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ANALYSIS OF DUST SAMPLES FROM URBAN AND RURAL OCCUPATIONAL ENVIRONMENTS IN CROATIA

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This study estimated the exposure to dust mites in various occupational environments in Croatia. In total, 29 occupational dust samples were collected: 10 from urban areas (offices, archive of an insurance company, tobacco, paper-recycling, fish-processing and textile plants, animal unit for experimental rats, winery), nine from rural areas (barley, hay, animal food and flour warehouses, tailor's shops, wood processing plant, swine confinement house, grocer's storeroom), and 10 samples from cabins of five fishing boats (five floor and five bed samples).

Mites were microscopically identified, and the levels of Der p 1, Der f 1, and Der 2 allergens measured using the DUSTSCREEN™ test (Heska AG, Switzerland). Microscopy showed no mites in urban areas. Pyroglyphid mites (*D. pteronyssinus*) were found in all bed samples from fishing boats. Non-pyroglyphid mites were found in samples taken from barley, hay and animal food warehouses, the swine confinement house, grocer's storeroom, and fishing boats. Pyroglyphid mite allergens were detected in eight of 10 dust samples from the fishing boats. Median levels of Der p 1, Der f 1, and Der 2 in cabin bed samples were 10 µg g⁻¹, 0.2 µg g⁻¹, and 3.5 µg g⁻¹, respectively.

Our findings on fishing boats suggests that pyroglyphid mites could be considered work-related allergens for fishermen. The results of this study confirmed non-pyroglyphid mites as occupational risk factors in various rural environments.

KEY WORDS: *Acaridae, allergens, agricultural workers, dust mites, fishermen, occupational exposure, occupational hazard, Pyroglyphidae*

Mites from various taxonomic suborders and families are well known and described as an important occupational hazard in rural population. Dust mites from the taxonomic suborder *Astigmata*, families *Glycyphagidae* and *Acaridae*, also known as "storage mites", are mostly studied as sources of occupational allergens causing work-related symptoms in the upper and lower airways and skin (1-3). Recent studies also suggest that spider mites from the suborder *Prostigmata*, family *Tetranychidae* and some species of predatory mites from the suborder *Mesostigmata* are potential sources of occupational allergens (4, 5). All these mites are established rural occupational allergens, particularly for farmers and greenhouse

workers, but were rarely studied as occupational hazard in urban industrial environments.

House dust mites (*Pyroglyphidae*), especially genus *Dermatophagoides*, are confirmed to be an important source of potent allergens in house dust. Due to their common presence in rural and urban households and other public indoor environments such as daycare centres or cinemas (6-9), they were rarely examined as potential work-related or occupational allergens.

Numerous studies have shown the relationship between the levels of environmental exposure to dust mites and the prevalence of allergy markers and allergic diseases such as asthma and rhinitis

in the exposed population (6, 7). In 1988, WHO proposed a threshold level of 2 µg of Der p 1 (main *Dermatophagoides pteronyssinus* allergen) per g of dust for developing allergy to *D. pteronyssinus*, and 10 µg of Der p 1 per g of dust for developing symptoms in already allergic subjects (10, 11).

The aim of this study was to assess the exposure to pyroglyphid and non-pyroglyphid dust mites in several rural and urban occupational environments in Croatia.

MATERIALS AND METHODS

Collection of dust samples

Occupational environments analysed in this study are listed in table 1. In total, 29 occupational indoor dust samples were collected: 10 from urban areas, nine from rural areas, and 10 samples from cabins of five fishing boats (five samples from the floor, and five from the beds). All samples were collected from March through May in 2002 and 2003.

In urban areas, dust samples were collected from the floors of two offices and an insurance company archive room; from the working surfaces of a tobacco-processing plant, a paper-recycling plant, a fish-processing plant, two textile plants, and a winery; from cages with experimental rats from a laboratory animal unit. In the industrial settings, samples were taken from workplaces where dust was the result of the production process. In the tobacco plant, samples were collected from the area where tobacco leaves were manually selected, sorted and cut; in the paper-recycling plant from a place where paper was dried and cut; in the fish-processing plant from the fish meal production and storage areas; and in the textile plants from the cutting and sewing sections.

In rural areas, dust samples were collected from the floors of a swine confinement house, from warehouses storing barley, hay, and animal food and from a grocer's storeroom; from working surfaces of two tailor's shops, and from a wood-processing plant. Samples were also taken from the floors and beds (mattresses and pillows) in cabins of five fishing boats. Each boat had one cabin with three or four beds. The boats operated in the Adriatic Sea, and were used for fishing either tunas or sardines.

