APJAI
Asian Pacific Journal of
Allergy and Immunology

ORIGINAL ARTICLE

An unequal endotoxin distribution in typical house types of Ho Chi Minh city

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Abstract

Background: Endotoxin exposure may cause asthma exacerbations and contribute to non-atopic respiratory diseases. Viet Nam, a country with multiple house types, is lacking data on indoor contamination by endotoxin in regard with house types.

Objectives: The comparison of measured settled dust endotoxin levels among house types in Ho Chi Minh city will allow to classify the house types regarding health risks.

Methods: This study is a cross-sectional study. Five identified house types were selected: apartment (APA), rental (REN), rural (RUR), slum (SLU) and tube house (TUB). One hundred house's endotoxin contamination was evaluated by questionnaire and dust sampling. Endotoxin concentration was measured by kinetic chromogenic Limulus assay.

Results: Endotoxin concentration (geometric mean 126.0 EU/mg, 95%CI 118.3-133.7) is particularly high in settled house dust compared to western countries and is significantly associated with the house type. The highest level was found in RUR in each room ($p = 0.002$ for living room; $p < 0.0001$ for bedrooms and for kitchens). Concerning levels in the different rooms, APA and TUB form a low group while REN and SLU ($p < 0.001$) form a median group and RUR the highest ($p < 0.001$). Differences in endotoxin levels were associated to the presence of dog, chicken and farm animals, wood cooking, air-conditioning usage.

Conclusions: Further understanding of the relevant factors to endotoxin levels would contribute to prevent asthma exacerbations and chronic respiratory diseases. Public health interventions to reduce exposure to endotoxin include improving housing conditions, eliminating risk factors and a priority to high-risk house types.

Key words: Endotoxin, house dust, house types, room types, explanatory factors.

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Introduction

Endotoxin is a lipopolysaccharide produced by gram-negative bacteria.[1,2] Exposure to endotoxin occurs via inhalation of airborne or settled dust or dust trapped in materials, carpets, mattresses, pillows, … Some studies suggested endotoxin in settle dust is a better indicator to evaluate the endotoxin level of the house.[3–5] Measurement of endotoxin in settled dust reflects the accumulation of endotoxin and allows a stable way to access the endotoxin concentration. Although inhalation endotoxin in airborne dust is correlated to respiratory diseases, level could be very low in residential houses. Therefore, endotoxin in mattress and bedroom floor dust was suggested to indicate long time exposure.[3,5,6] Authors found a correlation between settled dust endotoxin level and room type,[7,8] place of house (urban or rural area),[9,10] number of people living in house,[11,12] pets,[13–15] wood cooking, smoking, temperature and relative humidity.[2,7,15] Those are called predictive factors. Some studies found that endotoxin is a protective factor against asthma and allergic sensitization developments during childhood (only for child with hay fever, atopy or highly frequent contact with animals)[16–19] while it is also

known being a cause of development and exacerbation of asthma and other chronic respiratory diseases (CRD).[2–4,6,17,18,20] The hypothesis is that endotoxin and other indoor environment parameters could play a role in the development of mentioned diseases and that their levels are characteristic to specific house types. In this present paper, we focused on the endotoxin levels in settled dust to characterize the house types representative of Ho Chi Minh City (HCMC).

Materials and Methods

A cross-sectional study was conducted on 100 houses randomly selected among 5 typical house types (20 houses per type). The types of houses were categorised as apartment (APA), rental (REN), rural (RUR), slum (SLU), and tube house (TUB). REN is also usually called "rental room for worker". REN is often built in a row as room next to room, has only ground floor and have a very small window or none. SLU is a small house presents along the canal of city. It is built with wood or semi-solid materials. It has only ground floor and a part of floor above surface of the canal. TUB is a house with narrow in width and long in length. It has at least two floors and is built as house next house. Selected APA did not have a modern ventilation system. RUR has one ground floor and present mostly in rural area. It locates independently with yard surrounding. The definition of the five types of house has been reported previously.[21] The figure showing the five house types is available in **Supplement Figure 1**.

