)	32 (9%)	< 0.05
IgE Der p23 +	34 (13%)	64 (19%)	< 0.05

	Rural	Migrant	Urban	p
n	227	165	218	
SPT Dpt +	26 (11%)	31 (19%)	52 (24%)	< 0.005
IgE Dpt +	66 (29%)	54 (33%)	84 (39%)	NS
IgE Der p1 +	16 (7%)	19 (12%)	40 (19%)	< 0.005
IgE Der p2 +	7 (3%)	10 (6%)	28 (13%)	< 0.001
IgE Der p23 +	29 (13%)	32 (19%)	37 (17%)	NS

Fig. 5 IgE sensitization among 610 patients with chronic respiratory disease. Mean (+95% CI), concentrations of sIgE to dust mite extract (Dpt), recombinant protein Der p1 and recombinant protein Der p2, according to the type of home (A: rural or urban dwelling) and the place of residence (B: native rural, migrant from rural to urban, native urban). Statistics: ANOVA and Mann-Whitney U tests. *$p < 0.05$; **$p < 0.01$. Tables C and D: the prevalence of positive IgE to Dpt, Der p1, Der p2 of patients according to their house types (C) and their residences (D). Statistics: Chi square test

The mean concentrations of specific IgE to Dpt, Der p1, Der p2 among urban patients were higher compared to rural patients (Fig. 5A). Among the urban patients, the concentrations of specific IgE to Dpt, Der p1, Der p2 were not significantly different in urban natives compared to the rural migrants (Fig. 5B).

DISCUSSION

In this paper, we have observed that the concentrations of endotoxin (but not mite allergens) in Vietnamese dust was higher in rural compared to urban dwellings. In parallel, the risk of mite sensitization was lower among rural compared to urban populations, but this rural putatively protective effect disappeared within the migrants from rural to urban areas.

The home typology was based on the visual aspect and characterized by significant differences of animal/pets and flushed/unflushed toilets.

The concentrations of mite allergen (Der p1) in Belgian dust (963 ng/g) was high though consistent with data from temperate countries such as Germany (Hamburg 540 ng/g) or Spain (Barcelona 600 ng/g)[18] and also with Hong Kong, a tropical developed area (320–610 ng/g).[19] In the present study, Der p1 concentrations were lower in southern Vietnam (257 ng/g) compared to Belgium. Previous investigations performed in the Asia-Pacific region[20] have shown that the dust mite Der p1 concentration ranged from 40 ng/g (in Pune, India) to 160 ng/g (Hong Kong), 700 ng/g (Klang Valley, Malaysia) and 15860 ng/g (Wellington, New Zealand), this wide variation being mainly associated with the presence of carpets or kapok mattresses.[20]

The endotoxin concentration was lower in Belgian compared to the Vietnamese dust, but it was higher compared to other European countries (Germany, Netherlands, and Sweden, ranging

	N (%)	Rural 227 (37.2)	Migrant 165 (27.0)	Urban 218 (35.7)	p
Gender					0.005[d]
Female	121 (19.8)	31 (13.7)	37 (22.4)	53 (24.3)	
Male	489 (80.2)	196 (86.3)	128 (77.6)	165 (75.7)	
Age (years)[a]	54.3 (±1.0)	56.6 (±1.7)	52.9 (±2.1)	53.04 (±1.5)	0.004[e]
Cumulative smoke					0.017[d]
<10 PY[b]	243 (39.8)	77 (33.9)	68 (41.2)	98 (45.0)	
≥10 PY	367 (60.2)	150 (66.1)	97 (58.8)	120 (55.0)	
Diagnosis					NS[d]
Asthma	340 (55.7)	123 (54.2)	92 (55.8)	125 (57.3)	
COPD	186 (30.5)	77 (33.9)	50 (30.3)	59 (27.1)	
Others	84 (13.8)	27 (11.9)	23 (13.9)	34 (15.6)	
Post SB[c]FEV1/FVC (%)[a]	60.2 (±1.0)	59.7 (±1.5)	60.5 (±1.8)	60.5 (±1.7)	NS[e]
Post SB FEV1 (%)[a]	59.6 (±1.6)	60.4 (±2.4)	59.8 (±3.0)	58.8 (±2.6)	NS[e]
DLCO (%)[a]	80.4 (±1.7)	79.6 (±2.8)	83.6 (±3.6)	78.8 (±2.8)	NS[e]
Rural life					0.000[d]
0 year	218 (35.7)	0	0	218 (100)	
<10 years	17 (2.8)	0	17 (10.3)	0	
≥10 years	375 (61.5)	227 (100)	148 (89.7)	0	
Urban life					0.000[d]
0 year	227 (37.2)	227 (100)	0	0	
<10 years	27 (4.4)	0	27 (16.4)	0	
≥10 years	356 (58.4)	0	138 (83.6)	218 (100)	

Table 3. Characteristics of the studied patients. *a. Express as mean (±95%CI). b. PY = pack-year. c. SB = salbutamol 400 µg. d. Trend chi - square. e. ANOVA (analysis of variance)*

from 10 to 20 EU/mg).[21] This can be due to the method used for dust extraction (warmed ultrasonic bath) that can denature several protein inhibitors, and consequently increases the performance of endotoxin measurement, as it was reported by Milton et al.[22] Consistent with the data for Belgium, based on the same procedure, Abraham et al.[23] reported endotoxin levels of 82 EU/mg dust in the United States.

