House dust mite allergens and allergic diseases - the Wellington Asthma Research Group studies

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Abstract

Allergens produced by house dust mites are known to induce sensitisation in susceptible subjects and in turn sensitisation is associated with the development of allergic asthma. Furthermore, exposure to house dust mite allergens is an established risk factor for exacerbation of allergic asthma.

In this paper we review published studies from the Wellington Asthma Research Group on house dust mite allergens over the last decade. These studies have shown that New Zealand has some of the highest levels of house dust mite allergens in the world with extremely high levels of allergens present in the new born infant’s environment; Tokelau is a virtual house dust mite allergen free environment; synthetic beddings contains significantly higher allergen levels than feather beddings due to their permeability to house dust mites; carpets are a significant reservoir for allergens; and domestic clothes dryers can significantly reduce house dust mites from duvets.

These studies from the Wellington Asthma Research Group have contributed significantly to the international literature on house dust mite allergens and have included novel findings.


Introduction

For centuries house dust has been known to lead to allergy and asthma symptoms in susceptible individuals. The Flemish physician, John Babtista van Helmont in 1662 described such symptoms in one of his patients “...as oft as any place is swept or the wind doth otherwise stir up the dust, he presently falls down, being almost choked”. In the 1960s researchers in the Netherlands and Japan independently showed that house dust mites belonging to the Pyroglyphidae family, namely Dermatophagoides pteronyssinus and Dermatophagoides farinae, were major sources of allergen in house dust (1,2).

Like spiders, house dust mites belong to the class Arachnida. Of at least 50 species of house dust mites that have been found in domestic house dust, the two mites of the family Pyroglyphidae, namely D. pteronyssinus and D. farinae, are the most important in temperate climates, both in terms of numbers and of clinical relevance. Clinically, these house dust mites are of relevance because most asthma symptoms in children and young adults are associated with both an immediate hypersensitivity to their inhaled allergens, and a familial tendency towards atopy (3).

Asthma is a major clinical problem worldwide and there is evidence that both prevalence and severity of asthma are increasing (4,5), although some recent evidence points to a halting or decline in asthma prevalence in some countries (6). In New Zealand, 30% of 13-year-old children are sensitised to house dust mites, and that sensitivity is a significant independent risk factor associated with the development of asthma (7). Thus it is not surprising that increased focus has been directed towards the link between house dust mite allergens and asthma and allergy over the last couple of decades. Indeed, a Medline search from 1985 to 2005 returned 2,139 articles on house dust mites, an average of about two articles per week.

House dust mites are about 1/4 to 1/3 mm long, are eight legged, have no eyes, and have no developed respiratory structures. External temperature controls their body temperature, and their moisture requirements are regulated by passive and active mechanisms, mainly through saturated KCl and NaCl channels that extract water from moist air and prevent body moisture loss during low humidity conditions (8). Figure 1 shows an adult D. pteronyssinus which is the dominant mite species found in New Zealand homes (9), although we have found evidence of allergens from D. farinae in New Zealand, but at much lower levels than those from D. pteronyssinus (10).

The house dust mite D. pteronyssinus produces at least seven major allergens (and another 7 minor allergens) officially recognised by the WHO/IUIS Allergen Nomenclature Subcommittee (11). The major group one allergen, Der p 1, has been the most studied allergen of this house dust mite over the years. It is a 25 Kda protein showing homology with cysteine proteases (12). Very large quantities of Der p 1 are found in house dust mite faeces, its nature reflecting the digestive processes of house dust mites through the enclosure of food and cell debris by a peritrophic membrane (13). House dust mite allergens are widely distributed in the domestic environment. They are found abundantly in carpets, furniture, mattresses, pillows, duvets, blankets, curtains, soft toys, clothing, and are even detectable in human hair (14), on human skin (15), and in cord blood (16).

Over the years, studies have consistently shown that sensitisation to house dust mite allergens is strongly associated with asthma in children and adults (17,18), that the development of atopy and asthma follows early childhood exposure (19), strict avoidance to house dust mite allergens reverses asthma symptoms (20,21), and that there is a dose-response relation between Der p 1 exposure and the severity of asthma in house dust mite sensitised children (22).
To prevent predisposed infants from developing atopy and subsequently asthma, and to reduce symptoms in house dust mite sensitised asthmatics, various techniques have been trialled to kill house dust mites and to reduce their allergens in domestic sites, with mixed results. These techniques have included reduction of indoor humidity by mechanical ventilation and heat recovery (23,24), chemical treatment of carpets, bedding and furniture with acaricides such as benzyl benzoate and tannic acid to denature allergens (25,26), intensive vacuum cleaning (27), use of barrier covers on bedding (28,29), exposure to direct sunlight (30), super-heated steam cleaning (31), liquid nitrogen (32), washing and dry cleaning of bedding (33), and hot tumble drying of duvets (34).