House dust sampling

We conducted house dust sampling and endotoxin extraction with the method adapted from Bouillard *et al.*[1] In each house, we collected three dust samples with a vacuum cleaner (Electrolux, China) equipped by a cloth filter. We used the vacuum cleaner to collect dust in three locations during 5 minutes each, one in the living room (arm-chairs, cushions, floor and corners), one in the bedroom (mattress, rug, cushion), and one in kitchen (floor, shelves, stove). Each dust sample was then transferred into a sealed plastic bag and stored at -80°C until endotoxin extraction. The filters were washed between houses.

Endotoxin measurements

We conducted dust extraction and measured endotoxin concentration by following the method of Bouillard *et al.*[1] Furthermore, to avoid the interference of an activating effect of glucan in house dust, we used β-1,3-Glucan Blocker (LONZA, Europe). The sensitivity of our assay was 0.005 EU/ml. All dilutions of the samples were duplicated. We expressed the concentration of endotoxin in mg of dust (EU/mg).

Other variables

Several factors related to indoor air and house characteristics were obtained by questionnaire and inspection of each house. The questionnaire included environmental characteristics of house, number of inhabitants and indoor activities. The questions were developed on the base of the factors identified in the literature.

Supplement Figure 1. Photograph of the 5 typical house types in Ho Chi Minh City: a) apartment, b) rental house, c) rural house, d) slum house and e) tube house. The rental house is a small room with poor ventilation, ground on floor, kitchen in the main room. It has one small main door. Some rental houses do not have windows. It usually presents in urban and sub-urban districts

In each room (living room, bed room and kitchen), temperature and relative humidity were recorded automatically every 5 minutes during 21 days by data logger (Testo 174H, India), positioned at 1.5 m from floor level.

Ethical approval

This study was a part of the larger study (The relationship between environmental risk factors in housing types and prevalence of chronic respiratory diseases) which was approved by Ethics Committee of Pham Ngoc Thach University of Medicine (approval number CS.2015.04). This study is not including human subjects. All hosts of researched houses completed informed consent.

Statistics

From our dataset, data on endotoxin concentrations were not normally distributed. Our data were transformed into log-normal to use statistical parametric tests. Therefore, means were showed as geometric means (GM) and 95% confidence interval (95%CI). Parametric ANOVA test was used to compare endotoxin concentration among groups and Bonferroni test (a kind of t-test for multiple comparison) was used to see which group was different from the other. The relationship between house characteristics and endotoxin concentration were defined by t-test (for category variables) and Pearson's correlation coefficients (for continuous variables) based on natural log transformed data. To compare the characteristics among five house types, we used Chi-square test (for category variables) and Kruskal Wallis test (for continuous variables without normal distribution). Multiple linear regression models were used to explain the relationship between endotoxin level and other related variables. A p-value under 0.05 was considered as significant. All data were analyzed with SPSS 22.0.

Results

For all samples

The geometric mean concentration of endotoxin in 300 house dust samples was 126.0 (95%CI 118.3-133.7) EU/mg.

The mean concentrations of endotoxin were significantly different ($p < 0.0001$, ANOVA one-way) among the 5 house types. RUR presents the highest concentration (294.5 EU/mg) and APA the lowest (53.1 EU/mg). RUR's endotoxin concentration is significantly different from APA's ($p < 0.0001$, Bonferroni test) and TUB's (68.5 EU/mg) ($p < 0.0001$, Bonferroni test). Both SLU's (208.2 EU/mg) and REN's (142.6 EU/mg) were higher compared to APA's ($p < 0.0001$, Bonferroni test). SLU's was higher compared to TUB's ($p < 0.0001$, Bonferroni test).

Comparing the rooms, the kitchens showed the highest level (149.1 EU/mg; 95%CI: 140.5-157.7 EU/mg) while the lowest level was in the living rooms (107.8 EU/mg; 95%CI: 100.3-115.2 EU/mg). Endotoxin concentration in bedroom was 124.5 (95%CI 117.5-131.6) EU/mg.

