Comparison of exposure to allergens in homes in Uppsala, Sweden, and Tartu, Estonia

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ABSTRACT
Allergen levels in homes in Uppsala and Tartu were compared. Dust samples were collected and analysed for cat (Fel d 1), dog (Can f 1), horse (Equ cx) and cockroach (Bla g 1) allergens. All Swedish homes had cat and most had dog and horse allergens. In Tartu, most homes had cat and dog allergen and 50% also had horse allergen. No cockroach allergen was found in Uppsala and only in two homes in Tartu. Horse allergen levels were significantly higher in Uppsala than in Tartu. Cat allergen levels were twice as high in Uppsala as in Tartu, which is in accordance with published prevalence figures for cat-associated asthma symptoms, 6.6 versus 3.2% and allergic rhinoconjunctivitis, 11.1 versus 4.0% in Uppsala and Tartu, respectively. Our results indicate that horse allergen is common in Swedish homes and the significance of this finding needs to be further investigated.

INDEX TERMS
Allergen; Allergy; Environment; Pets; Settled dust

INTRODUCTION
Allergy depends on both genetic and environmental factors. Environmental factors seem to play a greater role in the development of asthma and sensitization, as indicated by the higher prevalence of allergic disease in western as compared to eastern countries (von Mutius *et al.*, 1992; Jogi *et al.*, 1996, 1998; Julge *et al.*, 1998). The reasons for the east–west differences in atopy and related respiratory diseases have remained mostly unexplained. Changes in lifestyle and the indoor climate, which could also facilitate allergen exposure, have been pointed out as possible contributing factors. In this study, we have compared the indoor allergen levels in homes in Uppsala, Sweden, and homes in Tartu, Estonia. These two countries have similar populations, geography and climate but the prevalence of allergic disease differs greatly.

METHODS
Sample Collection
Thirty homes from each country were chosen from the ECRHSII study. Collection of dust samples was performed in February–May 2002 in both countries. Settled dust was collected with a vacuum cleaner fitted with a special dust collector (ALK Abello, Copenhagen, Denmark) equipped with a Millipore filter (pore size 6 μm). The floor and furnitures in the bedroom were vacuum cleaned for 2 min each. The filters were sealed in plastic bags and stored at –20°C until extraction.

Analysis of Allergen Concentration
The total amount of dust in each sample was weighed. Samples of settled dust (100 mg) were

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extracted in 2 ml of phosphate buffered saline containing 0.05% Tween 20 (1/20 w/v) by rotating mixing for 2 h at room temperature. Samples were then centrifuged at 4500 rpm for 10 min followed by another centrifugation of the supernatant at 10 000 rpm for 10 min. The final supernatant was transferred to Microtubes (Sarstedt, Germany) and stored frozen at –20°C until analysed for the content of allergen. Allergen levels were determined using two-site sandwich ELISA for cat (Fel d 1), dog (Can f 1), cockroach (Bla g 1) (INDOOR Biotechologies Ltd., USA) and horse allergen (Equ c x) (MABTECH, Stockholm, Sweden) (Emenius *et al.*, 2001) using monoclonal antibodies. The assays were basically performed according to the protocols provided by the manufacturer except in the dog assay where the horseradish peroxidase labelled goat anti-rabbit Ig was from DAKOPATTS, Denmark. The dust samples were diluted in phosphate buffered saline containing 0.05% Tween and 1% bovine serum albumin (BSA) in serial dilution starting with 1/5 and assayed in duplicate. Allergen concentrations were expressed as μg/g dust, except for horse allergen concentrations which were expressed as kUnits/g dust, where 1 Unit is equal to 1 ng protein of a horsehair and dander extract used as standard (Allergon, Valinge, Sweden and NIBSC, Hertfordshire, UK). Protein determination was performed on the standard with the micro BCA method (Pierce, Rockford, USA) using BSA as standard.

Statistical Methods

Since the allergen levels were not normally distributed, median levels, geometrical mean and min–max values were calculated. Mann–Whitney *U*-test was used to calculate two-tailed *p*-values for the difference in allergen levels between the two countries.

RESULTS

The mean level of dust was 0.7 g in both countries, with a range of 0.06–4.02 g in Uppsala and 0.02–2.32 g in Tartu. The median level of Fel d 1 was 3.2 μg/g dust (range: 0.4–1653 μg/g dust) in Uppsala and 1.9 μg/g dust (range: <0.1–114 μg/g dust) in Tartu ($p = 0.14$). The median level of Can f 1 was 0.95 μg/g dust (range: <0.2–725 μg/g dust) in Uppsala and 1.6 μg/g dust (range: <0.2–677 μg/g dust) in Tartu ($p = 0.5$). The median level of horse allergen was 0.83 kU/g dust (range: <0.2–609 kU/g dust) in Uppsala and 0.2 kU/g dust (range: <0.2–2 kU/g dust) ($p < 0.001$). Cat allergen was detected in all bedrooms in Uppsala, of which 70% had levels of >1 μg/g, which is regarded as the risk level for sensitization. Of bedrooms in Tartu 94% had detectable levels of cat allergen of which 52% were over the risk level for sensitization. Twenty percent of the bedrooms in both countries had no detectable levels of dog allergen. In Uppsala, 30% of the bedrooms had dog allergen levels of >2μg/g as compared to 45% in Tartu. In Uppsala, 84% of the bedrooms had detectable levels of horse allergen, as compared to 48% in Tartu.

DISCUSSION

The study compares allergen levels measured in two cities during the same season. The sampling method was the same in both studies and the allergen analysis was performed at the same laboratory and with the same methodology. Thus, the results should be comparable with respect to sampling and immunological analysis.

