

Asthmatic symptoms and indoor levels of micro-organisms and house dust mites

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Summary

As a part of a worldwide investigation on the prevalence of respiratory symptoms, we have performed a study on the relationship between the indoor environment and asthma-like symptoms in the population of a central Swedish municipality. The study comprised 88 individuals, aged 20–45 years who underwent a structured interview, spirometry, a methacholine provocation test, skin-prick tests and blood samples for measurements of serum concentrations of eosinophil cationic protein (S-ECP), blood eosinophil count and total immunoglobulin E (S-IgE). In the homes, the room temperature, air humidity, respirable dust, house dust mites (HDM) and airborne micro-organisms were measured. The relative humidity in all the homes was found to be above 33%. HDM were found in 13% of homes. In the homes of the 47 subjects with asthma related symptoms, significantly higher total levels of bacteria and mould ($P < 0.05$) and a higher proportion of detected HDM ($OR = 5.3$) was found than in subjects with no asthma related symptoms, after adjustment for age, sex, smoking, indoor temperature and air humidity. HDM were found to be an independent risk factor for asthma related symptoms ($OR = 7.9$) and nocturnal breathlessness ($OR = 6.2$) ($P < 0.05$), while the total level of bacteria was a risk factor for asthma related symptoms and wheezing ($P < 0.05$). We conclude that although HDM is relatively infrequently found in the homes of central-Sweden, the presence of HDM is related to asthmatic symptoms. A relation between levels of airborne bacteria and asthma related symptoms was also found.

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Introduction

These days, interest in the impact of our environment upon respiratory diseases such as asthma is increasing. Asthma is the most common lung disease associated with indoor pollutants [1]. It affects about 5% of the Swedish population [2] and appears to be increasing in prevalence [3–5]. Since modern man spends 90% of his time indoors [6], the impact of the indoor environment is highly relevant.

Several studies have demonstrated a relationship

between damp housing conditions and the prevalence of airway symptoms [7,8]. Various types of exposure are related to dampness in buildings. Moulds or bacteria may grow on exposed surfaces inside the building or within the construction frame and many investigators have found mouldy environments to be related to respiratory symptoms [9,10]. Humid surroundings also enable the house dust mite (HDM) to thrive its faecal pellets comprise a major indoor allergen. Exposure to HDM has been considered to be an important determinant in the development of asthma, especially in early childhood [11,12] and epidemiological studies have suggested that levels of mite exposure exceeding $2 \mu\text{g}$ group I allergen/g of dust are relevant with regard to this [13].

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There is sparse information in the literature about the effects of bacteria on asthma and also about the relationship between indoor allergen exposure and serological markers of allergic inflammation.

The present study is a part of the European Community Respiratory Health Survey (ECRHS). The aim of this paper was to study the relationship between symptoms and objective signs of asthma and indoor exposure to micro-organisms, house dust mites and indoor dampness.

Materials and methods

Study population

A study of the prevalence of allergies and asthma is being conducted in 48 centres in 23 countries throughout Europe and in other parts of the world as part of the ECRHS [14]. Each study area has clearly defined geographical and administrative boundaries and a total population of at approximately 150 000 inhabitants (Fig. 1). Sweden contributes with data from three such areas, one of which is the municipality of Uppsala.

In December 1990, a screening questionnaire was mailed to 3600 men and women, aged 20–44 years, randomly selected from the population register of Uppsala. A random sample of 600 from this cohort, was selected for further investigation at the Department of Lung Medicine. This latter group was examined for signs of atopy and bronchial hyperresponsiveness (BHR), with skin-prick tests for 10 allergens and a bronchial provocation test with methacholine. Blood

samples were obtained and the subjects were all interviewed and examined by specially trained nurses. All subjects gave their informed consent.

For the present study of indoor environment two groups of subjects were chosen from the random sample of 600: (i) all those who in the screening questionnaire had reported one of the following: attacks of asthma in the last 12 months, nocturnal breathlessness in the last 12 months or current use of asthma medication. This comprised a total of 74 individuals; and (ii) 80 subjects who were randomly chosen from those that gave a negative answer to these same questions.

These 154 individuals were contacted by phone and offered an assessment of the indoor air in places of their dwelling. The field study was conducted during the period October 1991–April 1992, mostly in the evenings and on weekends, and parallel to the in-hospital investigations.

Laboratory results and the assessment of the subjects' lung health were not linked until the data collection had been completed. The protocol of the study was approved by the Ethical Committee of the Medical Faculty of Uppsala University.