From our data, the endotoxin level raises in a sequence from the living room and the bedroom to the kitchen. However, there is no significant difference in endotoxin levels among the three kinds of room ($p = 0.244$, ANOVA one-way). But in RUR, endotoxin concentration in kitchens was 490.1 (485.9-499.3) EU/mg; higher statistically than in living rooms (175.1 EU/mg; 95%CI 168.6-181.2) and bedrooms (297.6 EU/mg; 95%CI 291.6-303.6). (see **Table 1**)

Table 1. Average endotoxin concentration in house dust samples

	Room type	N	Geometric mean (95% CI) (EU/mg)	p-value*
All house types	Living room	100	107.8 (100.3 - 115.2)	0.024
	Bed room	100	124.5 (117.5 - 131.6)	
	Kitchen	100	149.1 (140.5 - 157.7)	
Apartment	Living room	20	49.8 (44.9 - 54.8)	0.546
	Bed room	20	66.1 (59.5 - 72.7)	
	Kitchen	20	45.5 (39.0 - 51.9)	
Tube house	Living room	20	66.0 (59.7 - 72.2)	0.247
	Bed room	20	51.7 (46.7 - 56.8)	
	Kitchen	20	94.0 (86.4 - 101.6)	
Rental house	Living room	20	131.6 (123.1 - 140.1)	0.900
	Bed room	20	137.7 (131.1 - 144.3)	
	Kitchen	20	160.0 (151.0 - 169.6)	
Slum house	Living room	20	192.1 (184.0 - 200.1)	0.939
	Bed room	20	213.5 (208.0 - 219.1)	
	Kitchen	20	220.0 (212.4 - 227.6)	
Rural house	Living room	20	175.1 (168.6 - 181.2)	0.011
	Bed room	20	297.6 (291.6 - 303.6)	
	Kitchen	20	490.1 (485.9 - 494.3)	

* ANOVA one-way

Among house types

The concentrations of endotoxin in rooms were measured and compared. There is a significant difference among living rooms ($p = 0.002$, ANOVA one-way), among bedrooms ($p < 0.0001$, ANOVA one-way), and among kitchens ($p < 0.0001$, ANOVA one-way) of the five types of house. Results in **Figure 1** show a pattern: APA is always different from RUR and SLU for the three studied rooms.

Among living rooms, TUB and APA present lower concentrations of endotoxin than other house types. The concentrations are not statistically different among REN, RUR and SLU. In APA and TUB bedrooms, endotoxin concentrations are lower than in REN, SLU and RUR. In REN bedroom, endotoxin level is lower than in RUR but not different from SLU. In kitchens, endotoxin concentrations of RUR are the highest and significantly different from others.