In this article we review studies undertaken and published by the Wellington Asthma Research Group on house dust mite allergens over the last decade. These studies have contributed significantly to the international literature and have included novel new findings.

**Methodological aspects**

Early methods for quantification of Der p 1 (originally termed antigen P₁) included radio allergen sorbent inhibition, counter immuno-electrophoresis and radioimmunoassay (35). These techniques were useful in assaying mite allergen levels in areas where *D. pteronyssinus* is the dominant mite species, but not where *D. farinae* is dominant, due to partial cross-reactivity between Der p 1 and Der f 1 (the major group one allergen of *D. farinae*). In order to overcome cross-reactivity with the major group two allergens of the *Dermatophagoides* spp., double-monoclonal antibody radioimmunoassays specific for Der p 1 and Der f 1 were developed and subsequently adapted to ELISA (36).

House dust mite allergen detection and quantification by ELISA has the advantage of being a non-isotopic immunosassay readily adaptable by most laboratories for use in epidemiological and interventional studies of house dust mite allergens and is the method of choice by the majority of research laboratories active in this field. An American-based company, Indoor Biotechnologies (Charlottesville, Virginia, USA) is the major manufacturer of these double-monoclonal antibody ELISA kits for the detection of Der p 1 and Der f 1. Additionally, the company also produces ELISA kits for the detection of *Dermatophagoides* group two allergens (from both *D. pteronyssinus* and *D. farinae*) and for the detection of the major allergen from the tropical house dust mite, *Blomia tropicalis*, which is the dominant mite species in tropical and semi-tropical areas (37). The Second International Workshop on House Dust Mite Allergens and Asthma set threshold levels for Der p 1 of 2 μg/g dust and 10 μg/g dust respectively for sensitisation in predisposed infants and acute exacerbation of asthma (38) although sensitisation can develop at levels as low as 0.5 μg/g dust (39).

House dust samples are extracted and the allergen quantified by double-monoclonal ELISA. In the 1990s, at the request of the Third International Workshop on Indoor Allergens and Asthma (12) we set up an external quality control program for Der p 1. After two rounds, in which two dust samples were sent to 23 laboratories worldwide, a six to seven-fold variability in Der p 1 results was apparent (40). In round three we sent six dust samples with a wide range of Der p 1 concentrations to these laboratories to test whether mean results from multiple samples were similar, or not. Results from this round re-confirmed the wide variability in results (41). This would imply that comparing Der p 1 levels between laboratories might not be valid. It also became apparent that laboratories extracting in the cold returned lower Der p 1 results than those extracting at room temperature. There also seemed to be differences in results depending on the buffer being used for extraction. This led us to study the effects of temperature and buffers on the extraction of Der p 1 from house dust (42). That study showed a mean reduction in Der p 1 levels from dust extracted at 4°C of 57.9% compared to room temperature extracts, and dust extracted with borate-buffered saline had Der p 1 values approximately twice the levels from phosphate-buffered saline or ammonium bicarbonate buffer, independent of pH (between 7.0 and 8.5) or ionic strength. Thus, when comparing results from different research centres, differences in extraction techniques must be taken into consideration.

Dust for house dust mite allergen analysis is usually collected by vacuuming. In order to present results as both an amount of allergen per amount of dust and a concentration per unit area (recommended for exposure assessment), standardised collection techniques are required. We usually collect dust samples by vacuuming a 1 m² area for 1 minute using the same brand vacuum cleaner for all studies. As uncarpeted floors generally result in small quantities of dust, we sample a 2 m² area from smooth floors. For irregular surfaces, such as furniture or pillows, the whole item is vacuumed for a set time, but for these items Der p 1 results can only be expressed as absolute concentrations but not as a concentration per unit area.

A previous study demonstrated a wide variation of Der p 1 levels within living room and bedroom floors (43). This has implications for epidemiological studies as normally a central area within the room is sampled with the assumption that this is representative of the whole room. As that study was conducted in the UK, where Der p 1 levels are generally 20-fold lower than in New Zealand, we determined the variability of Der p 1 (and of the cat allergen, Fel d 1) in domestic living room floors in Wellington (44). Mean coefficient of variation for Der p 1 from living room floors was 53.1% (range: 28.5-136.8), therefore a single sample from a floor is not suitable to assess an individual’s exposure risk but is representative of the whole room in large-scale epidemiological studies.

Vacuumed dust samples frequently contain larger particles of numerous origins; therefore dust samples are normally sieved before analysis. We sieve through a 425 μm steel mesh sieve to obtain fine dust. When collecting dust from pillows, normally only small quantities of fine dust are obtained. We therefor determined the effects of sieving on Der p 1 levels (45). Dust was collected from 24 living room floors and 24 mattresses, the dust samples split into two lots with one lot being sieved. Bland-Altman plots showed that, although yielding slightly lower Der p 1 levels, not sifting did not result in empirically different Der p 1 levels. Sieving dust takes time and may be an additional factor contributing to the total inaccuracy inherent in Der p 1 measurement.

