

Exposure to poultry dust and health effects in poultry workers: impact of mould and mite allergens

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Abstract

Purpose The aim of the study was to evaluate exposure to moulds and house dust mite *Dermatophagoides pteronyssinus* in poultry farms, and related health effects in poultry workers (PW).

Methods The study involved 41 PW and 45 control office workers. Working environment was evaluated for *D. pteronyssinus* allergen (Der p 1), moulds and endotoxin. In workers, eye, skin and respiratory symptoms, ventilatory lung function, atopy markers (skin prick test to inhalatory allergens, total IgE) and specific IgG to moulds were assessed.

Results Der p 1 levels ranged <0.1 – 3.3 $\mu\text{g/g}$, exposure to fungi was 4.9×10^3 – 6.8×10^4 cfu/m^3 , with prevailing *Aspergillus*, *Penicillium* and *Mucor* species, and endotoxin levels ranged 230–284 EU/m^3 . In comparison to control subjects, significantly higher prevalence of work-related nose, asthma, eye and skin symptoms, and slight decline in ventilatory lung function was found in PW. PW had significantly higher prevalence of IgG antibodies to moulds comparing to controls (63 vs. 36%, respectively, $P = 0.01$), especially to *Alternaria* and *Aspergillus* species. The prevalence of atopy markers in PW was lower than in population-based studies.

Conclusions Hazardous levels of Der p 1, endotoxin and moulds were determined in poultry houses. High prevalence

of work-related symptoms and IgG antibodies to moulds was found in PW. Healthy worker effect is proposed as an explanation of low atopy markers prevalence among PW.

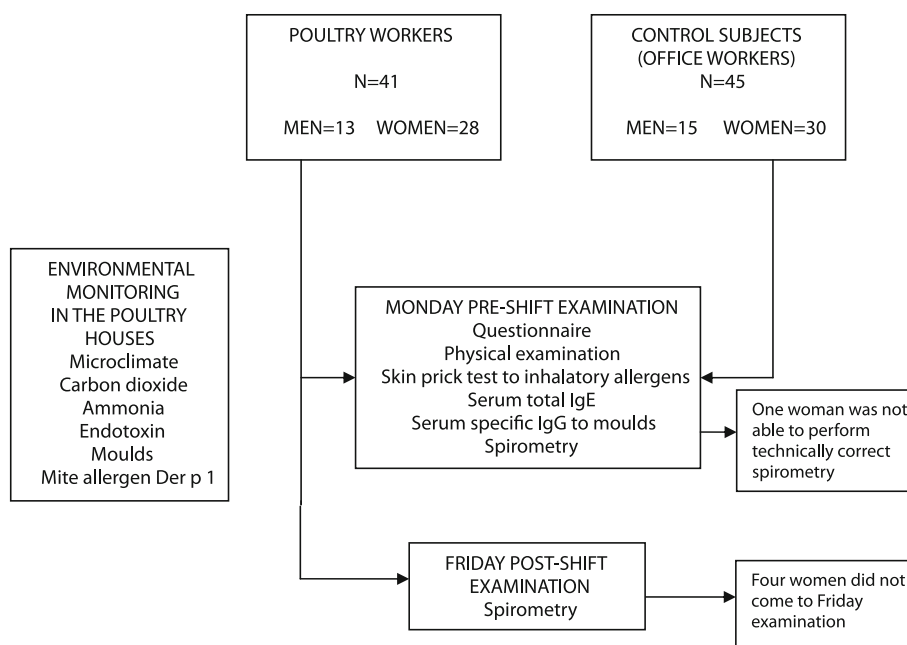
Keywords Poultry workers · Hypersensitivity · Moulds · Dust mites · Der p 1 · Endotoxin · Healthy worker effect

Introduction

Agricultural workers, especially pig and poultry farmers, are at increased risk of occupational respiratory diseases (Kogevinas et al. 1999; Senthilselvan et al. 1997; Novak 1994; Radon et al. 2001a). Exposure to organic dust is one of the most recognized respiratory hazards associated with animal production. Dust, bacteria, moulds, endotoxin and ammonia are considered central elements in daily exposure of agricultural workers (Omeland 2002). These substances are known to cause allergic and non-allergic rhinitis, asthma, extrinsic alveolitis, organic dust toxic syndrome and can also induce chronic bronchitis (Radon et al. 2001a). Epidemiological studies showed increased prevalence of respiratory symptoms and adverse changes in pulmonary function parameters in poultry workers (Radon et al. 2001b; Rylander and Carvalheiro 2006). Little is known, however, about the occurrence of sensitization to allergens common in poultry houses. Although pyroglyphid (“house dust”) mites may be present in bird nests, including poultry houses (Macan et al. 2005; Spiekma 1997), exposure to house dust mites in poultry farms is not well defined and occupational health risks of exposure to these ubiquitous allergens have been rarely studied so far. Additionally, data regarding sensitization to moulds in poultry workers are also scarce in the literature.

