

Domestic allergens in public places II: dog (Can f 1) and cockroach (Bla g 2) allergens in dust and mite, cat, dog and cockroach allergens in the air in public buildings

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Summary

Background Sensitization and exposure to indoor allergens are the major risk factors for asthma. It is possible that significant exposure to domestic allergens occurs outside the home.

Objectives To investigate the levels of Can f 1 and Bla g 2 in the dust from carpeted floors and upholstered seats in public buildings and public transport and the airborne concentrations of Der p 1, Fel d 1, Can f 1 and Bla g 2 in schools and offices.

Methods Can f 1 and Bla g 2 were measured in the dust collected by vacuuming a 1 m² area of carpet, as well as upholstered seats in five schools, six hotels, four cinemas, six pubs, three buses and two trains. Dust was also collected from the bedroom carpet, living room carpet, mattress and sofa in 20 homes with and 20 homes without a dog in the same area. Personal airborne sampling (2 L/min) was conducted for 8 h in offices ($n=16$) and classrooms ($n=9$). In addition, airborne samples in schools were collected using a high volume pump (60 L/min) for 1 h in three classrooms immediately after the children vacated the school. Can f 1, Bla g 2, Der p 1 and Fel d 1 were assayed using a two-site monoclonal antibody-based ELISA.

Results Can f 1 was detected in all dust samples from public places, ranging from 0.2 to 52.5 µg/g. Significantly higher levels were found in upholstered seats (geometric mean – GM 9.4 µg/g) than in carpets (GM 1.5 µg/g; $P<0.001$), and levels of Can f 1 >10 µg/g were found in 40% of upholstered seats in public places. Can f 1 was significantly higher in upholstered seats in public places than in sofas in homes without a dog (GM 1.8 µg/g; $P<0.001$). Detectable levels of Bla g 2 were found in all of the schools (GM 2.4 U/g, range 0.8–4.4 U/g). Bla g 2 concentration greater than 2U/g (provisional threshold level representing risk of sensitization) was measured in 65% of the classrooms sampled. Der p 1 and Bla g 2 were below the detection limit in all airborne samples. However, airborne Fel d 1 and Can f 1 were detected in schools and offices, albeit in low concentrations.

Conclusions Upholstered seats from public places constitute a reservoir for the accumulation of dog allergen, and a source of exposure to Can f 1 inside public buildings or on public transport. Exposure to cockroach allergens in schools may be important for cockroach sensitized asthmatic children.

Keywords: asthma, domestic allergens, Can f 1, Bla g 2, Der p 1, Fel d 1, airborne, reservoir, public places

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Introduction

The most common household pets in the UK are cats and dogs. Surprisingly, even people who are sensitized to pet allergens keep pets in their homes [1]. Allergic reactions to cats and dogs occur frequently and have been recognized since these animals were first domesticated [2]. Animal danders are also potential occupational sensitizers for laboratory workers who work with animals and for veterinarians [2].

Allergic disease caused by dogs appears to be less common than that caused by cats [3]. It is possible, however, that the reported differences in the prevalence of sensitization are due in part to less well standardized diagnostic extracts used in skin testing for the detection of dog sensitivity [3]. A recent study in Los Alamos, New Mexico, has shown that sensitization and exposure to dog allergen are major risk factors for asthma [4]. It is believed that there are 7.3 million domestic dogs in the UK (RSPCA estimate), of which some are likely to live in homes of dog sensitized patients. Nevertheless, allergy to dogs has received less scientific attention than cat allergy, partly because of the impression that dog sensitivity is a relatively minor clinical problem. Dander, pelt hair and saliva are the most important sources of dog allergens, and urine does not exhibit significant allergenic activity [5,6]. A major dog allergen Can f 1, has been purified [7], and is thought to play a role in taste reception [8]. Can f 1 induces a positive skin-test reaction in 92% of dog allergic patients [9].

Cockroaches are a common source of indoor allergen in some parts of the world, particularly in the homes of patients of lower socio-economic status [10]. The significance of sensitization to cockroach allergens in the UK is as yet unknown. Most of the data on cockroaches come from the USA. Although there are over 50 species of cockroaches in the USA, only a few of those occur indoors, the most common being *Blattella germanica* (German cockroach) and *Periplaneta americana* (American cockroach). Antigenic relationship between different species is not well understood.

We have previously reported low levels of mite allergen Der p 1 in public places, but found high levels of cat allergen Fel d 1 in upholstered seats from public buildings, trains and buses [11]. This study investigated the concentration of two other important domestic allergens (Can f 1 and Bla g 2) in the dust from public buildings and public transport, as well as the airborne levels of domestic allergens (mite, cat, dog and cockroach) in schools and offices.

