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Seasonal prevalence of allergenic mites in house dust of Kolkata Metropolis, India

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Abstract During the past few decades, house dust mites have attracted worldwide interest among medical entomologists and acarologists because of their importance in causing nasobronchial allergic disorders in human beings. House dust mites are present throughout the year; however, their relative densities differ in different seasons and habitats. Because the prevalence of house dust mite allergen is important epidemiologically and clinically, detailed knowledge on the seasonal abundance of important allergenic mites is of great importance for better understanding of the pathogenesis of the disease. In view of this, a systematic survey was carried out on the prevalence of total mites and four common allergenic mites in the city of Kolkata for two consecutive years. Both bed and bedroom floor dust were collected separately from homes inhabited by asthmatic patients situated in different corners of the city on monthly basis from

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Department of Zoology, Darjeeling Government College, Darjeeling 734101, India e-mail: skpzoo2@rediffmail.com January 2004 to December 2005. The population levels of total mites and four common allergenic mites, namely *Dermatophagoides pteronyssinus*, *D. farinae*, *Austroglycyphagus geniculatus*, and *Blomia tropicalis* separately, were highest during the pre-monsoon period (March–May), irrespective of habitat, whereas densities were low in all cases during winter (December–February). The study indicates that season had the most significant effect on the relative abundance of house dust mites except *Dermatophagoides farinae*, irrespective of habitat.

Keywords Allergen · Dust · Mite · Seasonal prevalence · Kolkata · India

1 Introduction

House dust mites are of immense importance to human beings because of their positive role in nasobronchial allergic manifestations as reported from different regions of the globe, including India in general and Kolkata city in particular (Saha 1993, 1997; Podder et al. 2006). The highest densities of house dust mites are generally found in beds, rather than elsewhere in houses, where shed skin scales are easily available to ensure a food source. Considering this, several epidemiological studies have been carried out to investigate the intricate association between allergen levels in homes and asthma (Sporik et al. 1990; Kivity et al. 1993; Ingram et al. 1995; Boquete et al. 2006). It is now well documented that mite populations fluctuate seasonally in different climatic regions (Arlian et al. 1983). In temperate regions mite density has a seasonal cycle that is directly related to changes of relative humidity (RH) in the different seasons (Dautartiene 2001), and a high mite population level occurs during summer and autumn whereas mite density drops during the dry season at low relative humidity in different countries. Identification of seasonal patterns could reveal the timing of peak allergen levels within homes. Thus an understanding of the timing of peak mite allergen levels might also be useful to provide sensitive patients with the best possible diagnosis and treatment.

Information in this regard is mostly available from western countries, but from third world countries like India it is still fragmentary and insufficient. This study was designed to monitor seasonal changes of total mites and, separately, four species of known allergenic mites in bed and bedroom floor dust in homes inhabited by asthmatic patients in Kolkata Metropolis, India. In India, the seasonal abundance of house dust mites is yet to be studied comprehensively.

2 Materials and methods

2.1 Study cohort

For this purpose 20 houses inhabited by patients suffering from bronchial asthma were selected on the basis of three criteria:

- houses were situated in five different zones representing different areas of the city (four houses each from east, west, north, south, and central);
- 2 the inhabitants of each house did not use any acaricides during the study period; and
- 3 the occupants did not change residence during the entire study period.

Before initiating studies, consent was obtained from the respective adults for their inclusion in this study and they affirmed that they would abide by all the above mentioned regulations.

2.2 Questionnaires

The residents in each of the 20 homes were asked to complete a questionnaire containing brief home

characteristics including building structure, home dampness, layers and types of bedding, presence of pets, frequency of cleaning, etc. An adult person from each home was given a digital hygrometer and thermometer to measure relative air humidity (RH) and temperature inside the room daily at 0600 and 1400 hours.

2.3 Dust collection

Dust samples from beds and corresponding bedroom floors were collected in each month by the same person during 2004 and 2005. Bed dust and the corresponding floor dust were collected by using a portable vacuum cleaner (model no. SA-300 DX) and kept separately in different polythene packets and labelled properly. Dust samples were collected from bedrooms situated on the ground floor, only, of concrete houses, usually provided with ventilation through at least two or three small windows per room, and the residents were advised not to use any floor carpets during the study period. They were also instructed to use traditional cotton mattresses (5 cm thick) with bed linen. All rooms were plastered properly and whitewashed. Being on the ground floor the rooms selected did not get proper sunlight and were of sufficient dampness. Bed linen was normally changed once a week.