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Fig. 1 Study protocol

In the present study, we evaluated the exposure to moulds and house dust mite *Dermatophagoides pteronyssinus* (*Pyroglyphidae*) on two poultry farms. Health effects of occupational exposure to organic dust in poultry workers, including allergic sensitization and the effects on respiratory system, were also examined.

Methods

Subjects and study protocol

This study was designed as a cross-sectional study in accordance with Helsinki Declaration, and approved by the Ethical Committee. Poultry workers from two poultry houses with laying hens for egg production from Zagreb and its surroundings were included, and a control group comprised office workers from the same region with no occupational exposure to organic dust. All subjects volunteered for the study. They were informed about the study protocol and had to sign a consent form. All participants were free to withdraw from the study at any time.

Study protocol is presented in Fig. 1. A total number of 58 poultry workers and 176 control subjects employed in an insurance company and a library were examined. Response rates were 77% for poultry workers and 30% for controls. The study involved 41 poultry workers who reported more than 20 h per week spent inside the poultry house. They all worked from Monday to Friday, 8 h per day, and had an additional 5-h shift on Saturday. Out of 176 control subjects, stratified random sample of 30 women and 15 men was frequency matched to poultry

workers by sex, smoking habit (active smoker or non-smoker) and age. Only subjects who never smoked were designated as non-smokers. Main characteristics of study subjects are presented in Table 1.

Environmental monitoring in poultry houses

Environmental monitoring was conducted in May on two poultry farms for egg production situated near Zagreb, each housing about 15,000 laying hens of Shaver breed. Each farm had several poultry houses sized 84.00 × 12.00 m. Hens were kept in cages sized 80 × 45 × 45 cm. Cages were placed in three levels with 8–10 hens per cage. Poultry houses had mechanical lateral and ceiling ventilation and automated feeding of hens. Fester cleaning was performed once a week.

Monitoring was performed in one poultry house on each farm (poultry farm I and II) as a single day measurement. Microclimate parameters were determined and air samples collected for airborne total dust, moulds and endotoxin determination from five locations in each poultry house. Sampling locations were chosen randomly through corridors at the level of the second cage floor in order to obtain a mean bioaerosol exposure in each analysed poultry house. Settled dust samples for measurement of dust mite allergen levels were collected with clean brush into clean plastic bags from floors and horizontal surfaces above the floor in poultry houses. Horizontal surfaces were located around the second cage level representing parts of the feeder and ventilation system. A total of 10 (five floor and five horizontal surfaces) and 7 (three floor and four horizontal surfaces) settled dust samples were collected from

Table 1 Descriptive data of poultry workers and control group

	Total			Women			Men		
	Controls	Poultry workers	<i>P</i>	Controls	Poultry workers	<i>P</i>	Controls	Poultry workers	<i>P</i>
Number of subjects	45	41		30	28		15	13	
Age [y, mean $\pm$ SD]	44.9 $\pm$ 8.74	46.0 $\pm$ 8.71	0.556	43.5 $\pm$ 8.08	44.0 $\pm$ 8.77	0.809	47.7 $\pm$ 9.59	50.3 $\pm$ 7.15**	0.434
Duration of work as a farmer [y, mean $\pm$ SD]	–	17.6 $\pm$ 8.00	–	–	17.1 $\pm$ 7.72	–	–	18.5 $\pm$ 8.75	
Smokers [N (%)]	17 (37.8)	21 (51.2)	0.210	8 (26.7)	10 (35.7)	0.573	9 (60.0)*	11 (84.6)**	0.221
Smoking index in smokers [median (range)]	480 (10–900)	200 (0.2–1600)	0.354	350 (100–600)	37 (0.2–600)	0.050	600 (10–900)	520 (100–1600)**	0.594

P: Significance of the difference between poultry workers and control subjects

* Significant difference between female and male controls, *P* < 0.05

** Significant difference between female and male poultry workers, *P* < 0.05

poultry house I and II, respectively. Air filters and dust samples were stored at -20°C until analysed.

Air temperature ($^{\circ}\text{C}$), relative humidity (%) and airflow rate (m/s) were determined with a TESTO device (Testo Inc., Germany). The concentration of ammonia and carbon dioxide was determined with a Dräger-Multiwarn II device (Dräger, Darmstadt, Germany). Dust was sampled onto glass fibre filters (Whatman International Ltd., Maidstone, UK) with an SKC pump (SKC Ltd., Blandford Forum, UK). The airflow rate was 4.0 l/min. Filters were weighed before and after sampling in a controlled laboratory at air temperature of 22°C and relative humidity of 45% ($\pm 5\%$). Air samples for determination of mould concentrations were collected with Merck MAS-100 (Merck KGaA, Darmstadt, Germany) device onto commercial Sabouraud medium (Biolife, Milan, Italy). Medium was then incubated for 5 days on 22°C . Grown mould colonies were counted with mechanical Colony Counter and additional correction (Anonimus 1998), and expressed as colony forming units per m^3 (CFU/ m^3). Mould species were identified microscopically in native preparations.