In each sampled occupational environment (working room, working area or fishing boat cabin), a single dust sample was collected as an overall sample from several spots on the floors, working surfaces, or beds.

In the offices, insurance company archive, tailor's shops, grocer's storeroom, and fishing boats, samples were collected using the standard method with a Dustscreen™ vacuum cleaner adapter (Heska AG, Frieburg, Switzerland) with a filter (10, 12). The dust was vacuumed until the filters were full. In all other industrial and farming environments where vacuum cleaning was not possible, samples were collected using a clean metal spoon and brush, and put in a clean plastic bag. Filters and plastic bags were kept frozen at -18 °C until analysis.

Methods

Two equal parts weighing 100±1 mg were taken from each dust sample. One was used for the microscopic identification of mite species and other for Dustscreen™ test.

Morphological analysis

All dust samples were checked for mites using light microscopy. The mites were separated from the dust using a modified floatation method by Hart &

Table 1 Occupational indoor environments from which samples were taken (N = number of sampled environments)

Urban occupational environments	Rural occupational environments
Offices (N=2)	Swine confinement house (N=1)
Insurance company archive (N=1)	Barley warehouse (N=1)
Tobacco plant (N=1)	Hay warehouse (N=1)
Paper-recycling plant (N=1)	Animal food warehouse (N=1)
Fish-processing plant (N=1)	Grocer's storeroom (N=1)
Textile plants (N=2)	Flour warehouse (N=1)
Winery (N=1)	Tailor's shops (N=2)
Animal unit for experimental rats (N=1)	Wood-processing plant (N=1)
	Fishing boats - cabin beds (N=5)
	Fishing boats - cabin floor (N=5)

Fain (13). Ten ml of 80 % ethanol was added to the dust sample which was then mixed and left for 24 hours. After 24 hours, ethanol was carefully decanted and 10 ml of saturated NaCl solution was added to the sediment. After 10 minutes, 10 samples of the supernatant (two drops per sample) were put with the Hoyer's medium (two drops per sample) on the microscope slides, covered with cover glass and left for at least 24 hours before microscopic analysis. Mite species were identified using light microscopy, comparing them to pictorial identification keys for domestic mites (14) and to permanent microscopic preparations from the mite cultures (C.B.F.LETI, S.A., Madrid, Spain).

Quantification of mite allergens

The DUSTSCREEN™ test (Heska AG, Frieberg, Switzerland) was used to measure the levels of Der p 1, Der f 1 and Der 2 (equal mixture of Der p 2 and Der f 2) allergens in all dust samples, applying the standard procedure described elsewhere (15, 16). 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 for each allergen. According to the quantity of dust sample and the volume of extraction buffer, the results were expressed as $\mu\text{g g}^{-1}$ of dust.

RESULTS

Morphological data

Mites were microscopically identified in five of nine rural dust samples, in all five samples taken from the

fishing boat cabin beds, and in one of five samples from the cabin floors (Table 2). Pyroglyphid mites (genera *Dermatophagoides* and *Euroglyphus*) were found in fishing boat samples, and non-pyroglyphid mites (genera *Lepidoglyphus*, *Blomia*, *Tyrophagus*, *Acarus* and family *Tarsonemidae*) in dust samples from the barley, hay and animal food warehouses, swine confinement house, grocer's storeroom, and fishing boats. No mites were microscopically found in occupational dust samples from the flour warehouse, tailor's shops, wood-processing plant, or in the ten analysed urban dust samples.

Allergen levels

Der p 1, Der f 1 and Der 2 were found in eight of the ten dust samples taken from fishing boats (in 5/5 bed samples and in 3/5 floor samples), and in three of the ten urban samples (an office, a paper-recycling plant, and a textile plant). The allergens were not detected in the nine analysed rural samples.

In the five fishing boat cabin bed samples, the median level of Der p 1 was $10 \mu\text{g g}^{-1}$ (range $0.1 \mu\text{g g}^{-1}$ to $15 \mu\text{g g}^{-1}$), median Der f 1 level $0.2 \mu\text{g g}^{-1}$ (range $0 \mu\text{g g}^{-1}$ to $6 \mu\text{g g}^{-1}$), and median Der 2 level $3.5 \mu\text{g g}^{-1}$ (range $0 \mu\text{g g}^{-1}$ to $10 \mu\text{g g}^{-1}$). In samples taken from the cabin floors, the median level of Der p 1 was $0.05 \mu\text{g g}^{-1}$ (range $0 \mu\text{g g}^{-1}$ to $0.65 \mu\text{g g}^{-1}$), median Der f 1 level $0 \mu\text{g g}^{-1}$ (range $0 \mu\text{g g}^{-1}$ to $0.1 \mu\text{g g}^{-1}$) and median Der 2 level $0 \mu\text{g g}^{-1}$ (range $0 \mu\text{g g}^{-1}$ to $0.1 \mu\text{g g}^{-1}$).

