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Exposure Levels and Skin Reactivity to German Cockroach (*Blattella germanica*) in Croatia

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Aim. To evaluate German cockroach (*Blattella germanica*) allergen levels in house dust and skin reactivity to German cockroach in adult atopic and non-atopic subjects in inland areas of Croatia.

Methods. *Blattella germanica* group 2 (Bla g 2) allergen was measured using the enzyme-linked immunosorbent assay (ELISA) on test strips (Dustscreen™ test, Heska AG, Switzerland) in 94 house dust samples collected from living room and bedroom floors, 35 from rural and 59 from urban areas. Skin prick testing with common inhalatory allergens, *Blatella germanica*, storage mites *Lepidoglyphus destructor* and *Tyrophagus putrescentiae* (ALK-Abello, Denmark) was performed in 187 adult outpatients, 131 from urban and 56 from rural areas. Total serum IgE levels were measured using the ELISA method. Subjects with relevant respiratory and/or skin symptoms, at least one positive skin prick test, and/or increased total IgE were considered atopic.

Results. Positive skin prick test to cockroach was observed in 18/187 subjects (9.6%). The frequency of skin prick test positive subjects to cockroach was higher in atopic than in non-atopic subjects, but not significantly (12.2% vs. 4.7%; p = 0.098). Of 15 atopic subjects with positive skin prick test to cockroach, 14 also had positive skin prick test to storage mites, 8 to house dust mites, 1 to pollens, and none to cockroach only. Positive skin prick test to storage mites was the only factor which significantly increased the risk for having positive skin prick test to cockroach (n = 187; odds ratio, 109.82; 95% confidence interval, 2.06- 5853.5; p = 0.020). In all 94 house dust samples, Bla g 2 was not detectable.

Conclusions. Our results suggest that there is no relevant exposure to cockroach allergen in house dust samples from inland areas of Croatia. Consequently, positive skin prick test to cockroach is rare in adult subjects, even in atopics. Positive skin prick test to cockroach in atopics is in the majority of cases related to positive skin prick test to storage mites, probably due to cross-reactivity.

Key words: *air pollution, indoor; allergens; Blattellidae; cockroaches; dust; enzyme-linked immunosorbent assay; hypersensitivity; skin tests*

Beside dust mites and pets, the cockroach seems to be an important source of indoor allergens all over the world (1-4). A number of US studies have established that cockroach is an important and independent risk factor for allergic asthma, particularly in urban, inner-city Afro-American or Hispanic population. The frequency of allergy to cockroach in asthmatic patients of this population is around 50% and is related to indoor exposure to cockroach allergen (1,5,6). However, in Europe, the prevalence of cockroach allergy in atopic or asthmatic population seems to be much lower, ranging between 4.2% in Germany (7) and 27.8% in France (8). Furthermore, the clinical significance of allergy to cockroach and its relation to cockroach allergen exposure is not clearly understood in Europe. As there are no data about cockroach allergy and exposure to cockroach allergens in Croatia, we wanted to investigate skin reactivity to cockroach in atopic and non-atopic subjects from rural and urban inland areas of Croatia, and the levels of exposure to cockroach allergen.

Subjects and Methods

Subjects

A sample of 200 consecutive outpatients examined in the Occupational and Environmental Health Unit of the Institute for Medical Research and Occupational Health, Zagreb, Croatia, was recruited for this study. All patients were examined regarding their health complaints related to environmental factors, particularly those from occupational exposures. After the exclusion of 13 patients with contraindications for skin prick testing, a total of 187 subjects (89 male and 98 female; mean age 34.9 ± 13.2 years) were included in the study. All subjects were residents of inland Croatia (Zagreb and its rural surroundings), 131 from the urban and 56 from rural areas. These subjects underwent a routine allergological checkup which included an interview, physical examination, skin prick testing, and total serum IgE measure-

ment. The subjects were divided into atopic and non-atopic groups. All subjects with at least one positive skin prick test to common inhalatory allergens and/or increased total IgE level, and relevant respiratory and/or skin symptoms were considered atopic (9) (Figure 1).

