MITE FAUNA AND MITE ANTIGEN DETECTION IN HOUSE DUST FOUND IN RESIDENTIAL AREAS IN LOS BAÑOS, LAGUNA, PHILIPPINES

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Abstract. Dust mites are a medically important group of animals commonly found in carpets and mattresses in houses. Antigens in their feces cause allergic reactions such as asthma and contact dermatitis. Dust samples were vacuum-collected in a special collecting bag from a one square meter area of living room floors of 100 randomly sampled houses in Los Baños, Laguna, Philippines for one minute. Chromato-immunoassay ELISA (Mitey Checker) was used to detect mite antigenicity. Twenty-three species of mites were identified belonging to 7 families. Of these, *Blomia tropicalis* (265 mites/g of dust in 87% of households) of Family Glycyphagidae and *Dermatophagoides farinae* (71 mites/g of dust in 58% of households) of Family Pyroglyphidae were the most prevalent and abundant species. Forty-eight percent of households were detected to have low levels of antigen (≤5 μg/m^2). There was a weak linear relationship between mean total mite intensity and antigen level ($r = 0.129$). Mean *Dermatophagoides* intensity and antigen levels were also found to have a weak linear relationship. More mites were found in carpeted living rooms (822 mites/g) when compared to non-carpeted living rooms (645 mites/g). Different floor types did not show any difference in mean mite intensity. Likewise, mite intensity did not show correlation with household size.

Keywords: house dust, mite fauna, mite antigen, chromatoimmunoassay ELISA, Philippines

INTRODUCTION

Mites are chelicerated arthropods belonging to Subclass Acari under Class Arachnida (Evans, 1992). House dust mites live and feed on dust. Mites living in human areas occur most frequently on floors and in mattresses (Evans, 1992). Dust is mostly composed of shed skin scales, on which the dust mites feed along with other materials in the dust, such as spoiled foodstuff, fungi and pollen (Krantz, 1978). Dust mites are medically important as they cause allergic reactions among certain people due to the antigens in their fecal matter (Evans, 1992). Healthwise, it is important to know if the mite fauna has an undesirable effect on people. Of the mites, *Dermatophagoides* sp (commonly called house dust mites) cause public health concern as they are sources

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of allergic diseases due to their proximity to humans and are commonplace in human dwellings (Kozo and Mariko, 2007).

Most people are unaware of the presence of mites in their homes. Also, a number of people in the population are susceptible to mite antigens present in dust. Asthma and rashes are usually attributed to other factors, such as pollen, weather or food consumed (De Luna, 1981). Several studies on dust mites were done in other countries, such as Japan and Indonesia. However, not many studies have been conducted in Philippines regarding mite fauna and antigen levels in households.

This study aimed to determine mite fauna and the level of mite antigens from house dust collected from residential areas of Los Baños, Laguna, Philippines. In order to achieve this goal, the study was designed to identify the mites collected in house dust, to detect the level of mite antigen using ELISA chromato-immunoassay method, to compare the detected mite intensity with the level of mite antigen, and to compare the detected mite intensity with various physical factors.

The study was limited only to the investigation of mite fauna in sampled homes of Los Baños residential areas. The antigens detected were limited to those specific to *Dermatophagoides* sp as the ELISA used detects only these specific antigens and no other commercial kits are available for other mite antigens, and those of *Dermatophagoides* sp have been implicated as a major allergy causing agent. The collection time of mites was from April to June. The study would provide important information on mite fauna and mite antigen levels in Los Baños, Laguna providing a foundation for mite sensitization and mite allergy alleviation studies.