Endotoxins were analysed using the end-point *Limulus* amoebocyte lysate method (LAL), as previously described (Varnai et al. 2004). A commercial kit for endotoxin assay was supplied by Endochrome (Charles River Endosafe, USA). Extraction procedure of filter samples and endotoxin analysis were performed on the same day according to general recommendations (Anonymous 1987). Sterile and pyrogen-free glassware and microplates (Greiner Labor-technik, GmbH, Germany) were used. Each sample was analysed in duplicate. Optical density was read at 405 nm on a Personal Lab microplate analyzer (Iason, Graz, Austria). Endotoxin concentrations were read from the standard curve and expressed in endotoxin units per 1 m^3 (EU/ m^3).

Blank filters revealed a very low level of background endotoxin. Recovery of the assay was 95%. The assay limit of detection was 0.008 EU/ml.

Concentrations of dust mite allergen Der p 1 (major *Dermatophagoides pteronyssinus* allergen) were measured by double monoclonal antibody (mAb)-based enzyme immunoassay (ELISA) as described previously (Prester et al. 2007). Set of monoclonal antibodies (5H8 and 4C1) and standard of Der p 1 were purchased from Indoor Biotechnologies Ltd (Cardiff, UK). The intensity of the colour was measured by a microtiter plate reader (absorbance at 450 nm) on PersonalLab (IASON, Graz, Austria). The concentrations of mite allergens were calculated from the standard curve. Each allergen sample was measured in duplicate. The low limit of detection was 2 ng/mL. The intra- and interassay variations were determined to be 6.9 and 13.1%, respectively.

Questionnaire

A questionnaire based on “Organic dust questionnaire” (Rylander et al. 1990) was completed by a physician interviewing each subject. Smoking habit was expressed as dichotomous variable (smokers and non-smokers) and as smoking index (number of cigarettes smoked per day $\times$ number of smoking years). Subjects who never smoked were designated as non-smokers. Questionnaire recorded reports on occurrence of work-related eye, respiratory, skin and general symptoms during the last 12 month. Eye symptoms included eye itching and lacrimation; nasal symptoms included sneezing, rhinorrhea, nasal itching and nasal obstruction (not related to common cold); asthmatic symptoms included wheezing and dyspnea; skin symptoms included itching, erythema, rash,

eczema (vesicular rash and itching) and urticaria; and general symptoms included fever and joint pain. The variable “work-related symptoms” was defined as a presence of at least one eye, nasal, asthmatic, skin and general symptom.

Contraindications for clinical tests included in the study were evaluated for each subject during the questionnaire completion and physical examination.

Skin prick testing

In all subjects, skin prick testing (SPT) was performed by standard method (Dreborg and Frew 1993) with a panel of common inhalatory allergens: grass pollen mixture, birch, hazel, weed (*Ambrosia elatior*, *Artemisia vulgaris*) pollens, mites *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae* and *Lepidoglyphus destructor*, cat, dog and moulds *Cladosporium herbarum* and *Alternaria alternata* (Allergopharma, Germany). Poultry workers were additionally tested with hen egg, hen feather and moulds *Aspergillus fumigatus*, *Penicillium notatum* and *Mucor mucedo*. SPT included testing with positive control solution (10 mg/mL of histamine hydrochloride) and negative control solution (buffer). Skin reaction (wheal) was evaluated after 15 min. The mean skin reaction (mean wheal diameter) was calculated according to the formula $(D + d)/2$, where D represents the largest longitudinal diameter and d its mid-point orthogonal diameter in mm. For statistical evaluation of SPT results, difference between mean skin reaction to each allergen and negative control solution was used as a parameter of SPT reactivity. The results of SPT were considered positive when mean wheal diameter was larger than the negative control for more than 3 mm.

Total IgE

Serum total IgE antibodies were measured from venous blood samples by the ELISA method (IASON, Austria) (Aberer and Kranke 2002). IgE levels were expressed in kIU/L.

Specific IgG to moulds

Precipitating IgG antibodies to moulds *Aspergillus species*, *Mucor racemosus*, *Penicillium species*, *Rhizopus nigricans*, *Fusarium moniliforme* and *Alternaria alternata* were detected in sera from all poultry and control workers by two-way immunodiffusion in agar gel (Ouchterlony 1958). Mould antigens were obtained from Institute of Immunology, Zagreb, Croatia. Variable “IgG to moulds” was expressed as dichotomous variable (positive or negative).

Spirometry

As common ventilatory function parameters, forced vital capacity (FVC), forced expiratory volume in one second (FEV₁), FEV₁/FVC ratio (Tiffnaoud index), forced expiratory flow at 50% of FVC (FEF₅₀), forced expiratory flow between 25 and 75% of FVC (FEF_{25–75}) and forced expiratory flow at 75% of FVC (FEF₂₅) were determined by standard method (Quanjer et al. 1993) using the spirometer Pneumoscreen II (Jaeger, Würzburg, Germany). At least three measurements were recorded per subject, and the best value was used for analysis. Ventilatory function parameters were expressed and analysed as measured values, percentage of referent values, and number of subjects with ventilatory function parameters below referent range (<80% for FVC and FEV₁, <70% for FEV₁/FVC ratio, and <60% for FEF₅₀, FEF_{25–75} and FEF₂₅).