As well as differences in sampling times and areas sampled, and use of vacuum cleaners with different wattage, there are also various dust collection devices in use that may influence dust yield and thus Der p 1 levels. We use a nylon mesh bag (25μm pore, developed by Dr. E. Tovey, Sydney) inserted between the vacuum hose and the vacuum cleaner furniture attachment to collect dust. We are also involved in the International Study of Allergies and Asthma in Childhood (ISAAC) where for phase 2 centres around the world are collecting dust in a standardised manner using dust collection devices (ALK, Copenhagen, Denmark) consisting of a filter dish pre-loaded with 70 mm Whatman no.4 25μm filter paper. We collected duplicate dust samples from 37 carpeted living room floors and from longitudinal halves of 37 mattresses with the two dust collection devices using the same vacuum cleaner. These dust samples were analysed for Der p 1 (as well as for...
cat allergen and bacterial endotoxin). Results showed that the use of nylon mesh bags resulted in more dust and thus higher absolute concentrations of Der p 1 than with the ALK device (46). Floor Der p 1 levels, expressed as μg per gram of dust, were also significantly higher using nylon mesh bags, but this was not so for mattress samples. Thus, in order to have confidence that the comparison of Der p 1 levels between centres is valid, not only Der p 1 analysis methodology, but also the standardisation of dust sampling equipment is essential.

Although double-monoclonal antibody ELISA remains the gold standard for measuring Der p 1, it is essentially a research tool and relatively expensive due to labour intensive collection and preparation of the dust samples. Various simple ‘dipstick’ semi quantitative systems have been developed to allow homeowners to test their house dust for house dust mite and other allergens. One such system has recently been developed by Indoor Biotechnologies, the major manufacturer of the ELISA Der p 1 research kit. It is a simple rapid test (30 to 60 min) using lateral flow technology and gold-labelled monoclonal antibodies to Der p 2, and can thus detect allergens from both D. pteronyssinus and D. farinae. We took part in a multi-centre evaluation of this rapid test. Archived dust samples (n=349) from homes in nine centres from eight countries (the Netherlands, USA, Brazil, Sweden, France, UK, Australia, New Zealand) were analysed for group 1 and group 2 house dust mite allergens and also compared with the rapid test (47). Significant correlations were obtained between Der p 1 and Der f 1 with Der p 2, with the strongest correlation from New Zealand. Significant differences were obtained between Der p 2 levels and the three rapid test scores (negative to low; low to medium; medium to high). The rapid test also showed a low rate (3.15%) of false negative reactions. The rapid tests also contain a convenient sampling and extraction device, allowing allergic patients to simply and rapidly test for house dust mite allergen exposure in their homes. The company has now developed ‘credit card’ systems using the lateral flow technology and gold-labelled antibodies for simultaneously detecting allergens from house dust mites, cat, dog and cockroach.

House dust mite allergens and the indoor environment

As previously mentioned, house dust mite allergens in the domestic environment can come from many sources. Over the years we have conducted a number of studies of house dust mite allergens in the indoor environment in New Zealand. Our first major study involved measuring Der p 1 on living room and bedroom floors and on mattresses of 474 children in Wellington (48). This study showed that Wellington has some of the highest house dust mite allergen levels in the world, and that higher floor Der p 1 levels were associated with older carpets and the presence of more than two children. Bedding Der p 1 levels were higher in beds with kapok and inner sprung mattresses, those with woollen under layers, and high relative humidity on the mattress. Reducing exposure to the very high levels of house dust mite allergen in New Zealand will be a major challenge, perhaps the most important being the removal of carpets and occlusive covering of bedding.

Having determined that carpets, especially older carpets, were the most and important determinant of floor Der p 1 levels, we set out to determine which housing characteristics explain Der p 1 variability. We re-sampled a subset of these houses with carpeted living room floors and selected those with the highest and lowest Der p 1 levels (49). The main findings were that lower levels of Der p 1 were associated with floor insulation, a thick layer of underlay, and the presence of more than two children. Also, although previously higher, Der p 1 levels had not significantly changed over a four-year period.

Having determined the levels of house dust mite allergens in the indoor domestic environment in Wellington, we were interested to see what the levels were in other indoor environments in New Zealand. In collaboration with the Canterbury Respiratory Research Group (Christchurch) we collected floor, bed and seat dust samples from hotels, hospitals, rest homes primary schools, child care centres,
House dust mite allergens and the bed room

We spend approximately one third of our lives in bed. Given its close proximity to the airways, bedding and mattresses are important sources of house dust mite allergen exposure. As exposure to Der p 1 is an important determinant of allergic sensitisation in the first year of life, we measured Der p 1 levels in infant bedding in Wellington. Bedding dust samples were collected from 154 newborn infants at a mean age of 11 weeks and again at a mean age of 15 months (51). At 11 weeks bedding Der p 1 levels were high, being approximately 10-fold higher than levels reported in other countries, and these levels increased significantly at 15 months. Bedding that included a sheepskin (used by a third of the infants) was associated with the highest levels of Der p 1. Thus in New Zealand newborn infants are exposed to levels of Der p 1 much higher than has been associated with sensitisation and exacerbation, and given these high levels it is not surprising that asthma in New Zealand is common, severe and dominated by house dust mite allergy.