2.4 Isolation of mites

All dust samples were sieved separately in a mechanical sieve shaker, using a stack of sieves with mesh sizes 2.36 and 1.00 mm, and 500, 75, and 45 µm. Dust collected on the two finest sieves was processed in accordance with the flotation method of ChannaBasavanna et al. (1984), with necessary modifications. One gram of dust from each sample was mixed with pure kerosene oil and vortex mixed for 10 min. The mixture was centrifuged at 2,000 rpm for 2 min and the supernatant was filtered using Whatman No. 1 filter paper. A mixture of kerosene oil and carbon tetrachloride of specific gravity 1.3 was added to the sediment in the tube and, after centrifugation, the sample was filtered through the same filter paper. This procedure was repeated with similar mixtures having specific gravity 1.4 and 1.5. The supernatant was again filtered and the residue collected on the filter paper was washed with a fine jet of 70% alcohol in a Petri dish. The mites were isolated from this solution and stored in 70% alcohol. For the next sample, the sieve was cleaned with running water and cotton soaked with 70% ethyl alcohol, so that no organisms remained on the sieve. After filtration, residues of dead mites only, at different stages, were found, but no exuviae. Mites were counted per gram of dust in accordance with the method of Dar et al. (1974) and Solarz (1997).

2.5 Mounting and identification of mites

Mites at all stages in each sample were counted and identified in accordance with Hughes (1961) and Colloff and Spieksma (1992). Temporary mounting was performed in lactic acid on a glass slide using a broken piece of coverslip. Monthly variation was calculated for total mite counts and, separately, for counts of four predominant and known allergenic species, viz. *Dermatophagoides pteronyssinus* (DP), *D. farinae* (DF), *Austroglycyphagus geniculatus* (AT), and *Blomia tropicalis* (BT).

2.6 Statistical analysis

Sampling seasons were categorized as pre-monsoon (March–May), monsoon (June–August), post-monsoon (September–November), and winter (December– February). Multivariate analysis was conducted to enumerate the possible effect of season and/or habitat on the four separate mite species. To explore the relationship between mite counts and RH and temperature, a simple product–moment correlation analysis was conducted. All data were analyzed using SPSS 10.0 for Windows.

3 Results

3.1 Monthly variation of allergenic mites in bed dust

During the first year study period, the monthly average total mite density in bed dust was maximum (282.5 \pm 17 per g dust) in May, when average temperature and RH were 27.5°C and 81.7%, respectively, and minimum (32.5 \pm 4.8 per g dust) in February, when average temperature and RH were 26.6°C and 61.2%, respectively (Table 1). The density

of DP, DF, and BT mites reached a peak in May $(150 \pm 12, 57.5 \pm 18.8, \text{ and } 55 \pm 16.5 \text{ per g dust},$ respectively) whereas the maximum density of AT (57.5 ± 14.9) was observed in June. In contrast, the lowest density of DP $(2.5 \pm 2 \text{ per g dust})$ and BT (7.5 ± 4.7) was observed in February. In November, however, when temperature and RH were 25.1° C and 69.8%, not a single DF was found. For AT, not a single mite was found in February, March, or December. A second peak of total mite density was observed in October when average temperature and humidity were 27.2° C and 81.7%, respectively (Table 1).

In the next year, the same trend was followed i.e. the monthly average of total mites and DP and BT $(327.5 \pm 16.5, 137.5 \pm 13.7, \text{ and } 135 \pm 9.2 \text{ per g})$ dust, respectively) reached their peak in May when average temperature and RH were 31.3°C and 89.7%, respectively. The maximum density of DF (32.5 \pm 16.5) was found in September when average temperature and RH were 29.4°C and 87%, respectively. The density of AT reached its peak (32.5 \pm 7.5) in October when average temperature and RH were 27.2°C and 83.9%, respectively. The lowest density of total mites and DP and BT (57.5 \pm 4.7, 20 \pm 7, and 10 \pm 3 per g dust, respectively) were found in February, when average temperature and RH were 25°C and 68.8%, respectively. The lowest density of DF and AT (0 and 2.5 ± 2 per g dust, respectively) were observed in November and June, respectively. The next highest peak of total and all four mite species density was observed in October when average temperature and humidity were 27.2°C and 83.9%, respectively (Table 1).