Statistical analysis

Statistical analysis was performed using software Stata/SE 9.0 for Windows (StataCorp LP, USA). Significant differences between control subjects and poultry workers were tested by Student's *t*-test for independent samples, Mann–Whitney *U* test and Pearson χ^2 or Fisher's exact test. To control for the possible effect of smoking on ventilatory function parameters, especially due to high number of smokers found in male poultry workers (11 out of 13), the analysis was performed separately in smoking subjects of both sexes and in non-smoking women. Two-way analysis of covariance (ANCOVA) with occupational exposure and sex as factors and age and smoking index as covariates was applied in smoking subjects, and one-way ANCOVA with age as covariate in non-smoking women. Analyses were done for measured values and percentage of referent values. Homoskedasticity and normality of residuals were checked for each model. Monday to Friday differences in exposed subjects were tested by Student's *t*-test for paired samples and McNemar's χ^2 test. Distribution of data was tested by Shapiro–Wilk normality test. The results were considered statistically significant at value of $P < 0.05$.

Results

Environmental measurements

Environmental parameters at two poultry farms are presented in Table 2.

Most prevalent moulds at poultry farm I were *Penicillium species*, *Fusarium species*, *Aspergillus species* and *Mucor species*. At poultry farm II, *Aspergillus species*,

Table 2 Environmental measurements (median and range)

Variable	Poultry farms			
	I		II	
	N ^a	Values	N ^a	Values
Temperature (°C)	5	29.7 (27.4–30.8)	5	18.9 (18.3–19.7)
Air humidity (%)	5	46.9 (44.4–47.2)	5	77.6 (74.4–81.1)
Air velocity (m/s)	5	0.17 (0.13–0.19)	5	0.11 (0.04–0.13)
NH ₃ (ppm)	5	7 (3–8)	5	2.71 (0–4.00)
CO ₂ (vol%)	5	0.03 (0.03–0.08)	5	0.03 (0.03–0.04)
Total dust (mg/m ³)	5	0.35 (0.20–0.50)	5	1.10 (0.70–1.50)
Total fungi (cfu/m ³)	5	1.27 × 10 ⁴ (4.90 × 10 ³ –3.12 × 10 ⁴)	5	2.59 × 10 ⁴ (7.60 × 10 ³ –6.84 × 10 ⁴)
Endotoxin (EU/m ³)	2	233.8 (230.0–237.5)	1	283.8
Mite allergen Der p 1 (µg/g)	10	0.78 (<0.10–3.30)	7	<0.10

^a Number of samples/measurements

Mucor species, *Rhizopus species*, *Scopulariopsis species* and *Penicillium species* prevailed.

Measurable levels of Der p 1 were determined only in poultry farm I with wide range from <0.1 to 3.3 µg/g. In 5 floor dust samples, Der p 1 levels ranged from <0.1 to 0.79 µg/g, and in five samples from horizontal surfaces from 0.42 to 3.3 µg/g.

Ventilatory function testing

Ventilatory function parameters were measured at the beginning and at the end of the working week (Monday

pre-shift and Friday post-shift spirometry) in poultry workers, and at the beginning of the working week in control subjects. Results are shown on Fig. 2 only for smoking subjects due to the high number of smokers, especially in males. In smoking subjects, two-way ANCOVA (with occupational exposure and sex as factors and age and smoking index as covariates) showed significantly lower values of FEF_{25–75} and near-significantly lower FEF₅₀ measured on Monday in poultry workers compared to controls. Significant difference for FEF_{25–75} was found both for measured values (L/s) ($P = 0.028$) and for percentage of predicted values ($P = 0.045$). Near-significantly lower

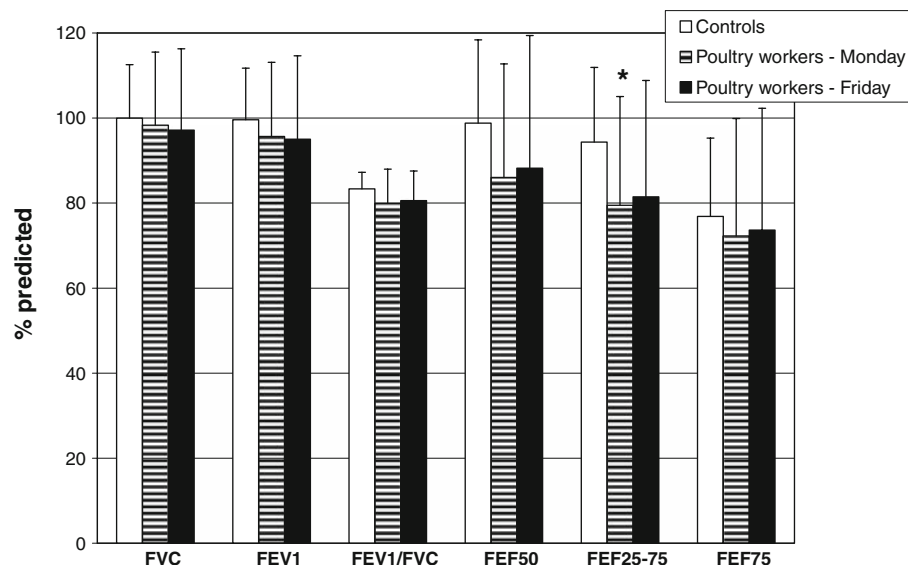


Fig. 2 Ventilatory function parameters in smoking controls and poultry workers of both sexes. Values are expressed as percent of referent value (% predicted). Spirometry was performed at the beginning and at the end of the working week (Monday pre-shift and Friday post-shift spirometry) in poultry workers, and at the beginning of the working week in control subjects. There were 17 controls (9