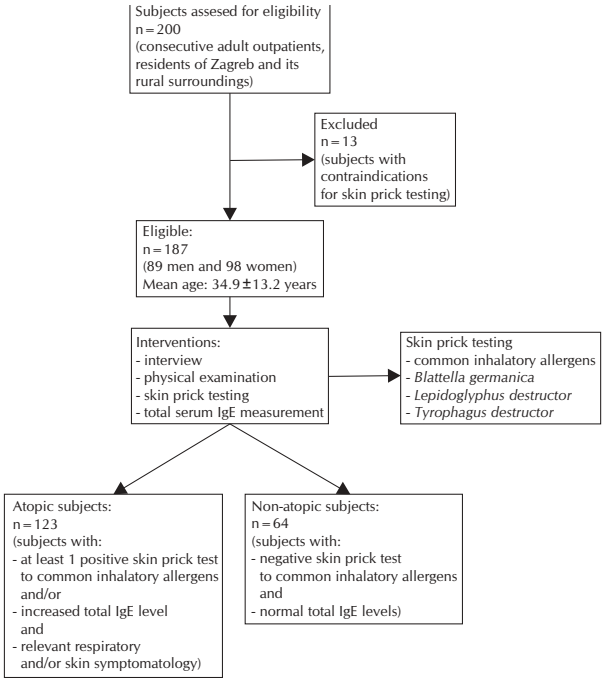

Figure 1. Selection and testing of subjects in the study.

Skin Prick Testing

Standard skin prick test (10) was performed in all 187 subjects using common inhalatory allergens (grass pollen mixture, birch, hasel, *Ambrosia elatior,* and *Artemisia vulgaris* pollens, *Dermatophagoides pteronyssinus* and *farinae*, *Alternaria alternata*, *Cladosporium herbarum*, cat and dog dander, feather mixture), *Blattella germanica* and storage mites *Lepidoglyphus destructor* and *Tyrophagus putrescentiae* (ALK-Abello, Denmark). Histamine hydrochloride (1 mg/mL) and buffer solution were tested as controls of positive and negative skin reactions, respectively. Skin prick test results were recorded after 15 min. The mean wheal diameter was calculated according to the formula (D+d)/2, where D represents the largest longitudinal diameter and d its midpoint orthogonal diameter. A mean wheal diameter which was larger than negative control (buffer solution) for 3 mm or more was considered positive. This criterion for positive skin prick test was chosen according to logistic regression analysis which showed a strong relationship between skin reactivity to buffer solution and cockroach (Figure 2). Skin reactivity was expressed and analyzed also as a mean skin reaction in all subjects and specified subgroups.

Total Serum IgE Measurement

Total serum IgE was measured from venous blood samples, using the ELISA method (IASON, Graz, Austria). Levels greater than 150 kIU/L were considered significantly increased.

Dust Sample Collection and Analysis

A hundred house dust samples were collected from the floors of bedrooms and living rooms in the rural and urban inland areas of Croatia, using the Dustscreen™ (Heska AG, Frieburg, Switzerland) vacuum cleaner adapter and a filter, and a standard procedure (11,12). The dust was vacuumed until the filters were full. Filters were kept frozen at -18 °C until analysis. Ninety-four house dust samples were included in the study: 35 from the inland rural area and 59 from the inland urban area. Six samples weighing less then 100 mg were excluded from the study. The Dustscreen™ test (Heska AG) was used to measure the levels of *Blattella germanica* group 2 allergen (Bla g 2) in all 94 dust samples applying the standard procedure described elsewhere (13,14,15). The test was performed in dust samples weighing 100±1 mg, which were extracted in 10 mL of extraction buffer. Optical density was read as arbitrary units using an original densitometer (FAG VIPDENS 111, FAG, Lausanne, Switzerland). Corresponding concentrations were obtained using a standard curve. The results were expressed as μg/g of dust.

Statistical Analysis

Statistica for Windows, Kernel release 5.5A was used to perform descriptive statistics, chi-square test, correlations and logistic regression in data analysis (16). The statistical significance was assumed at $p < 0.05$. Multivariate logistic regression models included variables that in univariate testing showed a significance level of $p < 0.10$.