It has been known for some time that sheepskins harbour house dust mites (52,53). Given the high levels of Der p 1 on sheepskins, we were interested in seeing how quickly new sheepskins accumulate house dust mite allergens, and the effectiveness of both washing and dry cleaning on its removal (54). Newly bought sheepskins were placed in the domestic environment (floors and mattresses) and monitored for Der p 1 accumulation over six weeks. They were then warm-washed, sampled for dust, returned to the same floors and mattresses for a further six weeks and then dry-cleaned and re-sampled. Der p 1 levels rose rapidly over time, while warm washing and dry cleaning reduced sheepskin Der p 1 levels by 79.2% and 95.3% respectively. Thus, sheepskins rapidly accumulate house dust mite allergens and should be discouraged as infant bedding for those at risk of developing sensitisation. If used, they should at least be regularly washed or dry-cleaned.

House dust mite allergens, and synthetic and feather bedding

For many years allergic patients with asthma, rhinitis and eczema have been advised to avoid feather pillows on the assumption that these are a source of large amounts of house dust mites as a great number of mites are found on bird feathers (55). Indeed, *D. farinae* means feather loving. However, modern pillow manufacturing processes ensure that through superheat steaming of feathers used for pillows and duvets, no mites survive and their allergens are denatured (56). In 1995 a study showed that the use of synthetic pillows was a significant indoor environmental factor associated with severe asthma in children, while feather pillows appeared to be protective (57). The authors hypothesised that synthetic pillows may release organic volatile compounds that would adversely affect the airways of children. We hypothesised that their findings could be due to differences in house dust mite allergen levels between different types of pillows. We set out to test this by measuring Der p 1 levels on pillows and collected dust from pairs of synthetic and feather pillows placed on the same bed for more than six months (58). To our surprise we found seven to eightfold higher levels of Der p 1 on synthetic, compared to feather pillows. Our findings, though interesting, were met with some scepticism in the research community. However, our English colleagues, at our urging, found the same applied in the UK, where house dust mite allergen levels are about 20-fold lower than in New Zealand (59). They also found that feather pillows contained much lower levels of cat and dog allergens than synthetic pillows.

We then set out to determine at what rate new synthetic and feather pillows accumulate house dust mite allergens. We placed 12 pairs of pillows, each pair consisting of a feather and synthetic pillow, on 12 mattresses and collected dust regularly over a 12-month period (60). After 12 months synthetic pillows contained on average five times as much Der p 1 than feather pillows, confirming our previous results (58) and those from the UK (59). The accumulation rate of Der p 1 on both types of pillows is governed by the environment, as there was a significant correlation between pillow Der p 1 accumulation and Der p 1 levels of the mattresses they were placed on. These findings attracted several leading Editorials/Reviews, including from our research group (61-63).

We hypothesised that the differences in allergen levels between pillows could be related to the weave of the covers. We had previously noted that the weave of the covers on feather pillows was much tighter than those on synthetic pillows (64). When contacted, a leading New Zealand manufacturer said the reason for the tighter weave of feather pillow covers was to keep the feathers inside the pillows. We therefore undertook a study to determine the permeability of feather and synthetic pillow covers to house dust mites and house dust (65). We seeded 20 live adult house dust mites on top of feather and synthetic pillow covers with adequate food supply underneath in sealed culture dishes kept at room temperature and high humidity. After 24 h all mites had penetrated the synthetic pillow covers while after 48 h no mites had penetrated the feather pillow covers. Dust permeability of the synthetic pillow covers was more than 12 times greater than that of the feather pillow covers. We believe these results provide a convincing explanation for differences in house dust mite allergens between feather and synthetic pillows.

The use of synthetic duvets has increased significantly over recent years. As the majority of these in use in New Zealand are synthetic, we undertook a study to look at house dust mite allergen levels in individual bedding items. We collected dust samples from 65 duvets, 81 pillows and 65 mattresses from 34 children and 31 adults in Wellington (66). As well as again showing that synthetic pillows contained much higher Der p 1 levels than feather pillows, synthetic duvets also contained much higher Der p 1 levels, 15-fold higher than feather duvets. This study, published in the *New Zealand Medical Journal* attracted intense media attention with newspaper articles throughout the country, radio interviews, and the lead story on TV 1.