Measurements

Room temperature, air humidity and respirable dust were measured in both the living room and the bedroom according to a standardized protocol. Also the concentration of viable moulds and bacteria and total concentrations of airborne moulds and bacteria and the levels of guanine in settled dust were measured in the bedrooms.

Room temperature and air humidity were recorded with an Assman psychrometer. Concentrations of respirable dust were measured with a direct reading instrument based on light scattering (Sibata P-5H2, Sibata Scientific Technology Ltd, Japan) calibrated by the manufacturer to 0.3 μm particles of stearic acid.

Airborne micro-organisms were sampled on a sterile 28 mm polycarbonate filter (Nuclepore, Millipore Corp., USA) with a pore size of 0.4 μm , the sampling rate being 2.5 l/min during 1.5 h. The total concentration of airborne micro-organisms was determined by the CAMNEA method utilizing epifluorescence microscopy [15] a method shown to yield similar or slightly lower estimates of micro-organisms than scanning electron microscopy or light microscopy [16]. Viable moulds and bacteria were determined by incubation on two different media. The detection limits of viable micro-organisms were 50 colony forming units (cfu)/ m^3 of air.

Settled bedroom dust was collected by standardized vacuum cleaning of both the mattress and the bedroom floor, using the same vacuum cleaner in all homes

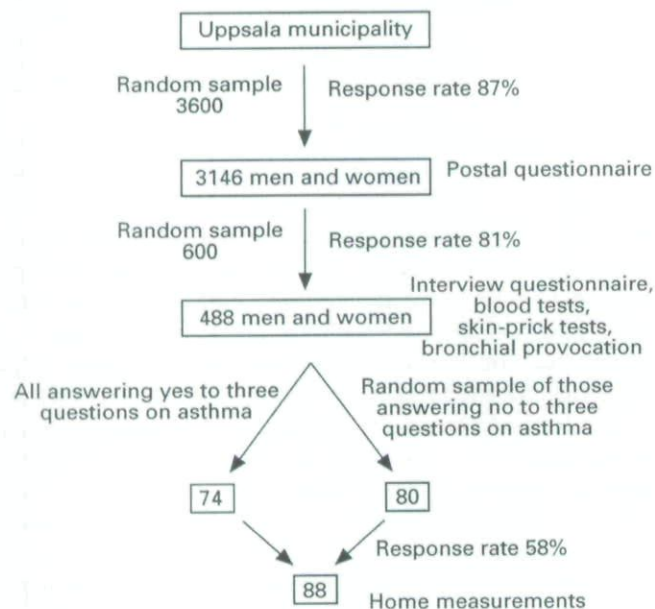


Fig. 1. The population studied and response rates.

(ELRAM HSS 09, ELRAM, Sweden). In each bedroom, a sampling time of 1 min/m² was applied to both the floor and the mattress. The presence of HDMs in the collected dust was reassessed by applying the semi-quantitative ACAREX test [17], which gives a reaction score from 0 to 3. It has been validated against a quantitative immunological test using a monoclonal antibody against HDM [18]. According to the suggestions of van der Bremer *et al.* [18] a ACAREX score of 0 and 1 was regarded as negative and 2 and 3 as positive for the presence of allergenic levels of house dust mites. The test is based on a reaction to guanine in faecal pellets from dust mites and is therefore not species specific [19]. Throughout this text, however, a positive ACAREX test is considered as evidence of HDM.

Skin-prick tests

Skin-prick tests were carried out in a standardized way with allergen-coated lancets (Phazets[®], Pharmacia Diagnostics, Uppsala, Sweden). The following allergens were used: *Dermatophagoides pteronyssinus*, cat, birch, dog, *Cladosporium*, olive, ragweed, mugwort, timothy and *Alternaria*. Histamine was used as a positive control. Subjects with dermatographism were excluded.

Lung function and bronchial hyperresponsiveness

Forced expiratory volume in one second (FEV₁) was measured on 82 subjects using the Spiro Medics computerized dry-rolling seal spirometer system 2130 (Sensor Medics, Anaheim, California, USA). The predicted values were calculated for each patient [20].

Methacholine challenge was performed on 73 subjects using a MEFAR inhalation dosimeter (MEFAR, Brescia, Italy) [21].