In one office, no Der p 1 and Der 2 were found, and the level of Der f 1 was $0.1 \mu\text{g g}^{-1}$. No Der f 1 and Der 2 levels were found in the textile plant, and the level of Der p 1 was $0.1 \mu\text{g g}^{-1}$. In the paper-recycling plant, the levels of Der p 1, Der f 1, and Der 2 were $0.45 \mu\text{g g}^{-1}$, $0.25 \mu\text{g g}^{-1}$, and $0.25 \mu\text{g g}^{-1}$, respectively.

Table 2 Dust samples with microscopically identified mites (N = number of analysed dust samples)

Samples	Number of mites	Pyroglyphid mites				Non-pyroglyphid mites					
		DP	DF	EM	Total	LD	Bs	TP	As	Tar	Total
Grocery store (N=1)	34	-	-	-	-	-	4	-	30	-	34
Barley (N=1)	10	-	-	-	-	4	-	4	3	-	10
Hay (N=1)	3	-	-	-	-	-	-	2	1	-	3
Animal food (N=1)	4	-	-	-	-	-	-	2	-	-	4
Swine farm (N=1)	2	-	-	-	-	-	-	2	-	-	2
Fishing boats											
Cabin beds (N=5)	34	23	-	1	24	1	-	-	1	8	10
Cabin floors (N=5)	1	1	-	-	1	-	-	-	-	-	-

Legend: DP-Dermatophagoides pteronyssinus; DF-Dermatophagoides farinae; EM-Euroglyphus maynei; LD-Lepidoglyphus destructor; Bs-Blomia species; TP-Tyrophagus putrescentiae; As-Acarus species; Tar-Tarsonemidae.

DISCUSSION

Dust mites are generally acknowledged as the most important sources of indoor allergens all over the world (6, 7). Pyroglyphid dust mites are common in households, settling mainly in mattresses, pillows and beddings (6, 17). Non-pyroglyphid dust mites are mostly described as exclusive sources of rural occupational allergens, but recent data also show their presence in rural households (18, 19) and a significant sensitisation to these mites was established in general urban population (20). Pennanen *et al.* (21) found non-pyroglyphid mites as occupational contaminants in animal laboratory facilities, particularly in animal food storages, but not in animal cages and rooms where they were kept, which is in accordance with our findings. Pyroglyphid mites seem not to dwell in mammal nests, including swine confinement houses, in contrast to bird nests and poultry farms where they are common (22, 23).

Another rarely studied issue is occupational exposure to dust mites in urban, particularly industrial environments. Our earlier study showed a significantly higher prevalence of positive skin-prick tests to the house dust mite *D. pteronyssinus* in certain groups of industrial workers (meat processing, animal food processing, swine farming, textile plant) in comparison with control subjects from a general population of the same region (24). These findings indicate that dust mites may cause work-related sensitisation and symptoms in industries where microclimate conditions and materials used in the working environment, particularly organic dust, are likely to favour mite growth. However, in diagnosing occupational allergy caused by dust mites, it is important to distinguish between the levels and duration of occupational and non-occupational exposure to dust mites. In this study, we found low or non-detectable exposure to pyroglyphid and non-pyroglyphid dust mites in the examined industrial environments, which suggests a significantly lower occupational than non-occupational exposure in the same region. Our earlier study investigated general environmental exposure to dust mites in the floor dust samples from households in two different climatic regions in Croatia (coastal region with the Mediterranean climate and inland region with the continental climate) (25). It seems that industrial indoor environments with ventilation systems and daily cleaning routine do not favour the reproduction and growth of dust mites. Everyday cleaning and low relative humidity are the probable causes of low

exposure levels to dust mites in offices, which was also confirmed by other authors (26, 27). However, the limitation of this study is in the small number of analysed dust samples. For each industrial setting included in this study, a single dust sample from only one working area was collected and analysed. Further studies with greater sample size and more working areas within industrial plants are needed to test our findings about industrial environments.