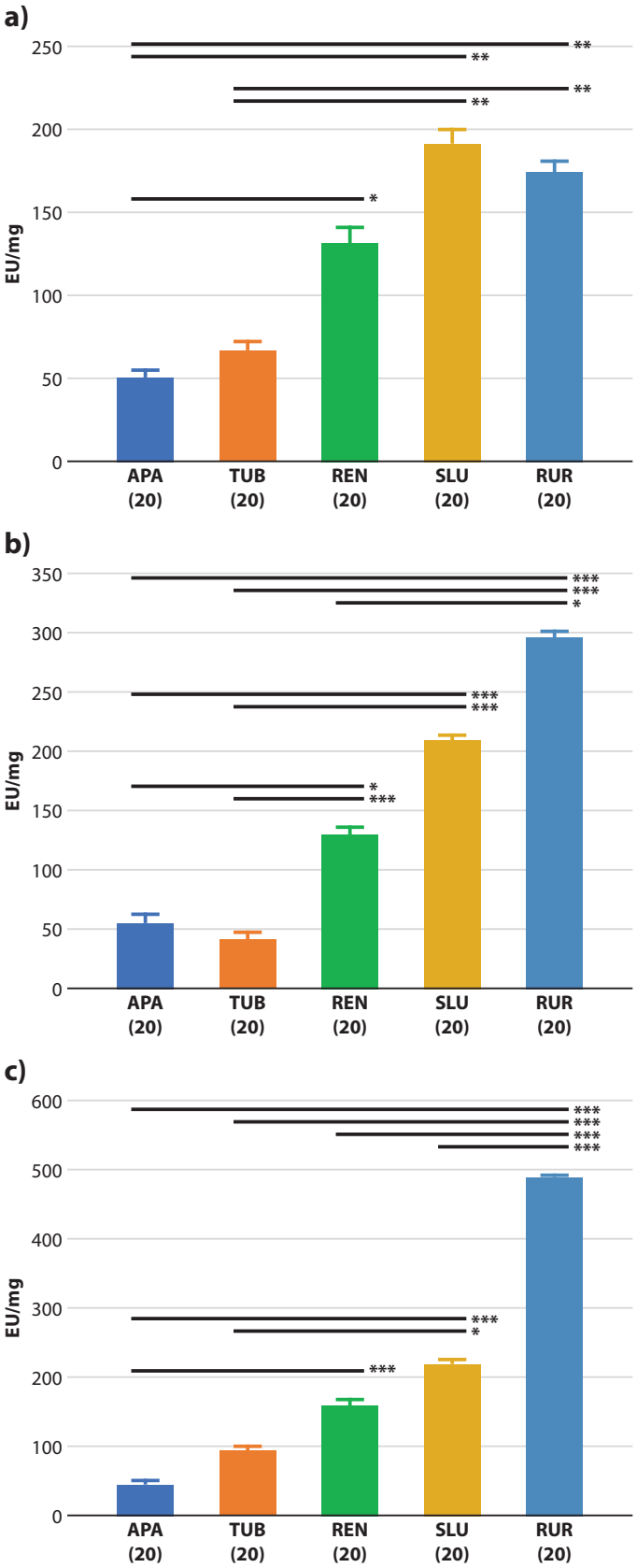

Figure 1. Comparison between the house types for endotoxin level in house dust among 5 house types. a) Living room area; b) Bedroom area; c) Kitchen room area. ANOVA one-way test was applied. * p < 0.05; ** p < 0.01; *** p < 0.001

Explanatory factors to the variations in endotoxin concentrations

When confronting the predictable factors presented in the introduction to the results of the questionnaire, we observed that some of them related to the endotoxin levels measured in our samples. Associations between endotoxin levels and predictors were found with a regression model using all house characteristics are shown in **Table 2**. Smoking influences the endotoxin level in living room. Air-conditioning usage is associated to endotoxin level in bedroom. Chicken and cow influence the endotoxin level in kitchen. Wood cooking relates to endotoxin levels of both the bedroom and the kitchen while dog and location of the house associated to endotoxin levels in the three types of room.

Table 2. Association between endotoxin levels in house dust in living rooms, in bed rooms and in kitchen of 100 houses and house characteristics.

Characteristic	n1 vs n2	GMR	95% CI	P-value
Living room				
Smoking (yes vs no)	36 vs 64	2.08	1.23-3.51	0.006
Dog (yes vs no)	25 vs 75	1.16	0.64-2.11	0.063
Cat (yes vs no)	16 vs 84	1.62	0.87-3.28	0.177
Chicken and cow (yes vs no)	14 vs 86	1.12	0.53-2.38	0.761
Wood cooking (yes vs no)	17 vs 83	1.67	0.84-3.31	0.142
Air-conditioner (yes vs no)	4 vs 96	0.33	0.08-1.23	0.100
Location (rural vs urban)	24 vs 76	1.98	1.09-3.58	0.024
Occupant (> 4 vs ≤ 4 people)	27 vs 73	1.40	0.78-2.50	0.261
Density (m^2/person)				
15 -< 30 vs < 15	21 vs 43	1.20	0.63-2.28	0.586
≥ 30 vs < 15	26 vs 43	0.99	0.50-1.97	0.976
Bed room				
Smoking (yes vs no)	36 vs 64	1.59	0.95-2.65	0.075
Dog (yes vs no)	25 vs 75	1.73	0.99-3.05	0.062
Cat (yes vs no)	16 vs 84	1.64	0.83-3.21	0.152
Chicken and cow (yes vs no)	14 vs 86	1.69	0.98-3.44	0.147
Wood cooking (yes vs no)	17 vs 83	2.45	1.29-4.66	0.005
Air-conditioner (yes vs no)	26 vs 74	0.56	0.32-0.98	0.043
Location (rural vs urban)	24 vs 76	2.74	1.58-4.74	< 0.0001
Occupant (> 4 vs ≤ 4 people)	27 vs 73	1.07	0.61-1.88	0.811
Density (m^2/person)				
15 -< 30 vs < 15	21 vs 43	1.13	0.61-2.09	0.586
≥ 30 vs < 15	26 vs 43	1.00	0.52-1.95	0.987

Data showed as geometric mean ratio (GMR) with 95% confidence interval (95%CI).

