

# House dust mite allergen exposure in infancy

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## Summary

**Background** Infancy may be a critical time for exposure to house dust mite allergens, when exposure to high levels can increase the risk of allergic sensitization and the development of asthma in later life.

**Objective** To measure house dust mite allergen (Der p 1) concentration in the infants' environment and examine lifestyle factors which may influence mite allergen exposure.

**Methods** Infants aged between 4 and 12 months ( $n = 134$ ) from the western region of Sydney, Australia, participated. Reservoir dust samples were collected from four sites within each home: infant's bed, second bed (adult or second child's bed), lounge floor and sheepskins (where available). Settling aeroallergen was collected for 10–14 d in Petri dishes in the infant's room. Der p 1 was measured by ELISA. A questionnaire on types of bedding, sleeping and playing patterns of the infant was completed by the parents at the time of dust collection.

**Results** All infants were exposed to at least one site with Der p 1 concentrations greater than  $10 \mu\text{g/g}$  fine dust. The mean settling aeroallergen level in the infants' room was  $24 \text{ ng Der p 1/m}^2\text{day}$  and this was weakly related to bed allergen levels ( $r = 0.21$ ,  $P < 0.05$ ). Mattress type had a weak effect on Der p 1 levels as measured in the whole bed ( $P = 0.07$ ), while bed cover and bed type had no effect ( $P > 0.6$ ). The mean product of time spent at a site and its allergen concentration was highest for beds in 69% of infants.

**Conclusion** The high level of allergen exposure in the environment of this group of infants places them at an increased risk of early sensitization and development of asthma. Any strategy to reduce asthma prevalence should address these high and avoidable levels.

**Keywords:** house dust mite allergen, exposure, infant, environment

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## Introduction

There is increasing interest in the role that early allergen exposure, occurring *in utero* and during infancy, might play in predisposing genetically susceptible infants to the later development of sensitization and asthma [1–4]. This has been stimulated by associations between early exposure to pollens and cat dander and the development of allergic sensitization [5–7], and a positive association between house dust mite allergen dose in infancy and the age of asthma onset [1].

It has been suggested that a primary cause of asthma in many humid temperate areas of the world is exposure

to house dust mite allergens (Der p 1) [1,8]. This includes studies which have shown that mean bed Der p 1 concentrations in different climatic regions correlate with community levels of airway hyperreactivity in atopic children [9]. In coastal Australia, Der p 1 levels are very high with at least 80% of homes exceeding  $10 \mu\text{g/g}$ , with beds containing higher levels than floors [9]. During infancy, beds represent a potentially major site for mite allergen exposure. Although there have been numerous studies on the concentration of mite allergens in adult's and children's beds, including the variations between bedding materials [10–12] and sites within beds [13], only a few studies have examined mite allergens in infant beds. An early study by Tovey *et al.* found lower numbers of live mites per site in infant mattresses than in adult mattresses [14]. However, sheepskins used in the beds contained

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significantly more live mites than the adult and infant bedding. In the UK, Carswell *et al.* reported that infant beds had less allergen than adult beds, but no values were given [15]. In The Netherlands, Van Strien *et al.* reported lower Der p 1 concentrations in mattress dust of infants of atopic parents than infants of non-atopic parents, and proposed that atopic parents may be more aware of allergen avoidance procedures [16]. Sporik *et al.* reported a mean of 18.4 µg Der p 1/g in the beds of 1–2 years olds, and this was much higher than the bedroom floor levels [1]. However, none of these studies have reported the allergen levels in infant beds and compared them with adult beds in the same house.

Allergen levels in infant bedding are likely to differ from both adult and children's beds for a number of reasons. Allergen levels in infant bedding may be lower because: they are more frequently washed, and the mattresses are more likely to be new or have waterproof covers. Alternatively, allergen levels may be higher, as infant beds are occupied for longer periods, leading to increased warmth and dampness, thereby providing a suitable microclimate for mite survival. In addition, they are more likely to contain unwashed soft toys and sheepskins which harbour numerous mites [14].

This study aimed to quantify allergen levels in the infant's environment during their first year of life and these figures were compared to other sites within the home. In addition, some aspects of infant lifestyle which may influence mite allergen exposure have been examined.