MATERIALS AND METHODS

Mite samples

Stratified random sampling with proportional allocation scheme was used in selecting households where dust was collected from each of the 14 barangays of Los Baños, Laguna making one stratum. Individual households were then randomly picked from Barangay Mass Index (BMI), a master list containing all households and their addresses in the barangay. Extra households were selected in case of refusal of the owners to take part in the study, and they were prioritized in the order they were picked. A square meter area in the living room was vacuumed for one minute using an electric vacuum cleaner and a special dust collecting bag. Another square meter area adjacent to the former was vacuumed for one minute for the dust to be used in antigen detection. Before vacuuming, all parts of the vacuum cleaner were wiped clean with a piece of slightly damp cloth. Also, presence of carpets in the living room, type of flooring, and household size were noted for each household.

Mite characterization

At most 50 mg of dust were taken from one of the bags. Two drops of methylene blue and a drop of liquid detergent were added to the samples to stain dust particles other than mites and to separate dust elements. Then, 20 ml aliquot of saturated saline solution (359 g/l) was added to float the mites due to differential specific gravity. The saline-dust solution was sieved and poured into a petri dish. Under a light compound microscope at 40x magnification, mites were separated individually using small needles and mounted in two drops Hoyer's medium on a clean glass slide. The species of mites

a b c

Fig 1–Most common mite species collected from house dust in Los Baños, Laguna, Philippines. a) *Blomia tropicalis*, b) *Dermatophagoides farina*, c) *D. pteronyssinus*.

were then identified using available key to mites commonly found in house dust (Colloff, 2009). Mites from each sample were counted and mite prevalence was expressed as the percentage of households positive for mites. Mite density was expressed as the number of mites extracted divided by the mass of dust in grams (mites/g). The species composition was computed using the following formula:

$$\text{Mite species composition} = \left[\frac{\text{Mite intensity of a particular species found in the household}}{\text{Total mite intensity found in the household}} \right] 100\%$$

Mite antigen detection

Ten ml of 0.1 M phosphate buffer solution was poured into the special collection bag inside a zip-locked bag. It was then pressed and shaken to release antigens into the phosphate solution. An ELISA strip was dipped into the solution for about 5 seconds. After 10 minutes, the intensity of the color on the test band was compared to that of the control band and relative antigen level was noted from one of four given values: <1 μg/m^2, 5 μg/m^2, 10 μg/m^2, >35 μg/m^2; based on the four standard color intensity comparisons given in the Mitey Checker Mite Antigen Detection Kit, an ELISA kit that uses monoclonal antibody, Anti-Der 2, specific for antigens from *Dermatophagoides* sp (Kozo and Mariko, 2007). It is a home-based assay which complements counting through flotation technique and is useful for examining mite contamination in the home. Its limitation however is that it cannot distinguish antigen of specific species of *Dermatophagoides*, only of the genus in general.

Statistical analysis

Leverne's test of equal variances was first computed to determine if the parametric statistical tests may be applied. One-way ANOVA was used to compare mean mite intensity across different antigen levels, and to compare mean mite intensity across different floor types. Two-tailed *t*-test of independent samples was used to compare the mean mite intensity between carpeted and non-carpeted living rooms. Simple correlation analysis of Pearson's sample correlation coefficient was used to determine if mite intensity correlates with household size.

Table 1
Mite fauna found in house dust collected from Los Baños, Laguna, Philippines.

Family	Species	Prevalence (%)	Mean density (mites/g)	Species composition (%)
Glycyphagoidea		90	419	60.99
	Blomia tropicalis	87	265	39.57
	Chortoglyphus arcuatus	51	75	10.92
	Austroglycyphagus sp	51	43	6.26
	Lepidoglyphus destructor	27	23	3.35
	Glycyphagus domesticus	8	3	0.44
	Glycyphagus privatus	5	2	0.29
	Goheria fusca	18	8	1.16
Pyroglyphidae		83	199	28.97
	Dermatophagoides farinae	58	71	10.33
	Dermatophagoides pteronyssinus	44	42	6.11
	Euroglyphus maynei	37	16	2.33
	Hirstia chelidonis	29	16	2.33
	Sturnophagoides brasiliensis	33	23	3.35
	Malayoglyphus intermedius	22	12	1.75
	Hirstia domicola	15	7	1.02
	Malayoglyphus carmelitus	14	5	0.73
	Hughesiella africana	14	5	0.73
	Gymnoglyphus longior	9	2	0.29
Cheyletidae	Cheyletid sp	17	20	2.91
Oribatidae (Order Oribatida)	Oribatid sp	23	17	2.47
Tarsonemidae	Tarsonemid sp	20	12	1.75
Acaridae		21	12	1.75
	Acarus sp	15	9	1.31
	Tyrophagus sp	6	3	0.44
Mesostigmatidae (Order Mesostigmata)	Mesostigmatid sp	18	8	1.16