Table 2. (Continued)

Characteristic	n1 vs n2	GMR	95% CI	P-value
Kitchen				
Smoking (yes vs no)	36 vs 64	1.46	0.81-2.65	0.211
Dog (yes vs no)	25 vs 75	1.96	1.02-3.76	0.044
Cat (yes vs no)	16 vs 84	0.99	0.45-2.17	0.980
Chicken and cow (yes vs no)	14vs 86	2.28	1.01-5.15	0,047
Wood cooking (yes vs no)	17 vs 83	3.13	1.50-6.52	0.002
Location (rural vs urban)	24 vs 76	3.83	2.06-7.13	<0.0001
Occupant (> 4 vs ≤ 4 people)	27 vs 73	1.64	0.86-3.11	0.132
Density (m^2/person)				
15 -< 30 vs < 15	21 vs 43	0.93	0.45-1.91	0.844
≥ 30 vs < 15	26 vs 43	1.08	0.50-2.32	0.849

Data showed as geometric mean ratio (GMR) with 95% confidence interval (95%CI).

Temperature and relative humidity (RH) present a weak or no correlation to endotoxin level in each room type when we look at R linear and p-value in Pearson's correlation test. Temperature and RH have a weak correlation to endotoxin in living room (R = -0.32, p = 0.001 for temperature and R = 0.21 p = 0.036 for RH) and in kitchen (R = -0.17, p = 0.088 and R = 0.21, p = 0.002). However, the scatter graphs do not show a clear correlation. In bedroom, there is no correlation between temperature, RH and endotoxin (p > 0.05).

Variations in the factors affecting the endotoxin levels could explain the difference in the measured concentrations in the 5 house types. We define the explanatory factors that are related to endotoxin concentration and difference among five types of house. Results in **Table 3** show that pets (including farm animals) in the house, wood cooking, air conditioner usage and location of house can explain the difference.

House type combination

As shown in **Figure 1**, both APA and TUB are different from REN, RUR and SLU. Moreover, APA and TUB show similar endotoxin levels in the 3 kinds of room. REN and SLU are also similar. This leads to a grouping hypothesis based on the endotoxin levels. In the urban area, we suggest to combine APA and TUB into a new group while REN and SLU are combined into another one. RUR is still kept in a separate group, as it is mainly present in the rural area. Therefore, we end up with 3 groups after such combination. (see **Figure 2**).

Results related to explanatory factors do not change after grouping (see **Table 3 and 4**)