men) and 20 poultry workers (11 men). Two poultry workers had no spirometry test on Friday. * Statistically significant difference between controls and poultry workers by two-way ANCOVA; $P_{\text{model}} = 0.021$, $P = 0.045$ for the effect of occupational exposure; the effect of sex was not significant ($P = 0.699$)

Table 3 The prevalence of subjects with ventilatory function parameters below referent values in poultry workers and controls

	Smoking controls (male and female)	Smoking poultry workers (male and female)		Female non-smoking controls	Female non-smoking poultry workers	
		Monday	Friday		Monday	Friday
Number of subjects	17	20	18	22	18	16
FVC < 80% predicted	1	3	3	1	3	1
FEV ₁ < 80% predicted	1	5	4	1	1	1
FEV ₁ /FVC < 70% predicted	0	3	2	1	0	0
FEF ₅₀ < 60% predicted	1	3	3	4	0	0
FEF _{25–75} < 60% predicted	1	4	3	5	0	1
FEF ₇₅ < 60% predicted	2	6	5	7	1	3

Results are present as number of subjects with spirometric parameters below expected values

FVC forced vital capacity, FEV₁ forced expiratory volume in one-second, FEF₅₀ forced expiratory flow at 50% of FVC, FEF_{25–75} forced expiratory flow between 25 and 75% of FVC; FEF₇₅ forced expiratory flow at 25% of FVC

There were no significant differences ($P < 0.05$) between smoking controls and poultry workers. Near-significantly higher prevalences of FEF_{25–75} ($P = 0.054$) and of FEF₇₅ ($P = 0.053$) were found in female non-smoking controls compared to poultry workers (Fisher's exact test). Male non-smoking subjects were not analysed due to low number of non-smoking poultry workers (2 out of 13). There were no significant differences between Monday and Friday prevalences in poultry workers (McNemar's test)

FEF₅₀ ($P = 0.056$) was found only for measured values (L/s). In non-smoking women, one-way ANCOVA (with age as covariate) did not show significant differences in spirometric values between control and exposed group (data not shown). There was no increase in the prevalence of subjects with parameters below referent values in poultry workers compared to controls, neither in smoking subjects of both sexes nor in non-smoking women (Table 3). No significant differences in ventilatory function parameters were found between Monday and Friday values in poultry workers (Fig. 2; Table 3).

Hypersensitivity markers

Table 4 presents the prevalence of positive SPT to common inhalatory allergens, dust mites, moulds, hen egg and feather and results of total serum IgE levels.

The prevalence of positive SPT with common inhalatory allergens including dust mites was higher in control group than in poultry workers (42.2 vs. 12.2%, respectively, $P = 0.003$). The prevalence of subjects with positive SPT to dust mites was similar in poultry workers and control subjects. Positive SPT to moulds was found in two female poultry workers (positive SPT to *Mucor mucedo*), and one male control subject (positive SPT to *Alternaria alternata*).

Total IgE values were similar in both groups, but the prevalence of subjects with elevated total IgE was higher in controls than in poultry workers, with near significant difference (26.7 vs. 10%, respectively, $P = 0.057$).

Serum-specific IgG to moulds are presented in Table 5. The prevalence of subjects with positive IgG to one or more tested moulds was significantly higher in poultry workers than in controls (63.4 vs. 35.6%, respectively,

$P = 0.010$), as well as the prevalence of subjects with positive IgG to *Alternaria alternata* (22 vs. 2.2%, respectively, $P = 0.015$). When analysed separately in women and men, these differences were found only in females. The prevalence of subjects with positive IgG to *Aspergillus* species was also significantly higher only in female poultry workers in comparison to female controls (21.4 vs. 0%, respectively, $P = 0.009$).

Symptoms

The prevalence of self-reported work-related symptoms including eye, nose, skin and asthma symptoms was significantly higher in poultry workers in comparison to control group (Table 6). No general symptoms were reported by poultry workers or control subjects.

Discussion

In this study, we evaluated potentially hazardous exposures in poultry houses, emphasizing the presence of mite and mould allergens and related health effects in poultry workers.