In Vietnam, the endotoxin concentration was lower in urban (126 EU/mg) compared to rural (352 EU/mg) dwellings. Lower endotoxin levels had been previously reported in Singaporean urban (18.4 EU/mg), Thai rural (26.3 EU/mg),[24] and Brazilian urban (1.6–158 EU/mg),[25] though levels of 388 EU/mg were observed in dust mattresses from Taiwan.[26] Consistent with our data, endotoxin levels were higher on the kitchen floor (reaching 602 EU/mg), compared to the bedroom[26] and the same difference between the rooms was also reported in the United States.[23]

What is the source of endotoxin? In Malawi and Nepal, burning biomass fuels was a risk factor for higher airborne endotoxin concentrations,[27] though this observation was not confirmed in our study, due to the limited user and the type of used biomass (wood versus dung). In our study, the high endotoxin concentration in Vietnamese rural settings were not related to the presence of smokers, animal/pets, and unflushable toilets, each being a possible source of Gram negative bacteria, the microbial source of endotoxin. The inhabitants density has been associated with higher endotoxin exposure[28] and the farming activity itself could also be associated with exposure to endotoxin, since in European rural areas, the concentrations of endotoxin were higher in farming households (37.8 EU/mg) compared to non-farming households (22.8 EU/mg).[9]

Among patients sensitized to Dpt, 36% had positive IgE to Der p1 and 22% to Der p2, suggesting that they are minor allergens in Vietnam. The prevalence of mite-sensitized patients (i.e. positive IgE to Dpt, Der p1 and Der p2) was higher in urban compared to rural dwellings, and the migration from rural to urban areas was associated with a rise of mite sensitization. Since the exposure to mite allergens was comparable in Vietnamese urban and rural homes, one or several other factors could be contributing to the risk of allergic sensitization in the urban setting. Obviously, the intensive migration from a rural to the urban center of Ho Chi Minh City induced a quick and dramatic change of lifestyle of the inhabitants. Indeed, one considered that the changing epidemiology of allergy and asthma in Asia is due to 3 environmental risk factors: air pollution (motor traffic and opened fire for cooking), frequent cigarette smoking, particularly in male subjects, and the disappearance of the rural environment.[29] In the present study, the first 2 factors were probably not involved because the cumulative smoking was slightly higher in the rural population, and both the rural and urban people were exposed to the pollution of biomass and traffic, respectively. The rural environment is characterized by exposure to abundance and diversity of microbes. During the lifetime, exposure to airborne allergens in the early-life has been associated with the risk of allergen sensitization as recently reviewed by Burbanck et al.[30] This rural effect was also sustained by the study on similar genetic populations of Amish and Hutterite.[31] The Amish environment, characterized by high early-life exposure to endotoxin, provided protection against asthma and allergic sensitization. The mechanisms of this protective effect are based on the switch of regulatory T cells by farm exposure during a critical "time window" before the age of 6 years.[32] In rural southern Vietnam, the present data showed particularly high levels of endotoxin independently of the room, reaching more than 654 EU/mg in the kitchen of rural homes. In urban dwellings, the concentration of endotoxin was low and comparable to western dwellings. Thus, the lower prevalence of sensitization to Der p1 and Der p2 in rural compared to urban area, suggested that endotoxin could be a protective factor. This hypothesis was reinforced by a rising risk of positive mite SPT among rural compared to urban migrants, associated with a lower endotoxin exposure.

Though one cannot exclude a simultaneous effect of chronic helminthiasis associated with rural life, that has been reported as a protective factor for the risk of allergy. Indeed, in southern Vietnam, geohelminthic hookworm and ascaris infections with poor sanitation (unflushable toilets) protected

against allergic sensitization.[33] Since the year 2010 the prevalence of parasitosis decreased due to the large use of albendazole treatment (400 mg every 4 months) which cured more than 70% of the persons affected.[34] In 2015, in southern rural of Vietnam, the prevalence of hookworm and ascaris were 22.8% and 2% [35] compared to 65% and 7% in 2006.[33] Thus, the decreasing prevalence of parasitosis involved both urban and rural areas and therefore could be an independent risk factor of allergic sensitization not restricted to urban life. Studies are in progress to evaluate the effects of helminthiasis both in rural and urban settings on the risk of allergy.

In conclusion, in southern Vietnam, associated with endotoxin exposure, the protecting effect of rural life on the risk of allergic sensitization could disappear among migrants to the urban area.

Abbreviations

APA: Apartment; CRD: Chronic respiratory diseases; Dpt: *Dermatophagoides pteronyssinus*; EU: Endotoxin unit; PBS: Phosphate buffered saline; REN: Rent building; sIgE: Specific immunoglobulin E; SPT: Skin prick test; TUB: Tube building; TRA: Traditional home

Submission declaration

We confirm that the paper is an original work and that it has not been published, in whole or in any part, in any other journal.

Statement of ethics

Participants have given their written informed consent. The study was approved by the Ethics Committee of Pham Ngoc Thach hospital (CS/PT/13/12) and was enrolled in the clinicalTrials.gov database (NCT02517983).

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Author contributions

HTC: conception and design, acquisition and analysis of the data, writing the paper; VD: acquisition and analysis of the data; endotoxin measurements; OD: acquisition and analysis of the data, allergen measurements; TNT, NTKD and TTTT: evaluation of the homes, collection of dust, acquisition and analysis of the data; CB, JMH, LHN, TTMH and NMX: design, interpretation of the data; FC: acquisition and analysis of the data, Immuno-CAP measurements; IG conception and design, interpretation of the data; OM: conception and design, analysis and interpretation of the data, writing the paper. All the authors have contributed to the conception and revision of the manuscript and have approved the submitted version.

Declaration of Competing Interest

The authors have no conflicts of interest to declare.

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