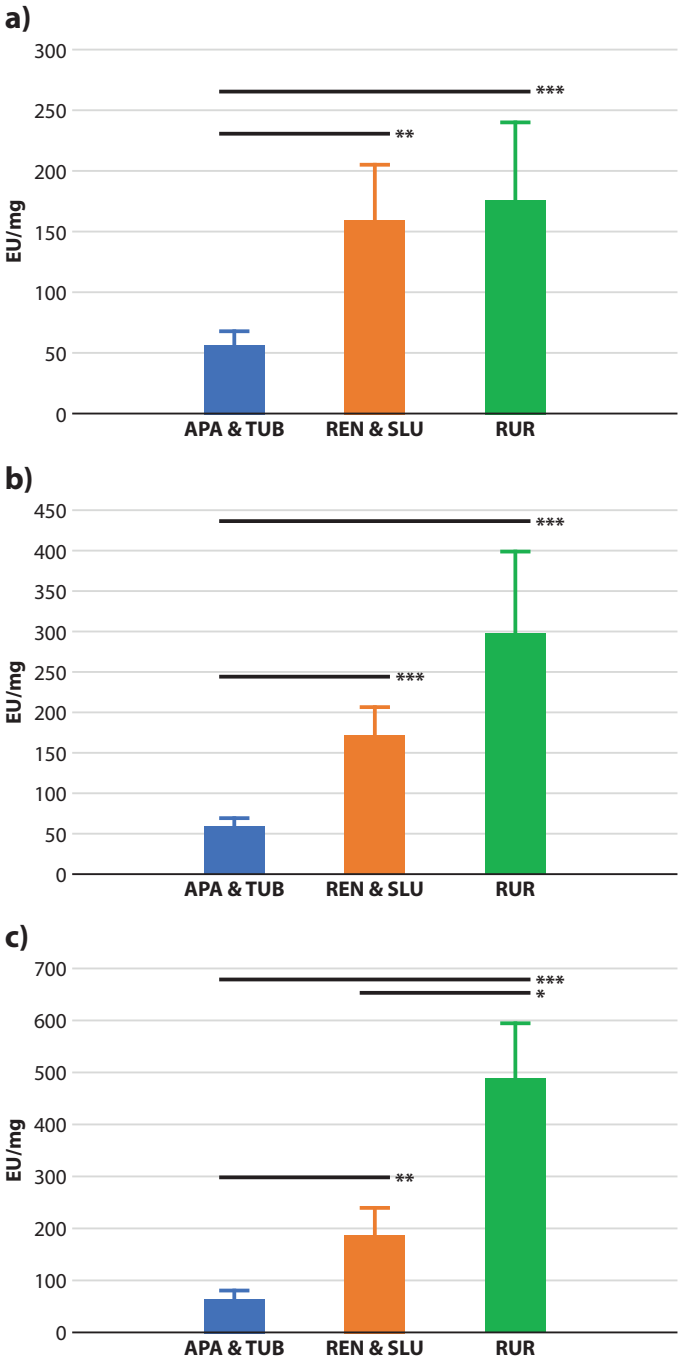

Figure 2. Comparison between groups for endotoxin levels in house dust among 3 groups of house types. a) Living room area; b) Bedroom area; c) Kitchen area. ANOVA one-way test was applied. * p < 0.05; ** p < 0.01; *** p < 0.001.

Table 3. Distribution of explanatory factors in 5 house types in Ho Chi Minh city.

	APA (N = 20)	TUB (N = 20)	REN (N = 20)	SLU (N = 20)	RUR (N = 20)	P
Smoker (people)	0 (0-1)	0 (0-1)	0 (0-1)	1 (0-2)	0 (0-1)	0.080*

Data was showed as mean (95% confidence interval) or median (1st quartile to 3rd quartile). APA: apartment; REN: rental house; RUR: rural house; SLU: slum house; TUB: tube house; LR: living room; BR bed room; KR kitchen; * Kruskal Wallis test. $ Chi-squared test.

Table 3. (Continued)

		APA (N = 20)	TUB (N = 20)	REN (N = 20)	SLU (N = 20)	RUR (N = 20)	P
Smoke (house)	No	15	15	14	8	12	0.109$
	Yes	05	05	06	12	08	
Pets (animal)	No	18	14	16	14	03	< .001$
	Yes	02	06	04	06	17	
Wood cooking	No	20	19	20	19	05	< .001$
	Yes	00	01	00	01	15	
Air conditioner	No	09	11	19	19	16	< .001$
	Yes	11	09	01	01	04	
Location of house	Urban	19	19	18	20	00	< .001$
	Rural	01	01	02	00	20	

Data was showed as mean (95% confidence interval) or median (1st quartile to 3rd quartile). APA: apartment; REN: rental house; RUR: rural house; SLU: slum house; TUB: tube house; LR: living room; BR bed room; KR kitchen; * Kruskal Wallis test. $ Chi-squared test.

Table 4. Distribution of explanatory factors in 3 groups of house type in Ho Chi Minh city.

		APA & TUB (N = 40)	REN & SLU (N = 40)	RUR (N = 20)	P
Smoker (people)		0 (0-1)	0 (0-1)	0 (0-1)	0.147*
Smoke (house)	No	30	22	12	0.162$
	Yes	10	18	08	
Pets (animal)	No	32	30	03	< .001$
	Yes	08	10	17	
Wood cooking	No	39	39	05	< .001$
	Yes	01	01	15	
Air conditioner	No	18	38	16	< .001$
	Yes	22	02	04	
Location of house	Urban	38	38	00	< .001$
	Rural	02	02	20	

Data was showed as median (interquartile) or number of houses. * Kruskal Wallis test. $ Chi-squared test.