RESULTS

Twenty-three species of mites belonging to 7 families were identified from 98 households of Los Baños, Laguna. The families identified were Family Glycyphagidae, Family Pyroglyphidae, Family Mesostigmatidae, Family Oribatidae, Family Tarsonemidae, Family Cheyletidae and Family Acaridae (Table 1) using the key of Colloff (2009). An average of 677 mites/g of dust were collected from the households. Families Glycyphagidae (90% of households) and Pyroglyphidae (83%) were the most prevalent and most abundant (409 and 188 mites/g of dust, respectively). Within Family Pyroglyphidae, *Dermatophagoides farinae* was found to be most prevalent (58%) and most abundant (71 mites/g of dust), and within Family Glycyphagidae, *Blomia tropicalis* was most prevalent (87%) and most abundant

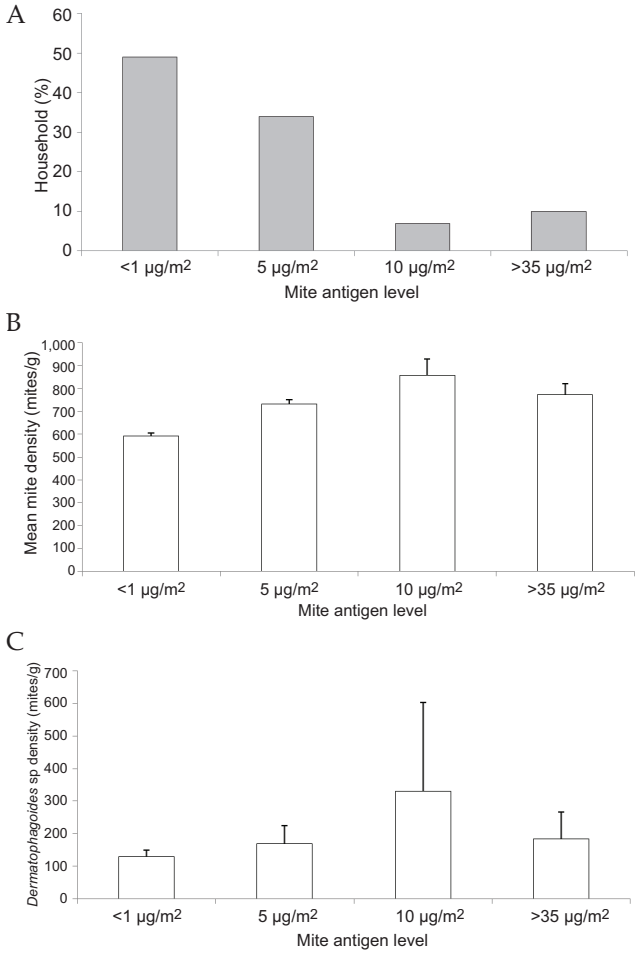

Fig 2–Percent households (A) mite density of 100 households (B), and density of *Dermatophagoides* sp in 100 households (C), with different mite antigen levels in Los Baños, Laguna, Philippines. The antigen levels were only for *D. pteronyssinus* and *D. farinae* using an ELISA technique.

(265 mites/g of dust) (Fig 1). It was also the overall dominant species.