## Materials and methods

### *Selection criteria of subjects*

A total of 134 infants aged between 4 and 12 months from the western region of Sydney Australia, participated. These infants were recruited from two different sources. The first group comprised 52 infants who had previously been recruited into a separate study of infant cough. Of these infants selected on the basis of symptoms, 84% had a first degree relative with an allergic disease. A second group was recruited to increase the sample size. Names of infants for the second group were obtained from the District Birth Registry after ethical approval was granted. The selection criterion was that the infant had a first degree relative with an allergic disease (defined as hayfever, asthma or eczema). Parents were invited to participate by phone and letter. A total of 82 infants of the 302 parents contacted by phone or letter fulfilled the selection criterion and participated in the study.

### *Reservoir house dust samples*

Samples were collected using a portable vacuum cleaner

(Makita, model 4071D) modified to collect the fine dust in a 25 µm pore size nylon bag. Sampling was conducted during the summer months of October through to December 1992. Four separate samples of fine dust were collected by vacuuming a 1 m<sup>2</sup>/min area of the infant's bed, parent or second child's bed (for comparison), lounge room floor, and infant nursery sheepskin (where available). Following collection, dust samples were stored at –20 °C to prevent mite allergen degradation and fungal growth. The samples were sieved and 50 mg of fine dust was extracted in 1 mL of 0.2% bovine serum albumin (BSA), phosphate-buffered saline (PBS), 0.05% Tween 20. Extracts were stored at –20 °C for 3 months until analysed for Der p 1.

### *Settling aeroallergen collection*

Plastic Petri dishes (150 × 15 mm) and their lids were coated with a 2% fish gelatine/0.05% azide solution for 10 min, after which excess gelatine was rinsed off with distilled water and the plates were left to air dry [17]. To collect the settling aeroallergen, both the dish and lid were left open for 10–14 d in an undisturbed area in the infant's bedroom, ≈1 m above the floor. Aeroallergen plates were returned from 122 of the 134 homes (91% response rate). The settling dust was eluted from both the lid and dish with 1 mL of 0.2% BSA/PBS/0.05% Tween/0.05% sodium azide. The extracts were stored at 4 °C for 3 months until analysed for Der p 1.

### *Mite allergen measurement*

The concentration of mite allergen Der p 1 was measured in the dust using a double monoclonal ELISA assay kit (University of Virginia) which contained a Der p 1 standard. The assay was performed essentially as described by Luczynska *et al.* [18], except that the primary antibody was used at half of the recommended concentration. To increase sensitivity for aeroallergen samples antigen incubation was extended to overnight and second antibody incubation to 3 h. This increased sensitivity from 4.0 ng/mL to 0.2 ng/mL. Plates were read and calculations performed using the BioRad model 450 plate reader (BioRad Richmond California, USA) and Microplate Manager software (BioRad), respectively. Reservoir house dust mite allergen concentration was expressed as micrograms Der p 1 per gram fine dust. Settling aeroallergen results are expressed as nanograms Der p 1 per metre squared per day and were calculated by dividing the mass of Der p 1 collected per day by the area of the dishes (including the lid).

### *Administration of questionnaire*

A questionnaire was completed by the infants' parents at the

time of dust collection. Items in the questionnaire related to the infant's bedding at ages 0–3 and 4–12 months. Questions included: presence of a sheepskin; type of bed (cot, cane, cradle, bed); mattress type (foam, tea tree, inner spring mattress, wadding); and bed cover type (quilt — duck feather, polyester; blanket — acrylic, wool, cotton). In addition, information was collected regarding general sleeping and playing patterns of the infant at 0–3 and 4–12 months. These questions included: the number of hours the infant spent in its own bed and the time spent playing on the floor.

#### Analyses of data

As the distribution of Der p 1 allergen levels was skewed, they were  $\log_{10}$  transformed to normalize the distribution and allow the use of parametric tests. Der p 1 allergen levels were expressed as geometric means with error bars showing 95% confidence intervals (95% CI). Analysis of variance, and the Duncans Multiple range test was used to determine statistical significance between Der p 1 allergen levels and the different collection sites. Linear regression analysis was used to test the effect of various bedding on allergen concentration. A *P*-value of less than 0.05 ( $P < 0.05$ ) was regarded as significant. Calculations were performed using the statistical packages SAS (SAS Institute, Cary, NC, USA).