When the data for both years were compiled seasonally it was observed that the densities of total mites and DP, DF, and BT separately were highest during the pre-monsoon period whereas AT was predominant during the monsoon season (Fig. 1).

3.2 Monthly variation of allergenic mites in floor dust

The maximum number of total mites in floor dust $(204 \pm 16.3 \text{ per g dust})$ was found during May and June 2004 when temperature and RH were 27.5°C, 32.1°C, 81.7%, and 73.9%, respectively, and minimum $(14 \pm 5 \text{ per g dust})$ during December and January when temperature and RH were 22.1°C, 20.4°C, 71.3%, and 57.6%, respectively. The maximum

Month	TM	DP	DF	AT	BT	<i>T</i> (°C)	RH (%)
2004							
Jan	62.5 ± 7	25 ± 6.5	5 ± 4	2.5 ± 2	17.5 ± 7.5	20.4	57.6
Feb	32.5 ± 4.8	2.5 ± 2	17.5 ± 4.7	0	7.5 ± 4.7	26.6	61.2
Mar	110 ± 18.1	50 ± 18	5 ± 4	0	42.5 ± 13.1	28.7	57.2
Apr	222.5 ± 4.7	120 ± 13	32.5 ± 11.8	30 ± 12.9	30 ± 12.2	30.3	68.4
May	282.5 ± 17	150 ± 12	57.5 ± 18.8	17.5 ± 2.5	55 ± 16.5	27.5	81.7
Jun	122.5 ± 7.5	37.5 ± 8.5	10.5 ± 4.7	57.5 ± 14.9	12.5 ± 4.7	32.1	73.9
Jul	87.5 ± 8.5	42.5 ± 8.5	10 ± 5.7	7.5 ± 4.7	20 ± 7	27	66.3
Aug	167.5 ± 8.5	55 ± 9.5	7.5 ± 4.7	35 ± 20.2	50 ± 25.4	29.2	78
Sep	187.5 ± 23.9	92.5 ± 31.1	17.5 ± 18.5	5 ± 4	42.5 ± 6.2	28.9	82.4
Oct	240 ± 7	112.5 ± 27.8	15 ± 11.9	30 ± 12.2	32.5 ± 14.3	27.2	81.7
Nov	72.5 ± 19.3	37.5 ± 11	0	10 ± 9	10 ± 9	25.1	69.8
Dec	32.5 ± 7.5	12.5 ± 6.2	5 ± 4	0	10 ± 9	22.1	71.3
2005							
Jan	127.5 ± 8.5	52.5 ± 8.5	5 ± 2.8	5 ± 4	65 ± 2.4	19.6	64.7
Feb	57.5 ± 4.7	20 ± 7	12.5 ± 7.5	12.5 ± 7.5	10 ± 3	25	68.8
Mar	145 ± 11.9	65 ± 6.4	20 ± 12.2	20 ± 12.2	37.5 ± 12	24	69.5
Apr	252.5 ± 11.8	110 ± 27	20 ± 9.1	15 ± 9.5	87.5 ± 14.3	30.5	75.1
May	327.5 ± 16.5	137.5 ± 13.7	25 ± 9.5	20 ± 12.2	135 ± 9.2	31.3	89.7
Jun	102.5 ± 12.5	35 ± 2.8	10 ± 5.7	2.5 ± 2	45 ± 8.1	26.4	75.9
Jul	90 ± 14.7	52.5 ± 11	2.5 ± 2	7.5 ± 7	27.5 ± 5.8	28.9	84.4
Aug	175 ± 6.4	80 ± 7	12.5 ± 7.5	25 ± 15	52.5 ± 5	30.6	82.1
Sep	207.5 ± 25.6	105 ± 10.4	32.5 ± 16.5	12.5 ± 11	45 ± 8.3	29.4	87
Oct	255 ± 10.4	137.5 ± 7.5	7.5 ± 7	32.5 ± 7.5	77.5 ± 7.4	27.2	83.9
Nov	80 ± 12.2	40 ± 7	0	17.5 ± 6.2	17.5 ± 11.3	23.7	70.3
Dec	127.5 ± 8.5	52.5 ± 8.5	5 ± 2.8	5 ± 4	65 ± 2.4	21	66.3