Discussion

Endotoxin results

The mean concentration of endotoxin in HCMC is higher than those measured in Taiwan (108.4 EU/mg).[22] There is still little data on endotoxin levels in Asia. The settled dust endotoxin level in HCMC was higher than western countries where concentrations range from 0.7 to 20 EU/mg.[13,16,23,24] Leung *et al.* found endotoxin levels between 12.4 and 24.2 EU/mg in Hong Kong dwellings. Holst *et al.* also found that floor dust composition (including endotoxin level) could differ depending on the geography in Europe.[10]

About endotoxin levels in the three main rooms, our finding (in South East Asia) is similar to other authors.[7,8] A study carried out in the US with 404 family rooms, 323 bedrooms, and 245 kitchens showed that the mean concentrations of endotoxin level are respectively 79 EU/mg, 63 EU/mg and 100 EU/mg.[7] However, a correlation between endotoxin levels among room types it is weak.[7,8,12]

From our data, the endotoxin concentration in the kitchen is always equal to or higher than that in the bedrooms and the living rooms. Our studies also present a similar pattern of endotoxin distribution that the other authors.[7,8,12] In Viet Nam, kitchens are also indoor places where housewives spend most of their time, especially in RURs. This supports the hypothesis that women more often non-smokers, but exposed to higher concentration of endotoxin, for longer period (in kitchen), could be at risk of CRD.

REN, SLU, and RUR were defined as the higher risk of endotoxin house types. Von Mutius *et al.* reported that endotoxin

values reaching 143.0 EU/mg for farmers versus 39.0 EU/mg for non-farming families in the kitchen floor dust in Germany.[9] The variations in endotoxin levels of the same room type among different house types support the hypothesis a role of the house type in the endotoxin level in HCMC (home effect). In Europe, dwelling type and dwelling location are shown as determinant of the endotoxin concentrations.[10] Barraza *et al.* found that the indoor and the outdoor concentrations in endotoxin are not different in Chile.[25]

Explanatory factors for differences in endotoxin level among the 5 house types.

These factors are the presence of dog, chicken and cow, wood cooking and air-conditioning usage. Smoking, which usually related to endotoxin, is not an explanatory factor. This implies that the indoor environment and the house characteristics could lead to difference in the prevalence of related endotoxin diseases among house types, besides smoking.

Pets: Pets in the houses was associated with PM10-2.5 but not with endotoxin concentrations in airborne.[26,27] However, the presence of pets, in particular, of cats and dogs was reported as an important determinant of endotoxin levels in house dust.[13–15] Eventually, dog's presence influences endotoxin levels in all kinds of rooms through our data. As us, Gehring et al. found that dog ownership, but not cat's, is a significant predictor of endotoxin concentration. Negative gram bacteria and cell-wall fragments produce endotoxin. They present naturally on the skin and colon of animal and human.[12] Thus, the presence of pets in the house could contribute to household endotoxin.

Farm animals: Chickens and cows, which presented more in RUR, correlated to endotoxin concentration. Cow cages and hencoops are often close to the kitchen area. Eventually, chickens are left free and they often come from outside into kitchen. Ivo Berger et al. found that the concentration of cow dander allergen (endotoxin included) was the highest in stables but also noticeable in living rooms and mattresses.[28] They also reported that endotoxin is bound to larger particles in the stables and could be transported from the shed to the living room. Thus, optimizing the hygiene of both farmers and family members could prevent allergen transport from the stables to bed. Especially, stables in the rural areas of Vietnam are mostly attached to the family home. Sometimes, they are even located within the houses. This could lead to a higher endotoxin concentration in the Vietnamese RURs than in other countries.