Most households have low levels of antigens detected (Fig 2A). Less than one mg of mite antigen per m^2 was detected in 49% of households. High to very high levels of antigens (10 µg/m^2 and >35 µg/m^2) were detected in only 17% of households. This trend was also seen in almost all barangays (Fig 3).

There was an increasing trend of mean mite intensity as mite antigen level increased, but only up to 10 µg/m^2 level (Fig 2B), but there is no significant difference among mean mite intensity across different mite antigen levels ($F_c = 0.724$; $p = 2.600$). Also, a weak positive linear relationship between mite intensity and mite antigen level was found ($r = 0.129$). The same trend ($F_c = 1.076$; $p = 2.600$) was seen when comparing mean *Dermatophagoides* sp intensity across mite antigen levels (Fig 2C). The ELISA kit used contained monoclonal antibodies specific to antigens of *Dermatophagoides* sp, as it is the most immunodominant among mite allergens (Sakata *et al*, 2004). No significant difference was found among the mean levels. There also existed a weak positive linear relationship between *Dermatophagoides* sp intensity and mite antigen level.

Carpets in living rooms were not commonly seen (18%) in the sampled houses, more likely as they are too expensive for the average household income. Nevertheless, more mites were found in carpeted living rooms (822 mites/g) than in non-carpeted living rooms (645 mites/g). Two-tailed *t*-test of independent samples confirms a significant difference between the two variables ($|t_c| = 1.138$; $p = 0.699$). There is no significant difference ($F_c = 0.623$; $p = 2.090$) in mean mite density among different floor types (Fig 4). There was a very weak positive linear relationship ($r = 0.043$; $p = 0.699$)

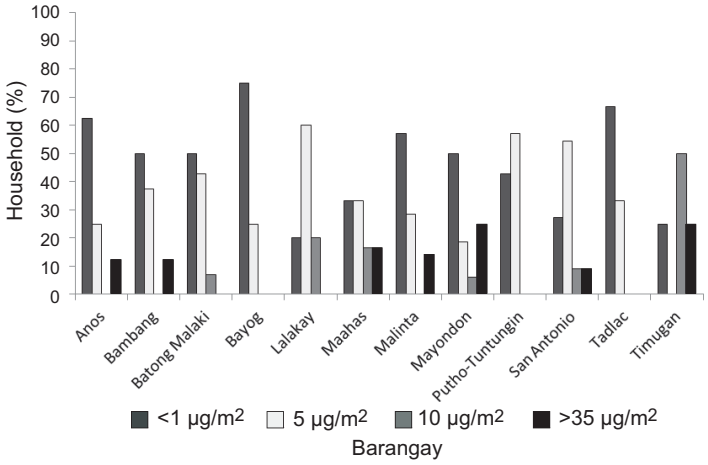

Fig 3–Percent households with mite antigen levels per baranggay in Los Baños, Laguna, Philippines. Antigen levels was determined using ELISA.

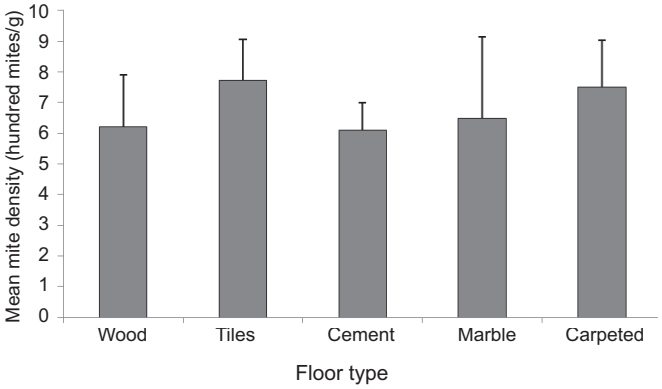

Fig 4–Mean mite density among different floor types in Los Baños, Laguna, Philippines. (Error Bar: + 1 SE).

between mite density and household size (Fig 5). However, using simple correlation analysis, no significant correlation exists between the two variables.