Table 1 Monthly variation of mite density in bed dust samples (number per g dust) collected from the homes of asthmatic patients inKolkata during 2004 and 2005

TM, total mites; DP, *Dermatophagoides pteronyssinus*; DF, *D. farinae*; AT, *Austroglycyphagus geniculatus*; BT, *Blomia tropicalis*; *T*, average temperature; RH, average relative air humidity

numbers of DP and DF (100 ± 15.8 , 32 ± 9.6 per g dust, respectively) were observed in May at the aforesaid temperature and RH, and the maximum density of AT and BT (60 ± 13.4 and 54 ± 23.1 per g dust, respectively) were found in June and April, respectively, when temperature and RH were 32.1° C, 30.3° C, 73.9%, and 68.4%, respectively. The lowest number of BT (2 ± 1 per g dust) was found in February and June when temperature and RH were 26.6° C, 32.1° C, 61.2%, and 73.9%, respectively. It is interesting to note that not a single DP, DF, or AT was found in December. The subsequent highest peak of mite density was observed in April when average temperature and humidity were 30.3° C and 68.4%, respectively (Table 2).

In the second year the maximum number of total mites $(224 \pm 15.6 \text{ per g dust})$ was in May and the minimum $(34 \pm 4 \text{ per g dust})$ was in February. The highest density of DP and BT $(94 \pm 7.4 \text{ and } 86 \pm 9.2 \text{ per g dust}, \text{ respectively})$ was found in May when temperature and RH were 31.3° C and 89.7%, respectively, whereas the maximum numbers of DF and AT $(18 \pm 11.1 \text{ and } 24 \pm 6.7 \text{ per g dust}, \text{ respectively})$ were found in August and October when the corresponding temperature and RH were 30.6° C, 27.2° C, 82.1%, and 83.9%, respectively. The lowest number of DP $(12 \pm 7.3 \text{ per g dust})$ was found in September. The minimum numbers of DF, AT, and BT $(0, 0, \text{ and } 4 \pm 3 \text{ per g dust}, \text{ respectively})$, however, were found in February, June, and



February, respectively. The next peak of mite density was observed in October when average temperature and humidity were 27.2°C and 83.9%, respectively (Table 2). Data obtained on the seasonal prevalence of mites in floor dust lie very close to the data obtained for bed dust, i.e. maximum densities of total mites and DP, DF, and BT were found in the premonsoon period whereas AT was encountered mostly in the monsoon period. In contrast, numbers of total mites, DP, AT, and BT were lowest during winter (Fig. 2).

3.3 Statistical analysis

Statistical analysis showed that season had a significant effect on the prevalence of DP, AT, and BT but that prevalence of DF did not depend on season (Table 3). Moreover, habitat (i.e. bed vs. floor) explained a significant amount of variation of DP density only, not that of the other species. Analysis of the correlation between mite densities (total and individual species) and temperature yielded only positive, though not statistically significant, correlation coefficients. The correlation between DP, DF, and BT densities and relative humidity was positive and significant (P < 0.01), however. For AT, the only positive correlation was between mite density and

relative humidity, and the correlation was not statistically significant (Table 4). The study indicates that the densities of DP, DF, AT, and BT are not associated with ambient temperature but relative humidity plays a vital role in regulation of the population structure of mites.