Methods

Can f 1 and Bla g 2 were measured in the dust collected in a previous study of carpets and upholstered seats in five schools, six hotels, four cinemas, six pubs, three buses and

two trains [10]. Four different classrooms were sampled in each of the schools and three rooms at different floors in each of the hotels. In addition, allergen levels were determined in dust from mattresses, bedroom carpets, living room carpets and sofas in 20 homes without a dog and 20 homes with a dog. Dust samples were collected using a Medivac vacuum cleaner (Medivac plc, Wilmslow, UK) with air-flow rate 45 L/s, adapted to collect the sample onto a 100 cm² of 5 µm vinyl filter (Plastok Associates Ltd, Wirral, UK). The filter was supported in a plastic dust trap located behind the cleaner attachment. A 1 m² area was sampled for 2 min. Dust was sieved through a 355 µm diameter mesh screen (Endecotts Ltd, London, UK) to remove large particles and fibres and thus obtain fine dust samples. One hundred milligrams of fine dust was extracted with 2 mL borate-buffered saline with 0.1% Tween 20, pH 8.0. The dust was resuspended using a vortex mixer. Samples were then mixed end over end on an orbital rotator for 2 h at room temperature before being centrifuged for 20 min at 2500 rpm, 1200 g, at 4°C. Supernatants were removed and stored at -20°C for future analysis of allergen content.

Air sampling

Personal air samples were collected using Casella AFC 123 pumps (airflow rate 2 L/min). Air samples using personal pumps were collected onto 25 mm Whatman GFA micro-glass fibre filter. Each sampling head was connected to the pump by wide-bore tubing. The pumps were pre-charged for a minimum of 8 h the day before sampling. The flow rate was adjusted immediately before sampling to 2 L/min using a spinning disk flow meter which had been calibrated against a soap bubble flow meter. Flow rate was rechecked immediately on cessation of sampling on site, and the volume of air sampled was calculated from the sampling time and the flow rates. Sixteen individuals working in six different offices (two to three persons/office) and nine children attending school wore personal samplers for 8 h (2 successive days with the same sampling head and filter for 4 h/day). All offices were carpeted and were situated in four different buildings, all of them mechanically ventilated. The children attended three different classrooms (three children/classroom) in the same school which was naturally ventilated. Pet ownership was recorded.

In addition, air samples were collected on three separate occasions using a high volume air sampler in different classrooms for 1 h immediately after the children vacated the school. The sampling pumps used were 60 L/min large volume dust samplers (Rotheroe-Mitchell, London, UK), and the air sample was collected onto a 37 mm Whatman GFA micro-glass fibre filter. Each sampler had a flow meter indicator and a time counter. Flow rates were measured at

commencement of the sampling, and then at 10 min, 30 min and 1 h. The early flow rate checks were performed in order to ensure that any early drop in flow rates which occurred due to the machine warming to full running temperature was measured. The volume of the air sampled was calculated by multiplying a geometric mean of flow rate by the sampling time at each interval.

Each filter was placed in a syringe and 1 mL 1% BSA PBS-T was added. After the overnight extraction at 4°C an extraction liquid was aspirated backwards and forwards several times through a three-way stop lock into a second syringe, then transferred into a test tube and centrifuged at 3000 rpm, 1500 g, for 30 min at 4°C. The supernatants were removed with a pasteur pipette, coded, and stored at -20°C.

ELISA measurement of indoor allergens

Can f 1 was measured by a two-site monoclonal antibody ELISA using anti Can f 1 MoAb 6E9 for allergen capture and polyclonal rabbit anti Can f 1 for detection [4]. Dust extracts were initially assayed at five-, 25- and 125-fold dilution for public places and homes without dogs and at 100-, 500- and 2500-fold dilutions for homes with dogs. The assay was quantified using doubling dilutions of dog allergen standard (UVA 94/02) from 500 IU/mL to 1 IU/mL Can f 1. The UVA 94/02 (10 000 IU Can f 1/mL) was substandardized against WHO/IUIS International Reference Preparation of dog hair and dander (NIBSC 84/685) which contains 100 000 IU/mL Can f 1. One International Unit is \approx 1 ng Can f 1 protein, and this value was used to calculate the results.

Bla g 2 was measured by a two-site monoclonal antibody based ELISA using anti Bla g 2 MoAb 7C11 for allergen capture and biotinylated 8F4 MoAb for detection. A control curve for Bla g 2 measurement was established using a reference *B. germanica* extract (UVA 93/04) which contained 30 Units/mL Bla g 2, and was diluted 1/6 to obtain 5 U/mL Bla g 2. Serial twofold dilutions of the extract were used to generate curves from 5 U/mL down to 0.01 U/mL. For cockroach allergen the relationship between units and protein has not yet been established. Thus, the results are expressed as U Bla g 2/g dust.