#### Results

Mean Der p 1 concentrations for the four collection sites are shown in Fig. 1. Mean allergen levels were greater than  $10 \mu\text{g/g}$  in at least one site in each home. The mean Der p 1 concentrations as micrograms per gram (95% CI) for each site were: infant bed ( $n = 134$ ), 12 (10.26, 15.10); second bed ( $n = 117$ ), 17 (14.32, 21.83); lounge floor ( $n = 134$ ), 7 (5.83, 8.40) and sheepskin ( $n = 33$ ), 29 (18.94, 43.70). The mean allergen concentration for sheepskins was significantly higher than both the infant bed and lounge floor ( $P < 0.0001$ ), and the lounge floor significantly lower than both the infant bed and second bed ( $P < 0.0001$ ). There was no statistical difference between allergen levels in the infant bed and the second bed (adult or second child).

The relation between settling aeroallergen levels and reservoir bed dust levels in the infant bed is shown in Fig. 2. The mean Der p 1 settling aeroallergen level was  $24 \text{ ng/m}^2/\text{d}$  (equivalent to  $0.85 \text{ ng Der p 1/d}$  per pair of dishes). A weak linear relationship was observed between settling aeroallergen levels and infant bed dust levels ( $r = 0.21$ ,  $P < 0.05$ ).

Completed questionnaires were obtained from 125 of the 134 parents (93% response rate). Mattress type had a weak effect on Der p 1 concentrations as measured in the whole

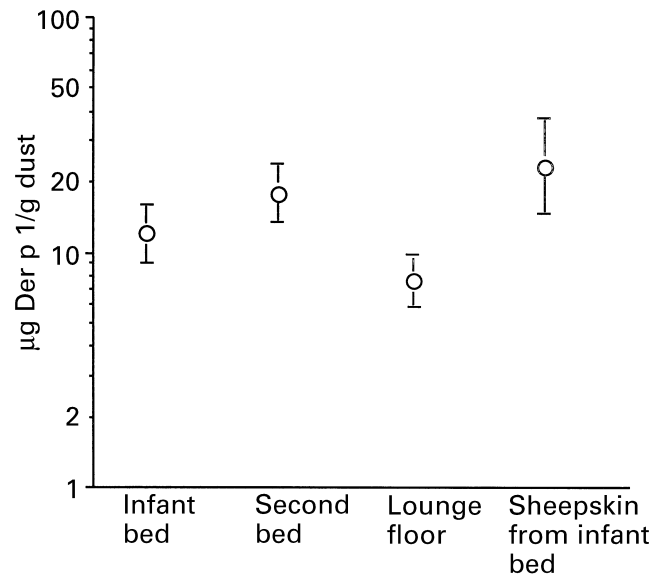


Fig. 1. Mean house dust mite allergen concentration (95% CI) at four collection sites.

bed ( $P = 0.07$ ), with 50% more allergen in foam mattresses than inner spring. The geometric means for the different bedding types were bed type: cot ( $n = 115$ ) 12.1 (9.8, 14.9); bed ( $n = 9$ ) 8.5 (4.1, 17.4). The means for mattress type: inner spring ( $n = 94$ ) 11.3 (8.9, 14.2); foam ( $n = 24$ ) 15.5 (9.9, 24.2); teatree ( $n = 4$ ) 5.8 (2.3, 14.2). The two 'other' mattresses included one, for which mattress type was not recorded ( $118 \mu\text{g/g}$ ) and one waterbed ( $2.7 \mu\text{g/g}$ ). The means for quilt type: polyester ( $n = 67$ ) 13.7 (10.5, 17.9); duck feather ( $n = 3$ ) 11.3 (2.4, 52.3) other ( $n = 4$ ) 4.2 (1.8, 9.6) and blanket type: acrylic ( $n = 37$ ) 11.1 (7.5, 16.5); wool ( $n = 29$ ) 15.1 (9.7, 23.4); acrylic and wool ( $n = 3$ ) 7.9 (1.7,