Ammonia, carbon dioxide and total dust concentrations were considerably below the Croatian occupational exposure limits (ammonia: 25 ppm for 8-h exposure and 35 ppm for short-term exposure; CO₂: 5,000 ppm for eight-hour exposure and 30,000 ppm for short-term exposure; total dust: 10 mg/m³) (Croatian regulation on maximal permissible concentrations of hazardous substances at the workplace and biological exposure limits, 1993) and below values usually observed in poultry houses with

Table 4 Markers of IgE-mediated hypersensitivity in poultry workers and controls

	Total			Women		Men	
	Controls	Poultry workers	<i>P</i>	Controls	Poultry workers	Controls	Poultry workers
Number of subjects	45	41		30	28	15	13
Positive SPT [<i>N</i> (%)]							
Common inhalatory allergens (≥ 1 positive) ^a	19 (42.2)	5 (12.2)	0.003*	10 (33.3)	3 (10.7)	9 (60.0)	2 (15.4)
Mites							
<i>Dermatophagoides pteronyssinu</i>	4 (8.9)	3 (7.3)	1.000	2 (6.7)	2 (7.1)	2 (13.3)	1 (7.7)
<i>Dermatophagoides farinae</i>	5 (11.1)	1 (2.4)	0.205	3 (10)	0	2 (13.3)	1 (7.7)
<i>Lepidoglyphus destructor</i>	3 (6.7)	1 (2.4)	0.618	2 (6.7)	0	1 (6.7)	1 (7.7)
Moulds							
<i>Alternaria alternata</i>	1 (2.2)	0	1.000	0	0	1 (6.7)	0
<i>Cladosporium herbarium</i>	0	0	–	0	0	0	0
<i>Aspergillus fumigatus</i>	–	0	–	–	0	–	0
<i>Penicillium notatum</i>	–	0	–	–	0	–	0
<i>Mucor mucedo</i>	–	2 (4.9)	–	–	2 (7.1)	–	0
Hen feather	–	0	–	–	0	–	0
Hen egg	–	0	–	–	0	–	0
IgE							
Total IgE [kIU/L, median (range)]	24.4 (0–1,000)	21.8 (0.7–743.4) ^b	0.625	15 (0–910.8)	18.9 (0.7–243.8)	168.6 (2.75–1,000)	40.8 (5.5–743.4) ^b
Elevated total IgE [<i>N</i> (%)]	12 (26.7)	4 (10) ^b	0.057	4 (13.3)	1 (3.6)	8 (53.3)	3 (25) ^b

P: difference between controls and poultry workers by Fisher's exact test. Analysis was performed for total number of subjects, and not separately in men and women

SPT Skin prick test

* Significant difference between poultry workers and control subjects, $P < 0.05$

^a Grass pollens, hazel, birch, *Ambrosia elatior*, *Artemisia vulgaris*, cat, dog, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Cladosporium herbarum*, *Alternaria alternata*

^b IgE was not measured in one male poultry worker

Table 5 Specific IgG to moulds in poultry workers and controls

	Total			Women			Men		
	Control group	Poultry workers	<i>P</i>	Control group	Poultry workers	<i>P</i>	Control group	Poultry workers	<i>P</i>
Number of subjects	45	41		30	28		15	13	
Positive specific IgG (≥ 1 positive of listed below) [<i>N</i> (%)]	16 (35.6)	26 (63.4)	0.010*	6 (20.0)	19 (67.9)	0.000*	10 (66.7)	7 (53.8)	0.700
<i>Aspergillus species</i>	3 (6.7)	10 (24.4)	0.069	0	6 (21.4)	0.009*	3 (20.0)	4 (30.8)	0.670
<i>Mucor racemosus</i>	0	2 (4.9)	0.494	0	2 (7.1)	0.229	0	0	–
<i>Penicillium species</i>	2 (4.4)	4 (9.8)	0.677	2 (6.7)	2 (7.1)	1.000	0	1 (7.7)	0.464
<i>Rhizopus nigricans</i>	13 (28.9)	17 (41.5)	0.503	5 (16.7)	10 (35.7)	0.136	8 (53.3)	6 (46.2)	1.000
<i>Fusarium moniliforme</i>	3 (6.7)	6 (14.6)	0.485	2 (6.7)	3 (10.7)	0.665	1 (6.7)	2 (15.4)	0.583
<i>Alternaria alternata</i>	1 (2.2)	9 (22.0)	0.015*	0	6 (21.4)	0.009*	1 (6.7)	2 (15.4)	0.583

P: difference between controls and poultry workers by Fisher's exact test

* Significant difference between poultry workers and control subjects, $P < 0.05$

Table 6 The prevalence of work-related eye, respiratory, skin and general symptoms in poultry workers and controls

	Total			Women		Men	
	Control group	Poultry workers	<i>P</i>	Control group	Poultry workers	Control group	Poultry workers
Number of subjects	45	41		30	28	15	13
Eye symptoms ^a	5 (11.1)	14 (34.1)	0.018*	5 (16.7)	12 (42.9)	0	2 (15.4)
Nose symptoms ^b	4 (8.9)	16 (39.0)	0.002*	3 (10.0)	14 (50.0)	1 (6.7)	2 (15.4)
Skin symptoms ^c	2 (4.5)	12 (29.3)	0.003*	2 (6.7)	10 (35.7)	0	2 (15.4)
Asthma symptoms ^d	0	16 (39.0) ^f	0.000*	0	11 (39.3)	0	6 (46.2)
General symptoms ^e	0	0	–	0	0	0	0