Exposure to dust mites in boats has seldom been studied. This study established high exposure to *D. pteronyssinus* with the median Der p 1 level of $10 \mu\text{g g}^{-1}$ in cabin beds on fishing boats operating in the Adriatic sea (coastal Croatia). Our earlier data showed a greater variety of dust mite species and a higher mite allergen levels in house dust from coastal than from inland Croatia. The levels of Der p 1 were significantly higher in the coastal than in the inland house dust samples (median levels $4.5 \mu\text{g g}^{-1}$: $0.85 \mu\text{g g}^{-1}$), and conversely, the levels of Der f 1 were greater inland than on the coast (median levels $0.88 \mu\text{g g}^{-1}$: $0 \mu\text{g g}^{-1}$) (25). These data suggest that high exposure to Der p 1 allergen is common in the beds of fishing boats, as well as in the households of the fishermen. However, fishermen have a more specific working schedule than average worker (28). Fishermen in this study spend on board three weeks a month, coming home only for five or six days when there is full moon. Therefore, their occupational exposure to *D. pteronyssinus* is more relevant for developing sensitisation or symptoms of allergic disease than non-occupational exposure at home. For comparison, King *et al.* (29) found pyroglyphid dust mites in dust samples from US naval ships, but the percentage of mite-infected samples was smaller on ships than in the households of military personnel. The high levels of dust allergens found in fishing boat cabins in our study are most likely due to the temperature and humidity which favour mite growth year-round, in combination with old furnishing and bedding, and poor cleaning practice.

CONCLUSIONS

According to these preliminary data, pyroglyphid and non-pyroglyphid dust mites were not established in a majority of examined urban dust samples where such exposure was expected, including samples from an insurance company archive, tobacco plant,

laboratory rat cages, fish-processing plant and winery. In the samples taken from offices, textile plant and paper-recycling plant, only low levels of pyroglyphid mite allergens were found ($<0.5 \mu\text{g g}^{-1}$). Non-pyroglyphid dust mites alone were established in several rural dust samples, including the warehouses of barley, hay, and animal food, a grocer's storeroom, and a swine confinement house. This suggests that these mites present occupational risk in various rural working environments.

Pyroglyphid dust mites were found in fishing boats, suggesting that pyroglyphid mites should be considered as work-related allergens for fishermen, not only because of high levels of the Der p 1 allergen ($\geq 10 \mu\text{g g}^{-1}$) established in cabin bed samples (mattresses and pillows), but also due to the fishermen's work schedule.

Further studies with greater sample sizes are needed to test our findings from industrial environments and fishing boats.

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Sažetak

ANALIZA UZORAKA PRAŠINE U GRADSKOM I SEOSKOM RADNOM OKOLIŠU U HRVATSKOJ

U ovome istraživanju ispitana je izloženost prašinskim grinjama u uzorcima prašina iz različitih radnih sredina u Hrvatskoj. Ukupno je analizirano 29 uzoraka prašine: 10 iz gradskih područja (uredi, arhiva osiguravajućeg društva, tvornica duhana, tvornica recikliranog papira, tvornica tekstila i prerade riba, eksperimentalni laboratorij, vinarija), devet iz seoskih područja (skladišta ječma, sijena, stočne hrane i brašna; krojački obrti, tvornica za preradu drva, skladište trgovine mješovitom robom, svinjogojilište), te 10 iz pet kabina ribarskih brodova (pet uzoraka s poda i pet s kreveta).

Grinje su identificirane mikroskopski, a razine alergena Der p 1, Der f 1 i Der 2 određene su DUSTSCREEN™ testom (Heska AG, Switzerland).

Mikroskopski grinje nisu nađene u uzorcima prašina iz gradskih sredina. Piroglifidne grinje nađene su u svih pet uzoraka s kreveta kabina ribarskih brodova, a nepiroglifidne grinje u uzorcima iz skladišta ječma, sijena, stočne hrane, svinjogojilišta, skladišta trgovine mješovitom robom i ribarskih brodova. Razine alergena piroglifidnih grinja utvrđene su u 8 od 10 uzoraka prašine s ribarskih brodova. Medijan razina alergena Der p 1 bio je $10 \mu\text{g g}^{-1}$, Der f 1 $0,2 \mu\text{g g}^{-1}$, a Der 2 $3,5 \mu\text{g g}^{-1}$.

Na ribarskim je brodovima utvrđena povećana izloženost piroglifidnim grinjama, što ukazuje da piroglifidne grinje mogu biti profesionalni rizični čimbenik za ribare. Rezultati ovoga istraživanja potvrdili su nepiroglifidne grinje kao rizični čimbenik u različitim seoskim radnim sredinama.

KLJUČNE RIJEČI: *Acaridae, alergeni, poljoprivredni radnici, prašinske grinje, Pyroglyphidae, radna izloženost, ribari*

REQUESTS FOR REPRINTS:

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