Two further pillow studies were undertaken. The first one was in conjunction with Korean colleagues. In Korea, buckwheat pillows are commonly used. We determined house dust mite allergen accumulation on new buckwheat and synthetic pillows in Korea. As the dominant house dust mite species in Korea is *D. farinae* we measured the major group one allergen, Der f 1, from this mite species. We found no difference in Der f 1 accumulation between the two different pillow types but the amount of endotoxin (from Gram-negative bacteria) from buckwheat pillows was approximately 12-fold higher compared to synthetic pillows (67). Thus buckwheat pillows may affect asthma severity as endotoxin is a pro-inflammatory compound that exacerbates asthma in house dust mite sensitised asthmatics (68).
In another study we determined the effects of lavender on house dust mite allergens in pillows as lavender oil is known to be acaricidal (69). Six new pairs of pillows, each pair consisting of identical synthetic pillows with and without a lavender sachet inside were placed on mattresses and Der p 1 accumulation studied over three months. Der p 1 accumulation was similar between the two types of pillows; thus, addition of lavender sachets (which smells nice and is supposed to have a soothing effect) inside pillows is unlikely to be a beneficial house dust mite reduction measure.

**House dust mite allergens in other environments**

House dust mite allergens are distributed throughout the home with the main exposure from floors and bedding. However, there are potentially other exposure routes. Tovey et al previously reported that adult clothing was an unrecognised source of allergen exposure (71). We studied house dust mite allergen levels of upper garments worn by 166 school children in Wellington (72). Although levels of Der p 1 were lower in clothing than normally found in the indoor environment, some still had levels associated with the exacerbation of asthma. Interestingly, we found that girls clothing had higher levels of Der p 1 than boys clothing. Perhaps boys clothing is washed more frequently or girls spend more time in direct contact with house dust mite allergen laden carpets and bedding. Also, the type of garment influenced Der p 1 levels, with woollen garments containing the highest levels. Thus house dust mite sensitised asthmatics should perhaps avoid woollen garments.

A report from Brazil suggested that human heads were an unrecognised reservoir of house dust mites (73). Their published data reported the number of mites per gram of vacuumed scalp dust. As the amounts of dust collected from scalps was small, we calculated that there was on average two mites per scalp, unlikely to be a major source of house dust mite allergens. We tested this out by collecting vacuumed dust from hair of 16 adults from our research group (14). House dust mite allergens were present in these samples, but at very low levels and thus hair has is unlikely to be a significant source of house dust mite allergen exposure.

Subjects with atopic dermatitis are frequently sensitised to house dust mites and reduction of exposure to its allergens in bedding has been shown to be of benefit (74). We wondered whether there were significant amounts of house dust mite allergen on the skin surface after rising from bed. We asked 25 subjects to vacuum their entire body after getting up and shedding any bedclothes, and also collected dust from their bedding (15). We found that, although the levels of Der p 1 on skin were low, these levels are known to elicit skin responses in atopic dermatitis. The major determinant of skin Der p 1 was bedding Der p 1.

House dust mites need a temperate climate and relative humidity levels of above 45% to proliferate and survive. This is why house dust mite allergen levels are very high in the temperate climates of New Zealand and Australia, compared to colder winter climates such as in the Netherlands (48, 75-76). We were interested to see if house dust mite allergens could be detected in the Antarctic, where outdoor relative humidity rarely rises above 20% and thus is not conducive for house dust mite survival. We hypothesised that any detectable allergen levels there would most likely to have been introduced passively from clothing. We obtained dust samples from the clothing of 11 recently arrived Scott Base personnel as well as from their mattresses and three living room areas (77). House dust mite allergens were undetectable in the living room areas and in all but one mattress (at a very low level). Six of the 11 sweaters from the Scott Base personal had detectable Der p 1 levels, albeit at low concentrations. This study confirmed passive transfer of house dust mite allergens in an area devoid of sustainable house dust mite populations. Interestingly, we also found significant levels of passively transferred cat allergen in the Antarctic, an area totally devoid of cats.

Many university students live in low cost, poorly maintained rental accommodation or in halls of residence with no choice of the type of bedding or floor coverings. We were interested to see whether university students were exposed to higher levels of house dust mite allergens. Dust samples were collected from bedroom floors and mattresses of 178 1st year students at the University of Otago in Dunedin (78). Student accommodation was grouped into family homes (n=61), student flats (n=43) and halls of residence (n=74). The highest levels of Der p 1 were from family homes, followed by student flats and halls of residence. The lowest levels found in halls of residence possibly is due to regular cleaning and washing of bedding in this type of student accommodation, and the vacuum of these premises for three month over summer. Domestic Der p 1 levels in Dunedin were lower than in Wellington. One reason for this could be the colder winter temperatures in Dunedin affecting house dust mite populations there. In support of this, Der p 1 levels in Christchurch (warmer than Dunedin but colder than Wellington in winter) are intermediate between those from Wellington and Dunedin (79). We also have unpublished data showing a concentration gradient of Der p 1 in New Zealand, with highest levels in the north (Kaitaia), progressively declining towards the south (Invercargill).