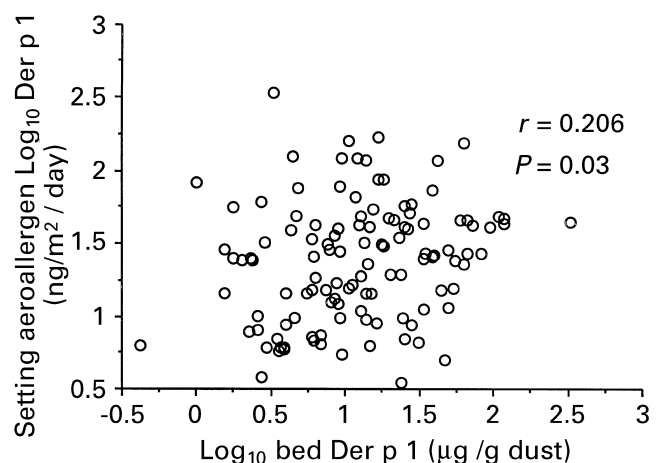


Fig. 2. Relationship between infant bed allergen and settling aeroallergen concentration ( $n = 122$ ).

38.4) and other ( $n = 22$ ) 10.9 (7.2, 16.4). Type of bed and bed cover had no effect on Der p 1 concentration ( $P = 0.6$ ) (Table 1). Infants aged 4–12 months, spent an average of 13 h/d in their own bed (CI 12.4, 13.5, range 0–20 h). Infants aged 0–3 months spent an average of 1 h/d on the floor (CI 0.8, 1.5, range 0–12 h), whereas by 4–12 months, infants spent an average of 5.5 h on the floor (CI 4.9, 6.2, range 0–12 h).

A simple model of relative exposure from beds and floors was calculated by multiplying the time spent by the infant (at age 4–12 months) at each site, by the mean reservoir dust allergen concentration of that site. The results expressed as micrograms Der p 1/g  $\times$  h (95% CI) were: bed, 149.9 (110, 189.1) and lounge floor, 72.4 (59.1, 88.9), with a bed: floor ratio 2.1 (1.6, 2.7): 1. On this basis, the mean bed level exposure was twice that of the floor, and  $\approx 69\%$  of infants aged 4–12 months had the greatest cumulative exposure from the bed.

## Discussion

This study found that infants were exposed to at least one

site with Der p 1 levels exceeding  $10 \mu\text{g/g}$  at an age when those genetically predisposed may be at risk for the development of sensitization and asthma later in life. There was no difference in allergen levels between the infant bed and adult bed in the same home, despite the fact that infants occupy their beds for longer periods. However, Der p 1 concentration was significantly higher in sheepskins than in beds. This confirms that sheepskins may present an exposure hazard to infants at risk of atopy. Tovey *et al.* had reported higher numbers of live mites/site in sheepskins than other types of bedding [14] and Abbott *et al.* also reported higher numbers of live mites/g dust in sheepskins than in foam, kapok and innerspring mattresses [12]. In contrast, Ingham and Ingham found sheepskins to contain lower numbers of live mites/site than infant mattresses, possibly due to the fact that the sheepskins were washed and aired regularly, and that they were newer than the mattresses tested [19]. Although a retrospective study conducted in New Zealand did not identify the use of sheepskins in beds with an increased risk for the development of asthma [20], it would seem unwise to introduce a source of

**Table 1.** Differences in bed Der p 1 concentration between different types of beds, mattresses and bed covers as measured in the whole bed ( $n = 125$ )

| Bedding type   | % Prevalence | Fold difference from reference | Lower 95% CI | Upper 95% CI | P for group | P for comparison with reference |
|----------------|--------------|--------------------------------|--------------|--------------|-------------|---------------------------------|
| Bed type       |              |                                |              |              | 0.6         |                                 |
| Cot            | 93           | *                              | *            | *            |             | *                               |
| Bed            | 7            | 0.78                           | 0.31         | 1.95         |             | 0.6                             |
| Mattress type  |              |                                |              |              | 0.07        |                                 |
| Inner spring   | 76           | *                              | *            | *            |             | *                               |
| Foam           | 19           | 1.51                           | 0.89         | 2.58         |             | 0.12                            |
| Teatree        | 3            | 0.53                           | 0.17         | 1.70         |             | 0.28                            |
| Other          | 2            | 14.63                          | 1.44         | 148.56       |             | 0.02                            |
| Quilt type     |              |                                |              |              | 0.8         |                                 |
| None           | 40           | *                              | *            | *            |             | *                               |
| Duck feather   | 2            | 1.27                           | 0.30         | 5.46         |             | 0.74                            |
| Polyester      | 54           | 1.40                           | 0.89         | 2.20         |             | 0.14                            |
| Other          | 3            | 0.41                           | 0.12         | 1.40         |             | 0.15                            |
| Blanket type   |              |                                |              |              | 0.61        |                                 |
| None           | 27           | *                              | *            | *            |             | *                               |
| Acrylic        | 30           | 1.15                           | 0.64         | 2.07         |             | 0.64                            |
| Wool           | 23           | 1.43                           | 0.76         | 2.68         |             | 0.26                            |
| Wool & acrylic | 2            | 0.93                           | 0.23         | 3.86         |             | 0.92                            |
| Other          | 18           | 1.19                           | 0.61         | 2.32         |             | 0.6                             |