Results are presented as [*N* (%)]

P: difference between controls and poultry workers by Fisher's exact test. Analysis was performed for total number of subjects, and not separately in men and women

* Significant difference between poultry workers and control subjects, *P* < 0.05

^a Itching and/or lacrimation

^b Itching, obstruction, rhinorrhea and/or sneezing

^c Itching, rash, erythema, eczema and/or urticaria

^d Wheezing and/or dyspnea

^e Fever and/or joint pain

^f Asthma symptoms were equally distributed among smoking and non-smoking poultry workers (eight subjects with asthma symptoms were smokers)

laying hens (Clark et al. 1983). Endotoxin levels in the air from poultry houses (230–284 EU/m³) were about 10 times lower than reported in other studies (Omeland 2002; Radon et al. 2002). However, comparison with other studies is somewhat impaired due to methodological differences in endotoxin measurement. Contrary to our study in which end-point LAL method was applied, majority of published data are obtained with kinetic LAL test. Other inter-laboratory variations could also influence the results (e.g. variations in extraction and collection, storing and handling of samples, high variability in potency of different batches of LAL reagents) (Varnai et al. 2004). Low endotoxin levels paralleled with low levels of total dust, confirming previous observations about the positive correlation between these indoor environmental parameters (Vučemić et al. 2008). No exposure limits are available for endotoxins so far. Several proposals were made for an exposure standard ranging from 5–200 ng/m³ (about 50–2,000 EU/m³). The National Health Council of the Netherlands has recently proposed an exposure limit of 4.5 ng/m³ (45 EU/m³), while the International Commission on Occupational Health proposed an occupational exposure limit of 12.5 ng/m³ (125 EU/m³) (Radon et al. 2002). The value of 10 ng/m³ (100 EU/m³) is suggested as a threshold for airway inflammation (Rylander and Carvalheiro 2006). Therefore, endotoxin levels established in this study can be considered hazardous for respiratory system.

Der p 1 levels measured in poultry houses, with median value 0.78, range <0.1–3.3 µg/g, were moderate and comparable to levels found in house dust samples from the

same region. Our previous study established Der p 1 median value 0.85, range 0.18–2.45 µg/g, in house dust from urban homes in Zagreb, and Der p 1 median value 2, range 0–20 µg/g, in house dust from rural homes in Zagreb surroundings (Macan et al. 2003). Although the exposure to house dust mite allergen in poultry houses did not exceed general exposures in homes, our results present the evidence that this allergen can be found in poultry houses and should be identified as one of the specific hazards present in poultry dust in levels hazardous for the induction of sensitization (>2 µg Der p 1/g of dust) (Dreborg 1998). According to our results, such hazardous levels were found only in dust samples from horizontal surfaces above the floor in the poultry house which were near hen cages, while floor levels did not exceed 1 µg/g. Such result was expected because bird nests, i.e. hen cages, should be considered as reservoirs of dust mites (Macan et al. 2005; Spiekma 1997). Therefore, allergens of the house dust mite *Dermatophagoides pteronyssinus* can be considered relevant work-related allergens for poultry workers with hazardous levels in and around hen cages. It should be expected that disturbances and movements in cages will contribute to the spreading of dust mites and their excreta in the air, making inhalable allergen levels higher.

In this study, high exposure to fungi was found in poultry houses, comparable to a number of previous findings in this type of animal confinement buildings (Omeland 2002). Dominating species were *Penicillium*, *Mucor* and *Aspergillus*, which were considered also by other authors as the most prevalent fungi species in occupational

environments (Lugauskas et al. 2004). Exposure levels established in this study (4.9×10^3 – 6.8×10^4 cfu/m³) were about 100 times higher than air levels established during summer in the same region (Segvić Klarić and Pepelnjak 2006). Some of the fungal species prevailing in this study (*Penicillium* and *Aspergillus* spp.) have been described to cause hypersensitivity reactions in humans, with clinical manifestations in the form of allergic rhinitis, asthma and extrinsic alveolitis (Halonen et al. 1997; Ostro et al. 2001; Horner et al. 1995).

It should be noticed that environmental monitoring for this study was limited to a single day measurement in one poultry house on each farm, and variations in levels of measured pollutants due to variations in animal and working activities should be expected. Nevertheless, recorded hazardous exposure levels of endotoxin, mite allergens and moulds should be expected to occur, at least intermittently.