Der p 1 and Fel d 1 were assayed using a two-site immunometric ELISA [12,13]. Air samples were assayed neat and at two-, four-, and eightfold dilutions. For concentrations lying off the linear portion of the standard curve, the assays were repeated at an appropriate dilution.

The allergen data were found to follow a log-normal distribution; the results are thus reported as geometric means (GM). All data were handled on a Compaq deskpro 386/20e computer using SPSS statistical package. Allergen levels in different sites were compared using log-

transformed data and the Student's *t*-test. Statistical significance was set at a 5% level.

Results

Reservoir dust

Can f 1 was detected in all dust samples from public places and public transport, ranging from 0.2 to 52.5 μ g/g. Figure 1a shows the concentrations of Can f 1 (GM and 95% CI) in the dust from different sampling sites in public buildings and homes with and without dogs. The overall concentration of Can f 1 in public places was significantly higher in the dust from upholstered seats (GM 9.4 μ g/g, 95% confidence intervals (CI) 6.4–13.9) than from carpeted floors (GM 1.5 μ g/g, 95% CI 1.3–1.7; $P < 0.001$); the same was found when comparisons in Can f 1 levels were made between the two sampling sites (upholstered chairs vs carpets) within each of the public buildings ($P < 0.001$). The concentration on chairs did not correlate with that on carpeted floors ($r = 0.18$). Can f 1 levels were greater than 10 μ g/g in 13/33 (40%) of the upholstered seats, but in none of the carpets sampled in public buildings and public transport.

Concentrations of Can f 1 were significantly higher in homes with a dog than in public places ($P < 0.001$; e.g. GM Can f 1 level in sofas from homes with a dog was 287 μ g/g). However, dust from upholstered seats in public buildings and public transport had significantly higher concentrations of Can f 1 than the dust collected from upholstered furniture in homes without a dog (GM 2.8 μ g/g, 95% CI 1.8–4.4; $P < 0.01$). Can f 1 levels were significantly higher in carpeted floors from public buildings than in bedroom carpets from homes without a dog (GM 0.9 μ g/g, 95% CI 0.6–1.2; $P < 0.01$).

Figure 1b shows the Can f 1 levels from different sampling sites in each individual type of public building and public transport. Concentrations of Can f 1 were significantly higher in the upholstered chairs from each of the public places (pubs, hotels, cinemas and transport) than in the homes without dogs ($P < 0.05$).

Levels of cockroach allergen Bla g 2 were below the detection limit of the assay (0.04 U/mL; 0.8 U/g) in the dust from all of the upholstered chairs in public buildings and public transport and in hotel mattresses. However, cockroach allergen was found in the samples from carpeted floors in five pubs (GM 1.4 U/g, range 0.9–1.6) and two hotels (GM 1.3 U/g, range 0.9–1.4) (Fig. 2), the levels being below 2 U/g in all these samples. Bla g 2 was > 2 U/g in one of the cinemas.

Surprisingly, detectable levels of Bla g 2 were found in all of the schools (GM 2.4 U/g, range 0.8–4.4 U/g), and only 4/20 classroom floors had Bla g 2 below the detection limit