4 Discussion

With regard to seasonal abundance and fluctuation of total number of mites and Dermatophagoides mites, several authors are of different opinion. This study reveals that total mite density and DP, DF, AT, and BT levels were highest in the pre-monsoon period (March-May) in both habitats, and decreased through the winter. Interestingly, a second peak was observed in October, in bed and floor dust, in the two consecutive years. This finding confirms the earlier observations of Solarz (1997), Chew et al. (1999), Bouquete et al. (2000), and Fernandez-Caldas et al. (2004) who recorded increased mite densities during April–November and the summer months. However, most workers throughout the world (Dar and Gupta 1979; Dar et al. 1974; Lang and Mulla 1978; Bronswijk 1981; Ranganath and ChannaBasvanna 1988; Banerjee 1988) reported that peak populations

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50 H 40 . 40 E

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Month	TM	DP	DF	AT	BT	<i>T</i> (°C)	RH (%)
2004							
Jan	14 ± 5	12 ± 3.7	4 ± 3	0	8 ± 4.8	20.4	57.6
Feb	18 ± 6.6	12 ± 4.8	0	7.5 ± 4.7	2 ± 1	26.6	61.2
Mar	84 ± 12	28 ± 8	6 ± 5	0	34 ± 12.4	28.7	57.2
Apr	174 ± 10	72 ± 16.2	24 ± 11.6	20 ± 9.4	54 ± 23.1	30.3	68.4
May	204 ± 16.3	100 ± 15.8	32 ± 9.6	16 ± 8.1	50 ± 8.3	27.5	81.7
Jun	204 ± 16.3	28 ± 10.6	4 ± 2	60 ± 13.4	2 ± 1	32.1	73.9
Jul	78 ± 11.5	32 ± 9.6	0	2 ± 1	26 ± 10.2	27	66.3
Aug	140 ± 7	36 ± 8.1	0	24 ± 8.1	46 ± 16.9	29.2	78
Sep	102 ± 20.5	42 ± 13.1	10 ± 6.3	0	30 ± 6.3	28.9	82.4
Oct	162 ± 14.9	74 ± 18	10 ± 6.3	32 ± 11.5	26 ± 12	27.2	81.7
Nov	52 ± 11.5	22 ± 3.7	8 ± 4.8	6 ± 5	16 ± 7.7	25.1	69.8
Dec	14 ± 5	0	0	0	8 ± 4.87	22.1	71.3
2005							
Jan	70 ± 15.8	25 ± 5.8	4 ± 3	12.5 ± 4.7	20 ± 2.2	19.6	64.7
Feb	34 ± 4	26 ± 6.7	0	4 ± 3	4 ± 3	25	68.8
Mar	106 ± 7.4	38 ± 3.7	10 ± 4.4	20 ± 8.3	34 ± 12	24	69.6
Apr	184 ± 10.2	58 ± 9.6	12 ± 7.3	8 ± 4.8	74 ± 14.3	30.5	75.1
May	224 ± 15.6	94 ± 7.4	16 ± 5	18 ± 9.6	86 ± 9.2	31.3	89.7
Jun	64 ± 8.1	32 ± 2	4 ± 2.4	0	26 ± 8.1	26.4	75.9
Jul	74 ± 9.2	40 ± 5.4	12 ± 7.3	8 ± 5.8	12 ± 5.8	28.9	84.4
Aug	134 ± 14.3	64 ± 15.3	18 ± 11.1	16 ± 7.4	36 ± 5	30.6	82.1
Sep	70 ± 15.8	12 ± 7.3	16 ± 10.2	8 ± 4.8	30 ± 8.3	29.4	87
Oct	182 ± 9.6	90 ± 6.3	12 ± 8	24 ± 6.7	36 ± 7.4	27.2	83.9
Nov	62 ± 10.6	24 ± 5	16 ± 6.7	6 ± 2.4	18 ± 11.3	23.7	70.3
Dec	70 ± 15.8	28 ± 5.8	4 ± 3	10 ± 4.4	22 ± 2.3	21	66.3

Table 2 Monthly variation in mite density in floor dust samples (number per g dust) collected from the homes of asthmatic patientsin Kolkata during 2004 and 2005

TM, total mites; DP, *Dermatophagoides pteronyssinus*; DF, *D. farinae*; AT, *Austroglycyphagus geniculatus*; BT, *Blomia tropicalis*; *T*, average temperature; RH, average relative air humidity

occur during different seasons of the year other than March–May and October. Apparently, it seems that mite population and incidence of disease did not always coincide. In those cases house dust must have contained other potent allergens, for example moulds, fungi, pollen, animal dander, etc., which might interfere in the pathogenesis of the disease, as suggested by Chew et al. (1999).