\* Reference category

high mite allergen levels into beds of infants who are at risk of developing sensitization and the attendant long-term sequelae.

The mean settling aeroallergen level in the infants' bedrooms was 24 ng/m<sup>2</sup>/d (equivalent to 0.85 ng Der p 1/d per dish). This value is higher than previously measured in the bedrooms of asthmatic children in the UK (0.44 ng/d) [21]. Settling aeroallergen represents reservoir allergen which has been disturbed and is available to be inhaled, although it cannot be directly compared with allergen per unit volume of air. The observation that reservoir dust and settling aeroallergen were weakly correlated is consistent with other findings [21]. Although reservoir dust is a useful index for community-based epidemiological studies, its variability between sites within a room, the variation in total quantity of dust and in the degree of dust disturbance, make reservoir values difficult to interpret as an index of short-term personal exposure.

In regard to infant bedding, mattress type had a weak effect on Der p 1 concentration as measured in the whole bed, with dust samples collected from beds with foam mattresses containing 50% more allergen than inner spring mattresses. This is in contrast to other studies which have found significantly higher levels in inner spring than foam mattresses [12,22]. Based on this discrepancy, it is difficult to suggest the use, or avoidance of any particular mattress type.

Bed cover and bed type had no effect on Der p 1 levels. Although separate samples were not collected from the mattresses and bed covers, it is plausible to use the whole bed dust measurement to represent these separate components. We have previously demonstrated that allergen levels at three separate sites within a bed are strongly related [13].

From the simple model of relative exposure, it was found that the mean bed exposure level was twice that of the floor, with 69% of infants having the greatest cumulative exposure from the bed. The assumptions made in this model were that exposure is a function of the sum of allergen concentration ( $\mu\text{g Der p 1/g dust}$ ) and time at the location. This finding suggests that during infancy, the bed is an important source of allergen exposure due to both the large proportion of time spent in bed, and their respective high allergen levels. Therefore, it is important to target this area for allergen reduction if the risk of sensitization and development of asthma later in life is to be reduced. Measures to reduce exposure in newborn infants can include providing new mattresses, blankets and top plastic sheets [16], or the use of mite-impermeable mattresses, quilt and pillowcases [23] both of which have been shown to be effective. There has been some concern over the use of polyvinyl chloride (PVC) plastic cover encasements and its association with sudden infant death syndrome (SIDS). However, studies have

concluded that sleeping on a mattress with a plastic cover encasement does not increase the risk of SIDS [24,25].

A recent study has confirmed that the risk of sensitization is a function of allergen levels, and that children with a positive family history of atopy, require less allergen levels to become sensitized at the same rate as those with no atopy in the family [26]. Further, a decrease in sensitization to house dust mites in a population of atopic children who were exposed to low levels of allergen was demonstrated, suggesting that sensitization over time may be transient if only low exposure occurs [27]. In the classic Isle of Wight, UK intervention study of infants at risk for atopy, there was a significant reduction in sensitization to house dust mites with only modest reductions in allergen levels, compared with controls. This was achieved by both reducing allergen exposure and avoidance of food allergens in infancy [28]. However, it should be noted that the pre-treatment levels in these studies were much less than those found in this study.

Therefore, in summary, the high levels of allergen to which these infants were exposed, place them at an increased risk of early sensitization and the development of asthma. Strategies to reduce asthma morbidity must address these high and avoidable levels.

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