The levels of cat and dog allergens in homes in Uppsala and in Tartu are in agreement with data from previous studies performed in Sweden (Munir *et al.*, 1993; Parvaneh *et al.*, 2000), Estonia (Julge *et al.*, 1998), Finland (Raunio *et al.*, 1998) and Japan (Sakaguchi *et al.*, 1993), but results are not directly comparable due to methodological differences. The presence of high levels of horse allergen in homes is a new finding. High levels of horse allergen have earlier been reported in public places such as schools in Uppsala region (Elfman *et al.*, 2002)

and in mattresses tried by customers in furniture stores (Egmar *et al.*, 1998). The main source of allergen contamination in homes is influx of allergens from the clothes of the residents and visitors. Allergens are stable proteins and accumulate in cracks on floors and walls, in textiles and in upholstered furnitures (Wood *et al.*, 1992; Smedje and Norbäck, 2001; Perzanowski *et al.*, 1999).

Table 1 Allergen levels in homes in Uppsala and Tartu

Home no.	Uppsala, Sweden			Tartu, Estonia		
Home	Fel d 1 (µg/g)	Can f 1 (µg/g)	Equ cx (kU/g)	Fel d 1 (µg/g)	Can f 1 (µg/g)	Equ cx (kU/g)
1	**26.0**	0.3	0.6	0.3	**3.0**	<0.2
2	**1653.0**	1.1	74.0	**3.2**	**3.8**	2.0
3	**30.0**	**37.5**	<0.2	0.75	0.3	0.2
4	**133.0**	0.5	<0.2	0.7	**8.7**	0.2
5	**3.8**	<0.2	1.6	**2.6**	1.6	0.3
6	0.6	0.9	0.3	1.0	1.2	0.2
7	**3.2**	<0.2	0.3	**2.0**	1.7	0.7
8	**2.1**	**36.4**	5.9	**6.4**	0.5	2.0
9	**9.2**	**266.4**	609.3	**74.7**	**3.9**	<0.2
10	0.5	**41.5**	1.9	0.45	1.4	<0.2
11	**7.2**	1.5	0.9	1.0	**60.7**	0.2
12	0.4	0.3	0.45	**86.7**	**5.8**	0.2
13	**1120.0**	**3.4**	2.4	0.9	**48.1**	0.5
14	1.0	0.5	<0.2	0.1	1.0	<0.2
15	**7.9**	<0.2	<0.2	**1.9**	1.5	<0.2
16	**2.8**	<0.2	2.8	**113.5**	**3.1**	<0.2
17	**1.2**	<0.2	1.7	**2.0**	**61.8**	1.7
18	0.7	**3.4**	2.0	**108.6**	<0.2	<0.2
19	1.0	0.9	0.9	0.8	<0.2	<0.2
20	**4.9**	**725.2**	0.8	**6.0**	0.3	0.6
21	0.6	**3.1**	2.1	**15.8**	<0.2	<0.2
22	0.4	**155.2**	0.7	**4.7**	**148.0**	<0.2
23	**1.1**	1.9	0.5	<0.1	<0.2	<0.2
24	**131.7**	0.5	1.6	0.9	**53.4**	<0.2
25	**174.6**	1.0	0.3	0.2	<0.2	0.2
26	0.5	0.6	0.4	0.9	0.2	0.3
27	**1215.0**	0.7	1.2	**108.4**	0.3	<0.2
28	**1540.5**	<0.2	0.6	0.4	**3.1**	<0.2
29	**1.3**	1.0	0.4	**86.9**	**677.0**	0.4
30	**3.2**	1.0	1.0	**38.2**	<0.2	0.2
31				0.5	**4.1**	0.2
Median	3.2	0.95	0.83	1.9	1.6	0.2
GM	7.3	1.8	1.1	2.8	2.2	0.3
Range	0.4–1653	<0.2–725	<0.2–609	<0.1–114	<0.2–677	<0.2–2

In general, cat allergen levels in homes were higher in Uppsala than in Tartu. This is in accordance with the prevalence figures for cat-associated asthma symptoms, 6.6 versus 3.2%, allergic rhinoconjunctivitis, 11.1 versus 4.0% and IgE antibodies to cat 14.3 versus 4.8% for Uppsala and Tartu, respectively. These figures were reported in part of the cross-sectional study ECRHS performed among young adults (age 20–44 years) in Uppsala and Tartu (Jogi *et al.*, 1998), from which study these homes have been choosen. Our results indicate that a higher total exposure to furry pet allergens in the home environment may be a contributing factor to the higher prevalence of allergic disease seen in Uppsala as compared to Tartu. However, as earlier reported ('Nordpet', Ahlbom *et al.*, 1998) exposure to pets cannot explain the increased incidence of allergies alone. Adjuvant factors of unknown species also appear to play an essential role.

CONCLUSION AND IMPLICATIONS

The cat allergen levels in homes were twice as high in Uppsala than in Tartu, but the difference was not statistically significant. This is in accordance with the prevalence figures for cat-associated asthma symptoms, 6.6 versus 3.2%, allergic rhinoconjunctivitis, 11.1 versus 4.0% and IgE antibodies to cat 14.3 versus 4.8% for Uppsala and Tartu, respectively. These data were previously published in part of the cross-sectional study ECRHS performed among young adults (age 20–44 years) in Uppsala and Tartu. The most striking difference was in the level of horse allergen, an allergen rarely measured in the home environment before. Our results indicate that exposure to horse allergen is common in the Swedish home environment, and the allergological significance of this new finding needs to be further investigated.

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