Peak expiratory flow (PEF) rate (best of three measurements) was recorded twice daily during one week with a Mini-Wright Peak Flow Meter (Clement Clarke, London, UK). Sixty-eight of the subjects did the PEF measurements acceptably, that is twice a day on at least 4 of 7 days. Peak flow variability was calculated by dividing the difference between the highest and lowest daily PEF reading by the daily mean PEF value [22].

Blood samples

The following venous blood samples were collected from each volunteer:

1. Five millilitres supplemented with EDTA (0.34 mol/l), which were used for the analysis of the blood eosinophil count on a Hemalog 2R (Technicon Chemicals Company, Tournai, Belgium).

2. Twenty millilitres from which serum was collected and thereafter kept frozen at -70°C until analysed for S-ECP. The concentration of S-ECP was assayed by means of double antibody radioimmunoassay (Pharmacia Diagnostics AB, Uppsala Sweden). The intra- and interassay coefficients of variation are less than 11%, and the detection limit is less than 2 µg/l [23].
3. Ten millilitres from which serum was collected and analysed for total serum immunoglobulin E (S-IgE) (Pharmacia CAP Systems, Pharmacia Diagnostics AB, Uppsala Sweden). The same investigation sequence was applied to all the participants. The blood was drawn just prior to the methacholine provocation test.

Questionnaire

The screening and interview questionnaire used in the ECRHS was a modified version of the IUATLD questionnaire [24,25]. All questions were translated into Swedish and then back to English in order to minimize translation bias. The structure of the questionnaire was such as to reflect the periodic prevalence of airway symptoms (symptoms in the last 12 months) as opposed to point, or cumulative, prevalence. The questionnaire also included questions concerning housing, social situation, education and environmental exposure.

Definitions

For this study the following definitions were used:

1. Bronchial hyperresponsiveness (BHR) was defined as a positive methacholine test, i.e. a reduction in FEV₁ by at least 20% with an accumulated methacholine dose of ≤ 2 mg (PD₂₀).
2. Asthma related symptoms were defined as in the interview reporting having had in the last 12 months: wheezing or whistling in the chest and/or; a day-time attack of shortness of breath during exercise or while resting and/or; a night-time awakening because of breathlessness or tightness in the chest.
3. Atopy was defined as: a skin-prick test reaction to at least one of the above mentioned allergens with a mean of diameters ≥ 3 mm, and no dermatographism [26].

Statistical analysis

The statistical analysis was performed on a Macintosh IIfx computer with the software package Statistica 4.0 (StatSoft Inc, Tulsa, USA). The unpaired *t*-test was used

for univariate analysis of relations between binary dependent variables and independent continuous variables that were approximately normally distributed while Mann-Whitney *U*-test was used for continuous variables that were not normally distributed. The chi-square-test was used when analysing the relationship between binary dependent and independent variables. Spearman rank correlation was used when testing correlation between continuous variables. When analysing the influence of multiple independent variables on binary variables multiple logistic regression was used. In this analysis the adjusted odds ratio was calculated for binary variables and the β -coefficient for continuous independent variables. The results are expressed as geometric mean or odds ratio (OR) with a 95% confidence interval (CI). A *P* value <0.05 (two tailed test) was regarded as statistically significant.

Results

Study population and response rates

The total response rate to the first part of the study was 87%. In the second part the response rate was 81%. Of the 154 persons chosen for the home study, 88 (57%) could be contacted and gave their approval. Of the remaining 66 (43%), 19 had moved during the study period and could therefore not be included, 14 did not

participate in the medical examination, 12 could not be contacted, seven were working/studying away during the study period and 14 refused to participate in the home study. The non-responders did not differ from the responders as regarded age, reported symptoms, smoking, bronchial hyperresponsiveness (BHR) or atopy. The participants consisted of 42 men and 46 women. Their mean age was 32 years, range 20–44 years. They had lived in their present dwellings for an average of 6 years (range: 0.5–31 years). From these 88 individuals, data could be obtained as follows: interview questionnaires in 88 individuals, spirometry in 82, skin-prick tests in 77, methacholine challenge test in 73, a peak flow diary in 68 and serum concentration of eosinophil cationic protein (S-ECP) in 74. The characteristics of the studied group and their homes are shown in Table 1.

General indoor hygiene

During the measurement period the relative humidity was above 33% in all the houses studied and above 45% in 75 houses (85%). House dust mites (HDM) could be detected in 12 houses (13%). HDM levels were positively related to air humidity ($r = 0.26$, $P < 0.05$) and increasing age of the house ($r = 0.36$, $P < 0.001$). Total levels of mould were related positively to air humidity ($r = 0.21$, $P < 0.05$) and total levels of bacteria ($r = 0.41$, $P < 0.001$).