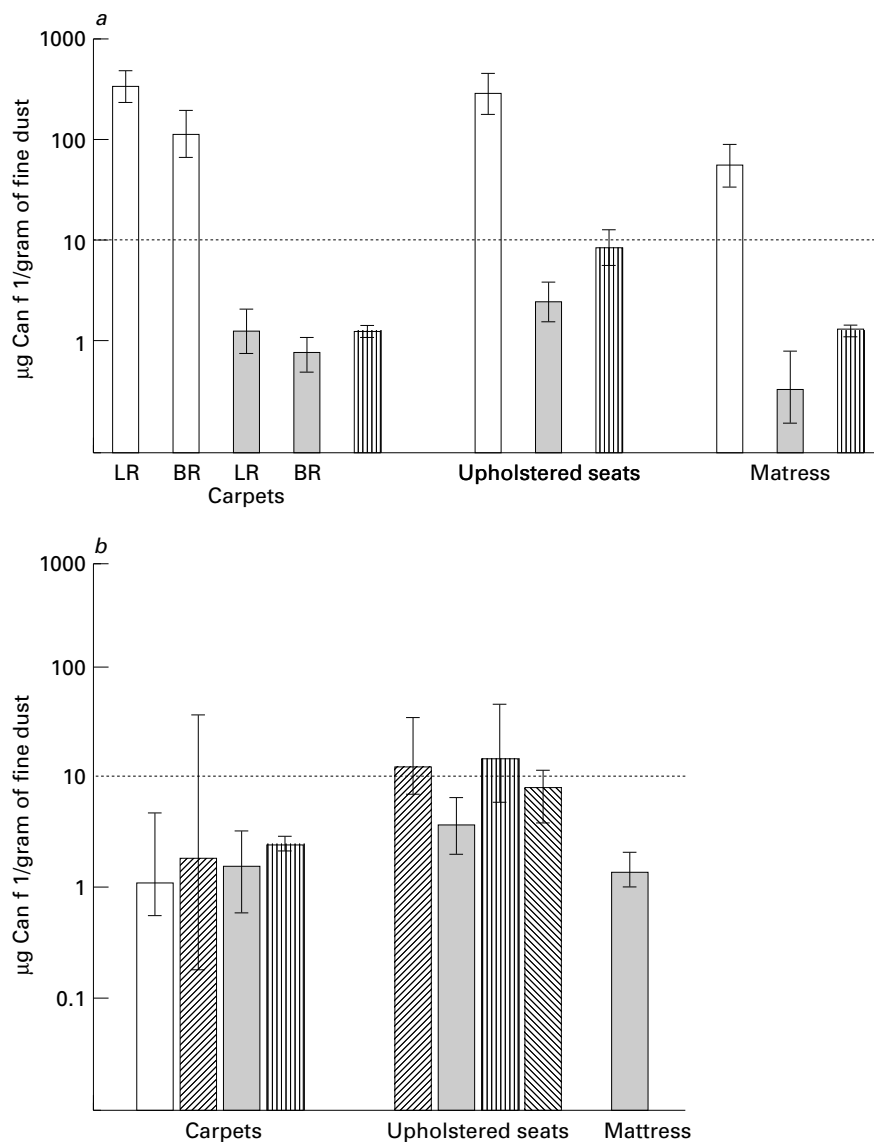


Fig. 1. (a) The overall concentrations of Can f 1 ($\mu\text{g}/\text{g}$; GM and 95% CI) in public places (▨) and homes with (□) and without (▩) dogs; LR = living room; BR = bedroom. The dotted line represents a proposed threshold for significant exposure (10 μg Can f 1/g). (b) Geometric means (bars) and ranges of Can f 1 levels (mg/g of fine dust) from different sampling sites in each individual type of public building and public transport. The dotted line represents a proposed threshold for significant exposure (10 Can f 1 $\mu\text{g}/\text{g}$); □ = school; ▨ = pub; ▩ = hotel; ▨ = cinema; ▨ = public transport.

of the assay. Bla g 2 concentrations greater than 2 U/g were measured in 65% of the classrooms sampled (Fig. 2). All classrooms had at least a part of the floor carpeted, and this was the area sampled. No visible evidence of cockroach infestation was observed in any of the public buildings.

Air samples

None of the air samples had detectable levels of Der p 1 or Bla g 2. All nine personal air samples from schools were

below the limit of detection for Can f 1 ($<1 \text{ ng}/\text{m}^3$). However, airborne Can f 1 was detected in all high volume samples (range 0.3–1.6 ng/m^3 ; detection limit 0.27 ng/m^3). Eight of the nine personal samples were also below the limit of detection for Fel d 1 (0.5 ng/m^3) and one was positive at 0.79 ng/m^3 . This had been collected by a child with a pet cat. None of the high volume air samples resulted in detectable Fel d 1 (limit of detection 0.22 ng/m^3).

Of the 16 personal air samples from offices one had a high level of Can f 1 (14.1 ng/m^3). Four personal samples were

Levels of cockroach allergen were very low. It is possible that they would be higher if the samples were collected from the kitchen areas in pubs and hotels. In domestic dwellings dust from the kitchen floor can contain up to 50 times more cockroach allergen than dust from the bedroom [28]. However, it was decided not to collect the dust from the kitchen areas, as this exposure could be considered to be an occupational rather than a community exposure. It is worth noting that significantly higher Bla g 2 levels were measured in the dust from schools than any other public building. One can only speculate at the reasons for this finding. It is possible that there is a sufficient food source for cockroaches in the classrooms, due to the fact that children often have snacks there and drop crumbs on the floors. Cockroach allergen was below the detection limit in all of the 20 homes in the same area. Children spend a significant part of their time in school. In half the classrooms sampled the level of cockroach allergen exceeded the proposed threshold of 2U/g above which there is an increased risk of sensitization. It is not yet possible to assess the effect of exposure to cockroach allergens in school on sensitization in the area where domestic exposure seems to be very low. The majority of homes in this survey were well maintained middle class houses. To estimate more accurately the importance of exposure to cockroach allergens, it will be necessary to target areas with poor standards of housing. Sensitization and exposure to cockroach allergens may prove a risk factor for asthma in socially deprived areas, and additional exposure in schools may also be important. Further work is needed to assess the level of sensitization to cockroaches in the UK.

In conclusion, upholstered seats from public buildings and public transport constitute a significant reservoir for the build-up of dog allergen, and the source of exposure to Can f 1 inside public buildings or on public transport. Exposure to cockroach allergens in schools may be important for cockroach sensitized asthmatic children.

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