Wood cooking: Besides animals, wood cooking also relates to endotoxin concentration in kitchen. In our study, 75% of RUR used wood for cooking while other house types nearly do not use any. Endotoxin concentrations in homes burning biomass fuels were considerably higher than those found in homes in the developed world and at levels comparable to agricultural-related occupations.[29] Airborne endotoxin generated from burning biomass may play an important role in the health effects. Other components emitted from burning wood such as particulate matter (PM2.5), carbon monoxide, nitric oxide (NOx) and a variety of polyaromatic hydrocarbons might also affect to respiratory health.[2]

Air-conditioning usage: It could change temperature, relative humidity, and ventilation of a house. This could affect the developing conditions of Gram-negative bacteria, the main source of endotoxin. Use of central air conditioning and operation of humidifiers have been reported as determinants of endotoxin levels.[7,15] Main heating source and temperature control were important factors for family room and bedroom floor endotoxin. The temperature in the family rooms between 18°C and 23°C or in the bedroom between 24°C and 29°C was associated with lower endotoxin levels compared with more extreme temperatures (> 29°C or < 18°C).[12]

House type combination

From our data, RUR has a higher level of endotoxin than others. This suggests that RUR is a high-risk type of house regarding endotoxin levels. Those explanatory factors such as place of house (rural), wood cooking and farm animals (chicken and cow) are also more popular in this house type. Those houses have these factors would have 2.28-3.83 folds in endotoxin levels higher than those do not. Because of these reasons, RUR should be kept as a separate group. In urban area, REN and SLU usually are higher levels of endotoxin than TUB and APA. Therefore, we could combine five types of typical houses in HCMC into three groups depending on endotoxin data. This makes the difference more clearly and there is no change on results of house characteristics and indoor air parameters after combination. The group of REN and SLU represents for high-risk house in urban area while RUR represents similarly for rural area. However, this classification needs to be collated with the prevalence of CRD in each house type or in each group of house type in a later study.

Limitations of the study

The dust samples in each house were collected during a single day. However, single endotoxin observation in settled dust was proved a good representative of long-term exposure and endotoxin levels in house.[5,6] Besides, taking three samples per house, as in this present study, could provide details on the endotoxin level of the house.

Conclusion

This is a first study that describes the concentration and the distribution of settled dust endotoxin concentrations in five types of dwelling in HCMC. The average concentration of endotoxin is 126.0 (118.3-133.7) EU/mg. There is a difference in endotoxin levels among five types of house. In general, RUR has the highest level. In urban area, SLU and REN present higher concentrations than TUB and APA. There is no difference in concentration among RUR, REN and SLU. The difference is clearer when combining APA and TUB; SLU and REN together. Pets (especially dog, chicken and cow), wood cooking, using air conditioner, are explanatory factors for the difference in endotoxin levels among types of house besides the building construction. Public health interventions to reduce exposure to endotoxin should include improving housing conditions, eliminating risk factors and a priority to high-risk house types

such in RUR, REN and SLU. These findings will have to be confirmed with studies on the prevalence of asthma and other CRD in the various house types.

Acknowledgement

We confirm that all the authors have contributed to the manuscript and approved the paper. These results have not been published previously nor are being considered by any other peer-review journals. All co-authors have contributed to the contents of the manuscript. Each and all authors declare no conflicts of interest.

This study has been supported by a Projet Interuniversitaire Ciblé (PIC), from 2012 to 2016, between the Université Libre de Bruxelles (ULB) and the Pham Ngoc Thach University (U-PNT). [PIC-CUD 2012-2016]

Special thanks to colleagues: Prof. Francis CORRAZA and Abdel BEKKOURI for access to the laboratory and endotoxin analyser equipment, Duc Huy Phong DINH and Thi Mong Hiep TRAN who coordinated the project in HCMC, Bao Ngoc TRAN, Tri Tuyen NGUYEN, Hoang Trung PHAM, Thi Tu Anh HUA, Nguyen Anh Thu HUA, Thi Mai Linh TRAN and Thanh Sang MAI for their help in contacting all the household and local officers during the data collection phase.

Funding Source

This study has been supported by a Projet Interuniversitaire Ciblé (PIC), from 2012 to 2016, between the Université Libre de Bruxelles (ULB) and the Pham Ngoc Thach University (U-PNT).

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