Results

Bla g 2 could not be detected in any of the 94 house dust samples. Positive skin prick test to cockroach was observed in 18/187 (9.6%) subjects. Hundred and twenty-three subjects met the criteria for atopy, and 64 were non-atopic. The frequency of skin prick test positive reactions to cockroach was higher in atopic than non-atopic subjects, but not significantly (12.2% vs. 4.7%; chi-square = 2.73, df = 1, p = 0.098; Pearson's chi-square). The mean skin reactivity to cockroach in all recorded skin prick test reactions was significantly greater in atopic then in non-atopic subjects (1.78±1.48 mm vs. 0.90±1.13 mm; U = 2466.5; Z = -4.2; df = 185, $p < 0.001$; Mann-Whitney U test). The mean skin reactivity in subjects with positive skin prick test reactions to cockroach was 3.8±0.94 mm. Of 15 atopic subjects with positive skin prick test to cockroach, 14 were also skin prick test positive to storage mites, 8 to house dust mites, and 1 to pollens. No atopic subject was skin prick test positive to cockroach alone. In contrast, three non-atopic subjects were skin prick test positive to cockroach alone, but their skin reaction had a mean diameter of only 3 mm. Positive skin prick test to storage mites was the only factor which significantly increased the risk for positive skin prick test to cockroach (n = 187; odds ratio, 109.82; 95% confidence interval, 2.06-5853.5; df = 180, p = 0.020; logistic regression) (Figure 2).

The number of atopic subjects was similar between the urban (n = 131) and rural (n = 56) populations (69.5% vs 57.1%, respectively; chi-square = 2.87, df = 1, P = 0.091, Pearson's chi-square), and so was the number of subjects with positive skin prick test to cockroach (9.2% vs 10.7%, respectively; chi-square = 0.11, df = 1, p = 0.741, Pearson's chi-square).

Discussion

We could not establish any exposure to Bla g 2 allergen in inland Croatia. Accordingly, there were no atopic monosensitized subjects to cockroach, and three non-atopic subjects had marginally positive skin prick test to cockroach alone. The skin reactivity to cockroach was very low and strongly dependent on the reaction to negative control solution. Positive skin prick test to cockroach was not related to atopy according to chosen criterion for skin prick test positivity. Positive skin prick test to cockroach was clearly related to positive skin prick test to storage mites