Table 1. Characteristics of the patients with and without asthmatic symptoms (* = $P < 0.05$), *** = $P < 0.001$)

	Asthma symptoms (<i>n</i> = 47)		No symptoms (<i>n</i> = 41)		
	<i>n</i>	%	<i>n</i>	%	
Asthma medication (<i>n</i> = 88)	14	30	0	—	***
Smokers (<i>n</i> = 88)	11	23	9	22	
BHR (<i>n</i> = 73)	12	32	3	9	*
Atopy (<i>n</i> = 77)	19	48	14	38	
Age of house					
Built before 1960	15	32	7	17	
1961–1970	13	27	10	24	
1971–1980	8	17	12	29	
After 1980	11	24	12	29	
Type of house					
Detached house	15	32	12	29	
Terraced house	7	15	9	22	
Apartment	25	49	20	53	
	Mean $\pm$ SD		Mean $\pm$ SD		
Duration of habitation (years)	6 $\pm$ 7		6 $\pm$ 6		
FEV ₁ (%pred) (<i>n</i> = 82)	105 $\pm$ 13		107 $\pm$ 12		
PEF variability (%) (<i>n</i> = 68)	5 $\pm$ 3		5 $\pm$ 4		

Table 2. Differences in indoor variables between subjects with and without asthma related symptoms (geometric mean and range) (* = $P < 0.05$)

	Asthma symptoms ($n = 47$)	No symptoms ($n = 41$)	
HDM (% detected)	21	5	*
Total bacteria ($10^3/\text{m}^3$)	87 (12–830)	52 (12–930)*	
Viable bacteria ($10^3/\text{m}^3$)	0.5 (0.1–8.5)	0.4 (0.1–8.8)	
Total moulds ($10^3/\text{m}^3$)	35 (12–330)	25 (11–570)*	
Viable moulds ($10^3/\text{m}^3$)	0.3 (0.1–3.3)	0.3 (0.1–1.3)	
Air humidity (%)	57 (33–75)	53 (36–75) *	
Indoor temperature ($^{\circ}\text{C}$)	20 (18–24)	20 (18–23)	

Asthma related symptoms

Forty-seven (53%) of the subjects reported having had at least one asthma-related symptom in the last 12 months. In the houses of these individuals, significantly higher total levels of bacteria and mould and a higher air humidity were found ($P < 0.05$). A higher proportion of detected HDM was found in the homes of subjects with asthma related symptoms compared with subjects with no such symptoms (OR 5.3, CI 1.1–26, $P < 0.05$) (Table 2).

Wheezing was reported by 33 subjects. Total level of bacteria was significantly higher in their houses than in the houses of subjects with no wheezing (91 vs $57 \times 10^3/\text{m}^3$, $P < 0.05$).

Daytime attacks of breathlessness were reported by 27 subjects. In their houses total levels of bacteria and moulds was found to be significantly higher than in the houses of subjects with no daytime breathlessness (97 vs 58 and 40 vs $27 \times 10^3/\text{m}^3$, respectively) ($P < 0.05$).

Nocturnal attacks of breathlessness or chest tightness were reported by 25 of the subjects. Total levels of bacteria and mould were found to be significantly higher in their houses than in the houses of subjects without nocturnal symptoms ($P < 0.05$). A higher proportion of detected HDM was also found in houses of subjects with nocturnal symptoms compared with subjects with no nocturnal asthma related symptoms (OR 5.5, CI 1.5–21, $P < 0.01$) (Fig. 2).

Multivariate analysis

In order to compare the influence of multiple indoor variables, logistic regression was applied with age, sex, smoking, total levels of bacteria and mould, HDM, air humidity and indoor temperature as independent variables. HDM was found to be an independent risk factor for asthma related symptoms (OR 7.9, CI 1.2–55) and nocturnal breathlessness (OR 6.2, CI 1.3–30)

Fig. 2. Differences in indoor exposure in individuals reporting symptoms of nocturnal dyspnoea and/or chest tightness as compared with individuals without these symptoms (% and median $\pm$ interquartile deviation).