A large percentage of Tokelauans now live in New Zealand through migration, particularly following hurricane damage to the Tokelau atolls in 1966. Asthma is rare in Tokelauans having lived all their lives in Tokelau while Tokelauans resident in New Zealand acquire atopie diseases, including asthma, at the same rate as New Zealanders (80). We hypothesised that the low rate of atopie diseases among native Tokelauans may be associated with low indoor allergen levels in Tokelau as the three small atolls sustain little fauna and flora, domestic cats and dogs were eradicated in the 1950s and Tokelauan homes have unglazed windows and uncarpeted floors. We therefore measured indoor floor and bedding allergens (house dust mites, dog, cat, cockroach and endotoxin) in 76 Tokelauan homes and compared these to homes of 30 Tokelauan families resident in Wellington (10). We found that house dust mite allergen levels in Tokelau were over 1000-fold lower compared to New Zealand. Dog and cat allergens were also significantly lower in Tokelau while cockroach allergens were very low in both locations. Thus, Tokelau is a natural low allergen environment at sea level that could explain the low prevalence of asthma and atopy on these atolls. Tokelau provides a unique natural environment to study secondary allergen avoidance among those with established atopie diseases. We are currently studying the effects of ‘back migration’ of atopie Tokelauans on asthma symptoms and severity.

**Intervention studies to reduce house dust mite allergens**

As sensitisation to house dust mites is associated with the clinical activity of asthma, it would seem logical that reducing exposure to house dust mite allergens would be of benefit clinically. Many studies have supported this assumption, although some have disputed this. We have conducted four studies looking at ways of reducing house dust mite allergens in the indoor environment. The first study looked...
at the effects of frequent carpet vacuum cleaning on house dust mite allergens. In this study nine bed rooms and three hallways in the residents medical officers quarters at Wellington Hospital were vacuumed daily (except weekends) for five weeks (27). Der p 1 levels expressed per unit area decreased on average by 68.5% after five weeks. We went back five weeks after completion of the daily vacuuming phase of the study and collected new dust samples. Der p 1 levels of these samples were higher than those at the start of the study. Thus, daily vacuum cleaning has the potential to significantly reduce house dust mite allergens in carpets, but returning to less regular vacuum cleaning causes this beneficial reduction to be abolished.

One potentially useful method to control house dust mite proliferation and thus allergen production is reduction of relative humidity in the indoor environment. Indeed, a study in Denmark showed that the use of mechanical ventilation and heat recovery was sufficient to significantly reduce absolute humidity and thus reduce or even eliminate house dust mites in mattresses (81). However, outdoor humidity in Scandinavian countries is much lower due to cold and dry winters and houses there are well constructed and air tight. This is not the case in other countries, such as in the UK where a study found no benefit of mechanical ventilation on house dust mite numbers and allergen levels (82). Given the generally poorly constructed houses in New Zealand and the very high house dust mite allergen loading here, we studied the effects of mechanical ventilation and heat exchange on house dust mite numbers and allergen levels. The study was conducted in Miramar, Wellington where thirty similar homes and their occupants were enrolled (24). The homes were split into three groups. Group A had mechanical ventilation and heat exchange units installed, they were insulated and draught proofed, and electric night store heaters were installed. Group B had identical insulation and draught proofing only, while group C was the control group with no interventions. The homes were monitored regularly for more than a year, and relative humidity, temperature, air exchange, house dust mite numbers and allergen levels recorded. Despite an overall reduction in relative humidity and an increase in temperature in the mechanically ventilated homes, this was not followed by reduction in either house dust mite numbers or in Der p 1 levels. Thus, mechanical ventilation and heat exchange is unlikely to have a significant impact and is not recommended for house dust mite and allergen reduction in New Zealand.

Duvets are widely used as bedding in New Zealand and contain high levels of house dust mite allergens (66). As the thermal death point for the house dust mite, *D. pteronynsinus* is 56°C we wondered whether domestic clothes dryers would be able to reach this temperature and kill house dust mites in duvets. Eight duvets, which had not been washed or dry cleaned for at least six weeks, were studied (34). Live house dust numbers were estimated by a heat exchange method (83) and dust obtained by vacuuming for Der p 1 analysis. The duvets were then individually tumble-dried for one hour with a data logging device recording temperature and relative humidity at eight-second intervals. After tumble-drying, the duvets were again assessed for live house dust mites and Der p 1 levels. Substantial numbers of live house dust mites (mean: 410/m²) were found in the duvets and these were significantly reduced after tumble-drying (mean: 6/m²). A mean maximum temperature of 59.3°C was reached during the 1 hr drying period and the mean time to reach the thermal death point was 22 min. House dust mite allergen levels were not significantly changed by the tumble-drying. Thus, tumble-drying with domestic clothes dryers is an easy and effective method of house dust mite reduction. Advice to atopic asthmatics is to first tumble-dry the duvet followed by cold water washing to remove the water-soluble allergens.