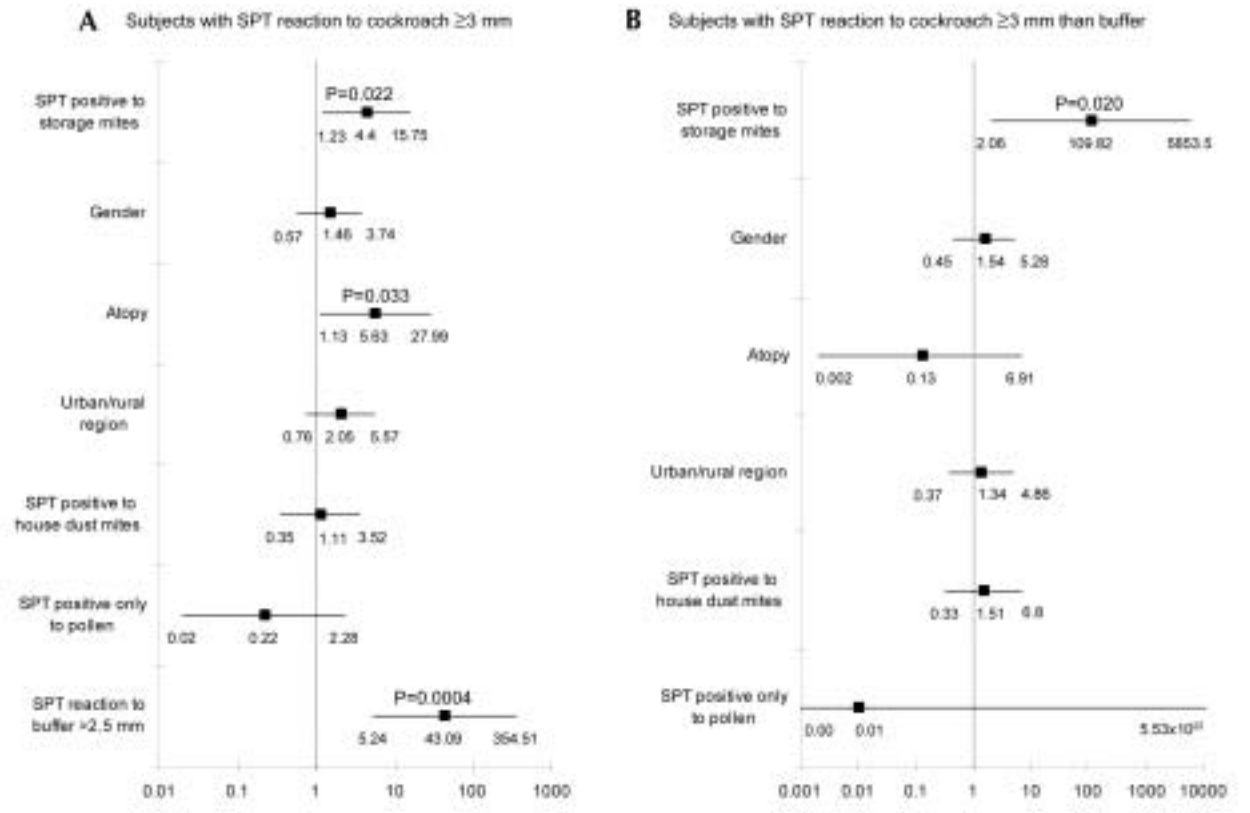

Figure 2. Multivariate logistic regression for skin prick test positive subjects to cockroach (n = 187). **A.** Subjects with skin prick test reaction to cockroach ≥ 3 mm. **B.** subjects with skin prick test reaction to cockroach 3 mm or larger then buffer solution (negative control). Black squares represent odds ratios (OR) and whiskers 95% confidence intervals (95%CI). SPT – skin prick test.

which partially confirm previous data about cross-reactivity between dust mites and cockroach (17,18). To our surprise, we have not found any relation between positive skin prick test to house dust mites and cockroach. Our earlier study showed that *Tyrophagus putrescentiae* allergen extract (also used in this study) was not specific enough (19), explaining why positive skin prick test to cockroach in this study may be related to non-specific skin reactions associated with cross-reactivity to the allergen extract of *T. putrescentiae*.

So far, the studies investigating allergy to cockroaches have included three species: *Blattella germanica* (European species), *Blattella orientalis* (subtropic and tropic species), and *Periplaneta americana* (North-American species) (20). Earlier studies confirmed that the three species shared common allergens, but also had species-specific allergens (21). Our study focused on *Blattella germanica* as a species that has been most studied in Europe (2,7,22). As we have not established any exposure to Bla g 2 allergen in inland Croatia, we believe that further investigation should focus on *Blattella orientalis*, which seems to be the most common cockroach in the Mediterranean (20). The connection between indoor exposure to cockroach and development of allergy and allergic diseases in exposed residents was most intensively studied in the USA. Exposure to high levels of cockroach allergens (> 8 μg/g) seems to be common in the inner-city US households, where the association between exposure to cockroach allergens and positive skin prick test to cockroach, as well as between positive skin prick test to cockroach and allergic asthma, have been confirmed (23,24). In addition, some studies showed that the symptoms and course of asthma were more severe and of longer duration when associated with allergy to cockroach than to the other allergens (6,24). Other data show that cockroach can be the first and the only sensitizing agent in early childhood, affecting about 14% of inner-city children (aged under 4 years) (25). According to some race-related differences in immune response to cockroach allergen, black people seem to run a higher risk of development of cockroach-related allergy and allergic diseases (8,26).

In Europe, the prevalence of cockroach allergy in atopic or asthmatic subjects is much lower than in the USA. In most studies it is around 25% (2,8,20,22), whereas monosensitization is even rarer, ranging between 1% and 10% (8,27). East Germany, Norway, and Italy show the lowest prevalence of allergy to cockroach in Europe (4.2%, 7.5% and 12.7%, respectively) (7,27,28) and our results (9.6%) suggest that Croatia belongs to that group of countries.

Indoor exposure to cockroach has not been studied extensively in Europe, but some relations between exposure to cockroach and allergy to cockroach can be observed. Rare allergies to cockroach in East Germany correspond to low exposure levels found there (cockroach allergen was found in 29% of kitchen dust samples and was below 8 μg/g of dust) (7), and the higher prevalence of cockroach allergy in Poland is associated with higher exposure levels (cockroach allergen was found in 55% of dust samples, its levels ranging from 0.1 to 389 μg/g of dust) (2). The absence of exposure and of allergy to cockroach in urban and rural inland population of Croatia in our study seems to confirm this relation.

In conclusion, our results suggest that even if inland population of Croatia is exposed to German cockroach, this exposure is not relevant. Consequently, positive skin prick test to cockroach may be extremely rare in adult subjects, even in atopics, and bears no relevance to routine allergological practice in this region. In our study, positive skin prick test to cockroach in atopics is largely if not entirely related to positive skin prick test to storage mites, quite likely due to cross-reactivity.

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