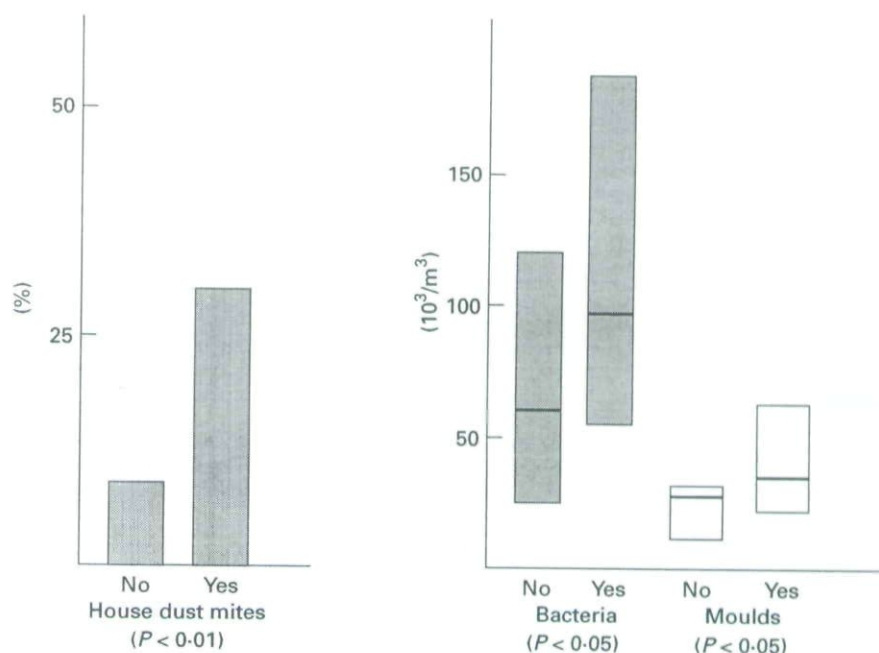


Table 3. The relationship between asthma related symptoms and total levels of bacteria, mould and detected house dust mites (HDM) after adjustment for age, sex, smoking, indoor temperature and air humidity (odds ratio [95% confidence interval], * = $P < 0.05$).

Symptoms	Measurements		
	Bacteria†	Moulds†	HDM
Asthma-related symptoms	5.1 (1.3–20) *	0.8 (0.1–5.1)	7.9 (1.2–55) *
Wheezing	4.2 (1.2–14) *	0.4 (0.1–2.4)	1.3 (0.3–5.8)
Daytime breathlessness	2.7 (0.8–9.6)	2.6 (0.5–14)	1.9 (0.4–9.2)
Nocturnal breathlessness	2.7 (0.8–9.3)	2.8 (0.5–15)	6.2 (1.3–30) *

† The odds ratio is calculated for a 10th-fold increase in bacteria or mould concentration.

($P < 0.05$), while total levels of bacteria was found to be a risk factor for asthma related symptoms ($\beta = 1.6 \pm 0.71$) and wheezing ($\beta = 1.56 \pm 0.67$) ($P < 0.05$) (Table 3).

Objective asthma-related measurements

There was no significant correlation between FEV₁ BHR or peak expiratory flow (PEF) variability and the indoor measurements of micro-organisms or HDM. S-ECP was positively correlated to levels of viable bacteria ($r = 0.23$) and total mould ($r = 0.26$) ($P < 0.05$). Total IgE was positively related to viable bacteria ($r = 0.25$) and total mould levels ($r = 0.24$) ($P < 0.05$). No correlation was found between the blood eosinophil count and micro-organisms or HDM.

Skin reaction to indoor allergens

Cats were kept by 17 persons, one of which had a positive skin-prick test to cat allergen. Dogs were kept by seven; none were allergic to dogs. Birds were kept by five persons. No correlations were found between the keeping of pets and respiratory symptoms. A positive skin-prick test to *Dermatophagoides pteronyssinus* was found in four persons. The ACAREX mite test was negative in the houses of all four.

Discussion

We have carried out measurements of indoor micro-organisms and house dust mites in a sample of the homes of participants in the European Community Respiratory Health Survey in Uppsala. Our aim was to establish whether a relationship existed between these variables and symptoms and signs of asthma. We found that exposure to airborne bacteria and house dust mites

were related to asthmatic symptoms. A positive correlation was also found between S-ECP and levels of viable bacteria and total mould.

Considerations of validity

Many methodological problems are inherent in an epidemiological study with regard to validity. In this particular study, selection bias due to low response rates is unlikely since the participation rate in the initial postal questionnaire was high (87%) (Fig. 1). In addition, the participants and non-participants in the clinical study did not differ significantly with respect to airway symptoms as reported in the postal questionnaire. Response bias due to awareness of exposure may cause a general overreporting of symptoms in exposed groups. In the present study, however, the exposure measurements were performed parallel to the medical investigations, and could thus not influence the reporting of symptoms.