Atopic asthmatics are often advised to cover all bedding with moisture permeable, but house dust mite excluding covers as these substantially reduce exposure to house dust mite allergens (84). However, little is known of the effects of occlusive covers on airborne house dust mite allergens. Airborne Der p 1 cannot be quantified using the established double monoclonal antibody ELISA due to their very low levels, however, Japanese researchers have developed a highly sensitive fluorimetric ELISA for air Der p 1 quantification (85). In conjunction with these Japanese researchers we set out to study the effects of occlusive bedding covering on airborne house dust mite allergen levels (28). Mattresses, pillows and duvets of 12 subjects were fitted with occlusive covers and dust samples were collected before, and one week after covering. Air samples were obtained with personal air samplers operating at 1 l/min over seven consecutive nights when the covers were in place, and for a similar period after their removal. We showed that bedding covers significantly reduced house dust mite allergens on each bedding type (5-fold, 16-fold and 76-fold respectively for pillows, duvets and mattresses). The reduction in air Der p 1 levels was more modest, a 6-fold decrease. As airborne Der p 1 may be more relevant for exposure assessment, further studies are required to determine if this modest reduction results in clinical improvement.

**House dust mite allergens and allergic diseases**

In house dust mite sensitised asthmatics, levels of Der p 1 predict the severity of symptoms (86). However, the link between house dust mite allergen exposure and prevalence of asthma within individual countries is debatable. We undertook a cross-sectional study of seven countries in the Asia-Pacific region (India, Hong Kong, Malaysia, Thailand, Japan, Chile and New Zealand) to determine whether exposure to different levels of house dust mite allergens in these countries was associated with prevalence of atopic diseases and asthma symptoms (87). Mattress and living room floor dust samples were collected from about 36 children in each of 10 centres in these seven countries and analysed for the house dust mite allergens Der p 1 and Der f 1. An ecological analysis was then conducted of allergen levels against asthma symptoms and severity data from the International Study of Asthma and Allergies in Childhood study (88). This study showed that asthma symptom prevalence and having at least four attacks of asthma was associated with house dust mite allergen (Der p 1 + Der f 1) levels. There is a need for further international prospective infant studies in centres with variable house dust mite allergens levels.

We undertook a study to examine the relationship between the indoor environment, atopy and asthma in 233 seven to nine-year old children diagnosed with asthma and 241 control children (89). Living room dust samples were collected for Der p 1 level, indoor exposure information (during the first year of life and currently) collected by questionnaires, and the children were skin prick tested for common indoor and outdoor allergens. Sensitisation to house dust mite allergens was independently associated with current asthma but current Der p 1 levels were not. Furthermore, use of sheepskins and exposure to carpets in the first year of life were also independently associated with current asthma. Thus, exposures in infancy may be more important than current exposure in explaining the prevalence of asthma in childhood. We are currently exploring early life exposures and development of allergic diseases in a multi-centre prospective infant cohort study.

As previously mentioned, there is evidence that both the prevalence and the severity of allergic diseases are increasing (4,5). Strachan
proposed that this may be due to reduced exposure to microbes (90), thus switching the immune system from predominantly Th1 to the allergic Th2 pathway, the so called “hygiene hypothesis” (91). Recently, various studies have shown that being born on a farm is protective for the development of allergic diseases (92, 93) and this protection seems to be mediated by exposure to higher amounts of endotoxin from Gram-negative bacteria (94). As farming practices differ significantly between New Zealand and Europe, we undertook a study to see if a similar protective effect of farm living was apparent for New Zealand children (95). We studied 494 children living in suburban Dannevirke or on farms in the surrounding region. Allergic symptoms and exposure information was collected by questionnaires, living room floor dust samples were collected for house dust mite allergen and endotoxin levels, and the children were skin prick tested to common allergens.

We found that currently living on a farm was associated with a greater prevalence of allergic diseases, but early life exposure to yoghurt and unpasteurized milk consumption, cats, dogs, and pigs was associated with a reduced prevalence of allergic diseases. We also found higher levels of Der p 1, but lower levels of endotoxin on farms. Levels of Der p 1 were not associated with any outcome variable studied.