Season is considered to be a compound measure for a combination of climatic factors that seem to affect the dust mite population. It has been recognized that temperature and relative humidity are the two most important predictors of dust mite allergen concentration in bed and floor dust samples. Cunnington (1965, 1967, 1969), Leupen and Verekamp (1966), Spieksma and Spiksma-Boezeman (1967), Bronswijk (1973), and Lopez-Rico et al. (2000) reported that the annual periodicity of mite numbers is possibly correlated with humidity cycle inside the house. Spieksma and Spiksma-Boezeman (1967) were of the opinion that the seasonal fluctuation in house dust mites corresponds to the seasonal rise in relative humidity of the air.

In our study, product moment correlation coefficients (r) agree with those from earlier studies by Murray and Zak (1979), Arlian et al. (1983), and Brandt and Arlian (1976). According to these workers seasonal fluctuation of mite abundance was positively correlated with seasonal changes in relative humidity and temperature. As temperature increases, dust mites





 Table 3 Multivariate analysis where Dermatophagoides pteronyssinus, D. farinae, Austroglycyphagus geniculatus, and Blomia tropicalis are dependent variables

Source of variations	Sum of squares	df	Mean square	F value	Remarks
Dermatophagoides pterony	essinus (DP)				
Seasons	23446.854	3	7815.618	10.111	P < 0.0001
Habitat	7129.688	1	7129.688	9.223	P < 0.004
Season × Habitat	2403.854	3	801.285	1.037	NS
Residual	30920.583	40	773.015		
Total	63900.979	47			
Dermatophagoides farinae	(DF)				
Seasons	1704.875	3	568.292	2.051	NS
Habitat	901.333	1	901.333	3.252	NS
Season × Habitat	518.208	3	172.736	0.623	NS
Residual	11085.750	40	277.144		
Total	14210.167	47			
Austroglycyphagus genicul	atus (AT)				
Seasons	1603.542	3	534.514	2.979	P < 0.04
Habitat	161.333	1	161.333	0.899	NS
Season × Habitat	153.542	3	51.181	0.285	NS
Residual	7176.250	40	179.406		
Total	9094.667	47			
Blomia tropicalis (BT)					
Seasons	10313.354	3	3437.785	6.694	P < 0.001
Habitat	1668.521	1	1668.521	3.249	NS
Season × Habitat	96.688	3	32.229	0.063	NS
Residual	20541.250	40	513.531		
Total	32619.813	47			

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Parameters	DP		DF		AT		BT	
	Bed	Floor	Bed	Floor	Bed	Floor	Bed	Floor
Temperature (°C)	0.501	0.688	0.009	0.569	0.577	0.876	0.748	0.751
RH (%)	0.976	0.912	0.356	0.948	0.275	0.273	0.940	0.922

Table. 4 Correlation coefficients of *Dermatophagoides pteronyssinus*, *D. farinae*, *Austroglycyphagus geniculatus*, and *Blomia tropicalis* with temperature and relative humidity in bed dust and floor dust

require a higher A_w to survive (Arlian 1975; Arlian and Vesclica 1981; Brandt and Arlian 1976; Fernandez-Caldas et al. 2003). However, small temperature changes (increase or decrease of 2-5°C in the range of normal ambient temperature of 20-25°C) have a negligible effect on dust mites. In our study it was noteworthy that the frequency of breathlessness of asthmatic patients became high in the pre-monsoon and post-monsoon periods, when the density of allergenic mites was higher, as also reported by earlier workers (Suto et al. 1992; Mumcuoglu et al. 1999; Ciftci et al. 2006). The observed seasonal patterns in dust mite allergens were as might be expected of homes in such a region, where the temperature and RH do not favour multiplication of the mite. However, different regions may have different seasonal patterns. Detection of these seasonal patterns in levels of dust mite allergens in different regions improves estimates of exposure to these allergens. This more precise estimation of peak, average, and season-specific allergen exposure may be useful for assessment of association between allergen exposure and development of different nasobronchial allergic disorders, especially asthma.

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