Concomitantly to environmental monitoring in poultry houses, we evaluated the health effects in exposed workers, especially atopy and sensitization to moulds and mites found as constituents of poultry house organic dust. According to recent atopy definition, this disorder is defined as “personal and/or familial tendency, usually in childhood or adolescence, to become sensitized and produce IgE antibodies in response to ordinary exposures to allergens, usually proteins”. As a consequence, these persons can develop typical symptoms of asthma, rhinoconjunctivitis or eczema (Johansson et al. 2004). The prevalence of subjects with positive objective atopy markers in our study was higher in controls than in poultry workers. Number of subjects with positive SPT to common inhalatory allergens was 4 times higher, and the frequency of subjects with elevated total IgE was 3 times higher in controls than in poultry workers. The interpretation of this difference is impaired by the fact that the prevalence of atopy in control group was higher than that reported in population-based studies in Croatia (Macan et al. 2007; Munivrana et al. 2007), indicating selection bias. The response rate was 30% in the control group, and it is highly possible that individuals with atopic symptoms had higher motivation for volunteering in the study than healthy subjects. However, the prevalence of positive SPT and elevated total IgE was about 2 times lower in poultry workers than that observed in recent population-based study about the prevalence of atopy among Croatian subjects who underwent pre-employment examination at the very end of the twentieth century (1995–1999) (Macan et al. 2007). Bearing in mind high response rate among poultry workers (77%), the results suggest that atopy status affects the selection of occupation, with less atopic workers employed in poultry farms than should be expected according to population-based studies. Our results are in line with

previous findings suggestive of the “healthy worker effect” in population occupationally exposed to respiratory and skin hazards, including allergens (Hollander et al. 1997; Zock et al. 1998; Drexler et al. 1999; Bünger et al. 2000; Portengen et al. 2003; Tabar-Purroy et al. 2003). Due to the low number of atopics among poultry workers, findings of IgE-mediated sensitization (positive SPT) to occupational allergens like mites, moulds, hen egg or hen feather were scarce or absent. Two female poultry workers were sensitized to mould *Mucor mucedo*, and one of them reported work-related atopic symptoms, indicating that this mould may cause an atopic disease in the exposed subjects.

Subjects were also analysed for precipitating IgG antibodies to moulds *Aspergillus species*, *Mucor racemosus*, *Penicillium species*, *Rhysopus nigricans*, *Fusarium moniliforme* and *Alternaria alternata*, revealing different results among species. In poultry workers, the prevalence of subjects with positive IgG antibodies to *Aspergillus* spp. and *Alternaria alternata* was significantly higher than in control subjects. Precipitating IgG antibodies were only scarcely found for *Mucor racemosus* in both tested groups, while for *Penicillium species*, *Fusarium moniliforme* and *Rhysopus nigricans*, similar prevalences of IgG antibodies were found in both groups. These results could suggest different immunological activity among mould species in humans, especially for *Aspergillus*, *Alternaria* and *Mucor* species. Additionally, we should consider insufficient specificity of the antigen preparations, particularly for *Rhysopus nigricans*. Significant difference in the prevalence of positive IgG antibodies to *Aspergillus* spp. and *Alternaria alternata* was found only in females, supporting earlier results showing sex differences in production and regulation of immunoglobulins (Macan et al. 2007; Simell et al. 2008). Suggested sex differences in the production of immunoglobulins G need additional clarification in larger sample. None of the poultry workers reported any sign or symptom of extrinsic alveolitis, a disorder known to be related to the precipitating IgG antibodies. As suggested also by other authors (Skorska et al. 2007), it is likely that precipitins to moulds should be considered only as markers of exposure, or perhaps markers of immunological tolerance.

Despite of the absence of sensitization markers, slight decline in ventilatory function parameters was observed in poultry workers compared to controls (Fig. 2), and nearly 40% of them reported work-related asthmatic symptoms. This finding supports previous evidence of decline in lung function due to exposure to poultry dust (Rylander and Carvalheiro 2006; Zuskin et al. 1995). The prevalence of other self-reported work-related symptoms was also significantly higher in poultry workers than in controls (3 times for eye, 4 times for nose and 6.5 times for skin symptoms). High prevalence of respiratory symptoms,

especially nasal irritation, among farming population was shown also by other authors (Radon et al. 2001a). In the present study, the evaluation of respiratory health was limited to spirometry and the prevalence of respiratory symptoms, which are rather crude biomarkers of airway inflammation. More sensitive methods capable of detecting early, subclinical inflammatory changes in the airways, such as airway responsiveness, induced sputum analysis, exhaled nitric oxide or exhaled breath condensate analysis, would probably reveal more distinctive results on respiratory health in poultry workers (Rylander and Carneiro 2006; Lemiere 2007; Do et al. 2008; Fireman et al. 2008).

Conclusions

This study confirmed that work environment of poultry houses involves variable exposure to respiratory and skin irritants, pro-inflammatory agents and allergens. Hazardous levels of endotoxin, main allergen of dust mite *Dermatophagoides pteronyssinus* (Der p 1) and moulds (particularly *Aspergillus*, *Penicillium* and *Mucor* species) were determined. In line with these occupational exposures, significantly higher prevalence of work-related respiratory, eye and skin symptoms was found in poultry workers in comparison to control subjects. Poultry workers also had higher prevalence of IgG antibodies to moulds, especially *Alternaria* and *Aspergillus* species, and slight decline in ventilatory lung function. Results indicate that poultry houses as hazardous working environment should be considered selective for individuals with atopy who are susceptible to irritants and allergens. Therefore, less atopy was found among poultry workers than should be expected according to population-based studies. Consequently, low prevalence of markers of IgE-mediated sensitization to occupational allergens like mites, moulds, hen egg or feather was found among poultry workers.

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Conflict of interest statement The authors declare that they have no conflict of interest.

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