DISCUSSION

Blomia tropicalis was found to be the most dominant mite species present in households, Los Baños, Laguna, Philippines. Similar observation were made by other studies done in the tropics (De Luna, 1981; Chew *et al*, 1999; Mariana *et al*, 2000; Ciftci *et al*, 2006; Malainual *et al*, 2006). Fifteen mite species were recorded in this study that were not found in the study of De Luna (1981), namely *C. arcuatus, E. maynei, G. fusca, G. privatus, G. domesticus, Austroglycyphagus* sp, *L. destructor, S. brasiliensis, H. chelidonis, H. domicola, M. carmelitus, H. africana, G. longior, Acarus* sp, and *Tyrophagus* sp. These new findings were most likely due to the salt floatation technique employed in the study that allowed more efficient harvesting of mites.

Low mite antigens were detected in most of the sampled households, owing to the fact that 86 % of the households clean their living room floor daily. Another possibility is that *Dermatophagoides* sp, to which the ELISA test was specific, was not the dominant species. However, when *D. pteronyssinus* has 97.6% prevalence, the geometric mean antigen level was found to be 13.1 µg/g of dust, which is considered a very high level (Bouquete *et al*, 2006). *B. tropicalis* outnumbered *Dermatophagoides* sp by 148 mites per gram of dust. In barangay Timugan however, 50% of the sampled households were detected to have high levels of antigens (10 µg/m^2), perhaps due to the floors of the sampled houses in barangay Timugan being less frequently cleaned (once a month) compared to other houses (eight times a month).

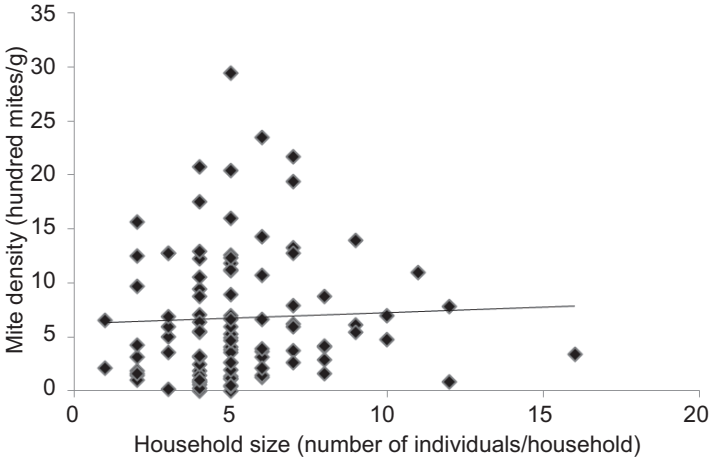

Fig 5–Scatter plot of mite density against household size in Los Baños, Laguna, Philippines.

In this study, there is a weak positive relationship between mite intensity with mite antigen level. This was contrary to the studies of Malainual *et al* (2006) and Boquete *et al* (2006) where antigen levels strongly correlate with mite counts. This may be due to the relatively low percentage of households detected with high levels of mite antigen, whereas the two aforementioned studies detected high levels of antigens (13.8 µg/g of dust). Moreover direct comparisons with these studies are difficult to make due to the differences in methods of mite collection.

This study also revealed that only 18% of households have carpeted living rooms. Carpets in living rooms are not frequently seen in Philippines as most households cannot afford them. Nonetheless, significantly more mites are found in carpeted than in non-carpeted living rooms, consistent with the results of Chew *et al* (1999). Carpets have gaps between fibers, which provide mites with dark humid spaces in which they can thrive. Dust can settle in carpets it they are not regularly cleaned.

Malainual *et al* (2006) found no significant difference in mean mite intensity among different floor types. Also, Ree *et al* (1997) reported no correlation between mite intensity and mite antigen level. Both results may be due to other factors such as carpeting and household activities that affect mite intensity. These factors are not independent of one another. Thus, we suggested more studies are needed, focusing on the ecology and microclimate indoors and how they affect mite population dynamics.

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