Not only house dust mite sensitisation, but sensitisation to the cat allergen Fel d 1 is also associated with asthma. Lately, intriguing findings have suggested that early life exposure to cats is associated with a reduced prevalence of atopic sensitisation (96). This is thought to be due to a modified Th2 response where, instead of producing cat-specific IgE when living with cats, children produce increased levels of IgG and IgG4 antibodies to cat allergen (97). We were also interested in seeing whether this was true for New Zealand with its high cat ownership rate and high levels of cat allergen (51). We also were interested in seeing whether a similar tolerance occurs with exposure to high levels of house dust mite allergens. We collected blood, and mattress dust samples from 112 wheezing and 112 control children in a nested case-control study in Hawkes Bay (98). Serum samples were analysed for specific IgE as well as IgG and IgG4 to house dust mite and cat allergens by colleagues in the USA. The dust samples were analysed for house dust mite and cat allergens and endotoxin. As expected, having a resident cat was associated with higher levels of cat allergen in the indoor environment. We found that children who had ever lived with a cat were less likely to have cat-specific IgE, but there was no similar effect on house dust mite-specific IgE. Interestingly, in those sensitised, cat-specific IgE levels were 10-fold lower than house dust mite-specific IgE levels. These results suggest that tolerance to cat allergen is an allergen-specific phenomenon and the strong IgE antibody response to house dust mites could contribute to the high prevalence and severity of asthma in New Zealand.

Conclusions

Levels of house dust mite allergens in New Zealand are high and most likely contributes to the high prevalence and the severity of asthma here. Over the last decade the Wellington Asthma Research Group has focussed on studying the distribution and risk factors for house dust mite allergens, and its association with asthma and allergic diseases. These studies have shown that New Zealand has some of the highest levels of house dust mite allergens in the world with extremely high levels of allergens present in the new born infant’s environment; Tokelau is a virtual house dust mite allergen free environment; synthetic high levels of allergens present in the new born infant’s environment; Tokelau is a virtual house dust mite allergen free environment; synthetic high levels of allergens present in the new born infant’s environment; Tokelau is a virtual house dust mite allergen free environment; synthetic high levels of allergens present in the new born infant’s environment; Tokelau is a virtual house dust mite allergen free environment; synthetic high levels of allergens present in the new born infant’s environment; Tokelau is a virtual house dust mite allergen free environment; synthetic high levels of allergens present in the new born infant’s environment; Tokelau is a virtual house dust mite allergen free environment; synthetic high levels of allergens present in the new born infant’s environment; Tokelau is a virtual house dust mite allergen free environment; synthetic high levels of allergens present in the new born infant’s environment; Tokelau is a virtual house dust mite allergen free environment; synthetic high levels of allergens present in the new born infant’s environment; Tokelau is a virtual house dust mite allergen free environment; synthetic high levels of allergens present in the new born infant’s environment; Tokelau is a virtual house dust mite allergen free environment; synthetic high levels of allergens present in the new born infant’s environment; Tokelau is a virtual house dust mite allergen free environment; synthetic high levels of allergens present in the new born infant’s environment; Tokelau is a virtual house dust mite allergen free environment; synthetic high levels of allergens present in the new born infant’s environment; Tokelau is a virtual house dust mite allergen free environment; synthetic high levels of allergens present in the new born infant’s environment; Tokelau is a virtual house dust mite allergen free environment; synthetic high levels of allergens present in the new born infant’s environment; Tokelau is a virtual house dust mite allergen free environment; synthetic high levels of allergens present in the new born infant’s environment; Tokelau is a virtual house dust mite allergen free environment; synthetic high levels of allergens present in the new born infant’s environment; Tokelau is a virtual house dust mite allergen free environment; synthetic high levels of allergens present in the new born infant’s environment; Tokelau is a virtual house dust mite allergen free environment; synthetic high levels of allergens present in the new born infant’s environment; Tokelau is a virtual house dust mite allergen free environment; synthetic high levels of allergens present in the new born infant’s environment; Tokelau is a virtual house dust mite allergen free environment; synthetic high levels of allergens present in the new born infant’s environment; Tokelau is a virtual house dust mite allergen free environment; synthetic high levels of allergens present in the new born infant’s environment; Tokelau is a virtual house dust mite allergen free environment; synthetic high levels of allergens presented in the new born infant’s environment.

Acknowledgements

We wish to thank current and past members of the Wellington Asthma Research Group for their valuable input in our studies. Our studies mentioned in this article have been supported by grants from the Health Research Council of New Zealand, Lotteries Health New Zealand, the Asthma and Respiratory Foundation of New Zealand, the Child Health Foundation of New Zealand, the Wellington Medical Research Foundation, the Marjorie Barclay Trust, and Otago University. We thank the Electricity Corporation of New Zealand for establishment of the allergen laboratory.

This article is dedicated to the late Juliette Lane, a former valued colleague, friend and member of the Wellington Asthma Research Group.

References

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1. Appendix

The Wellington Asthma Research Group house dust mite allergen publications.

Methodological aspects

House dust mite allergens and the indoor environment

House dust mite allergens and the bed room
- Siebers RW, Lane JM, Crane J. Lavender in pillows. No effect on Der p 1 accumulation. *Allergy* 2004; 59: 231-2.

House dust mite allergens, and synthetic and feather bedding

House dust mite allergens in other environments

Intervention studies to reduce house dust mite allergens

House dust mite allergens and allergic diseases

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