In order to achieve a sufficient number of symptomatic individuals the material was enriched with persons reporting symptoms in the postal questionnaire. The evaluation is, however, based on symptom reports from the interview which we believe are more reliable. Our study is therefore in a strict sense not a case-control study but rather a study on the relations of asthma-like symptoms and objective findings to home measurements of potential allergens and irritants. Finally, the size of the sample is rather small and through necessity limited by the design of the ECRHS. This may have caused some connections to be missed.

General indoor hygiene

Home dampness is by many regarded as a risk factor of its own as regards the development of respiratory symptoms. Dampness influences the level of potent allergens in our dwellings and several studies have

shown an association between home dampness and respiratory symptoms in children [7–9,27,28] and in adults [29,30]. To what degree this can be attributed to the growth of moulds and bacteria, the survival of HDM, to the admixture of the three, or to other factors is not clear. It has even been stated that the awareness of damp surroundings may cause people to report more respiratory symptoms [31]. In most of the houses in our study the relative humidity was high and was related to the measured levels of moulds and to the presence of HDM.

Relation of house dust mites to symptoms

Compared with many other countries HDM are a relatively infrequent finding in homes in central-Sweden. Some studies indicate, however, that HDM infestation is increasing in this area [32,33]. This may be due to better insulation causing a rise in relative humidity indoors. Most of the houses in our study had relative humidity higher than 45%, a threshold of mite viability [34]. In line with findings of previous investigators [35] relative air humidity correlated with the presence of HDM in our study. We found signs of HDM in 17% of the dwellings during the coldest part of the year and mite infestation is likely to be more widespread during the summer months.

A relationship between the presence of HDM in homes and asthma in children suffering from mite-allergy, has been demonstrated in earlier studies [32]. In our study HDM were a significantly more frequent finding in patients who reported asthma-related symptoms. Among isolated symptoms, the presence of HDM correlated best with nightly symptoms of chest tightness and breathlessness. This is reasonable since people come into closest contact with mites in their beds.

However, none of the inhabitants in the mite-infested houses, could be shown to have an immediate hypersensitivity to HDM as measured by a skin-prick test. Although there is evidence that specific IgE antibodies can be found in the airways without being manifest in the skin [36], the mechanisms responsible for the relationship may not be IgE-mediated. Contrary to a causal relationship is the possibility that a generally poor indoor hygiene is a common denominator as regards both the development of symptoms and the growth of mites. Finally, since the test used is not species-specific, we cannot exclude the possibility that some of the persons living in mite-positive houses were exposed and allergic to mites other than *D. pteronyssinus*.

Relationship between bacteria, moulds and symptoms

Studies on the impact of bacteria and their products on

respiratory symptoms have not focused on asthma or allergy as infectious asthma exacerbation have been thought to result mainly from viral infections [37]. Nonetheless, it has been shown that many bacteria commonly found in the respiratory tract can induce the release of histamine and other mediators of inflammation, possibly by means of both IgE mediated [38] and non-immunological mechanisms [39]. Thus, inhaled bacterial particles may cause an inflammatory reaction even without the occurrence of an infection. In our study the levels of airborne bacteria showed a significant correlation to many asthma-related symptoms (Tables 2 and 3 and Fig. 2). The total level of mould showed relationships to asthmatic symptoms using univariate analysis but was not found to be an independent risk factor when applying multivariate analysis.

Objective measurements and airway symptoms

Despite the connection of indoor variables to reported symptoms in our study, neither FEV₁, PEF variability or BHR showed any relationship to indoor micro-organisms or HDM. On the other hand S-ECP, a marker of eosinophil degranulation [40] was significantly related to viable bacteria and total moulds in the dwellings. Eosinophil infiltration, a characteristic pathological finding in allergies, is said to be a variation of the delayed type hypersensitivity reaction and S-ECP may here be an indicator of an on-going low-grade inflammation which, when other factors such as respiratory infections are superimposed, can cause lower airway symptoms. This theory, however, is speculative and is not supported by the lack of connection between home measurements and BHR.

Conclusions

We conclude that although HDM is relatively infrequently found in the homes of central-Sweden, the presence of HDM is related to asthmatic symptoms. A relationship between levels of airborne bacteria and asthma related symptoms was also found.

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