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A longitudinal study of sick building syndrome among pupils in relation to microbial components in dust in schools in China

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ABSTRACT

There are few longitudinal studies on sick building syndrome (SBS), which include ocular, nasal, throat, and dermal symptoms, headache, and fatigue. We studied the associations between selected microbial components, fungal DNA, furry pet allergens, and incidence and remission of SBS symptoms in schools in Taiyuan, China. The study was based on a two-year prospective analysis in pupils (N = 1143) in a random sample of schools in China. Settled dust in the classrooms was collected by vacuum cleaning and analyzed for lipopolysaccharide (LPS), muramic acid (MuA), and ergosterol (Erg). Airborne dust was collected in Petri dishes and analyzed for cat and dog allergens and fungal DNA. The relationship between the concentration of allergens and microbial compounds and new onset of SBS was analyzed by multi-level logistic regression. The prevalence of mucosal and general symptoms was 33% and 28%, respectively, at baseline, and increased during follow-up. At baseline, 27% reported at least one symptom that improved when away from school (school-related symptoms). New onset of mucosal symptoms was negatively associated with concentration of MuA, total LPS, and shorter lengths of 3-hydroxy fatty acids from LPS, C14, C16, and C18. Onset of general symptoms was negatively associated with C18 LPS. Onset of school-related symptoms was negatively associated with C16 LPS, but positively associated with total fungal DNA. In general, bacterial compounds (LPS and MuA) seem to protect against the development of mucosal and general symptoms, but fungal exposure measured as fungal DNA could increase the incidence of school-related symptoms.

1. Introduction

There is concern about various health effects of the indoor environment, including increased prevalence of a variety of non-specific symptoms often described as sick building syndrome (SBS) (WHO, 1983). The term SBS has been used to describe symptoms (including headaches, fatigue, and irritation in the upper respiratory tract, nose, throat, eyes, hands and/or facial skin) that can be influenced by the indoor environment (Redlich et al., 1997; WHO, 1983). Factors reported to be related to SBS include indoor chemical air contaminants, low ventilation rate, female gender, personality trait, and work stress (Burge, 2004; Hansen et al., 2008; Runeson et al., 2004; Takigawa et al., 2009). Moreover, building dampness and exposure to microbial components from molds and bacteria have been the focus with regard to both SBS and asthma (Fung and Hughson, 2003; Hardin et al., 2003; WHO, 2009). It has been concluded that there is sufficient evidence to show that the occupants of damp or moldy buildings have an increased risk of respiratory symptoms and SBS (WHO, 2009).

School is one of the most important environments for children, and there is evidence that the indoor air quality (IAQ) and ventilation in school buildings may affect their health (Daisey et al., 2003), but we have found few publications on associations between building dampness and measured microbial exposure in schools or about SBS in school children (Meyer et al., 2004; Saijo et al., 2010). Indoor microbial contaminants include bacteria, molds, yeast, and various components from these organisms. The most studied bacterial compound is endotoxin (LPS) (Seltzer, 1995). Endotoxins are integral components of the outer membrane of Gram-negative bacteria, and LPS is responsible for most of the immunological properties of bacterial endotoxins (WHO, 2009). Muramic acid (MuA) is a cell wall compound found in all bacteria, but since the walls of Gram-positive bacteria are much thicker than those of Gram-negative bacteria, it is considered to be mainly an indicator of Gram-positive bacteria (Sebastian et al., 2004). Ergosterol (Erg) and beta-1-3-glucane are cell wall compounds found in fungi (Rylander, 2004; Saraf et al., 1997). Moreover, analysis of fungal DNA by quantitative PCR (qPCR)

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has recently been applied in epidemiological studies on asthma in relation to mold exposure in homes (Vesper et al., 2007) and schools (Cai et al., 2011).

China has the largest population in the world, but we found no publication on SBS from mainland China, and, with respect to SBS in children, only a few from Taiwan. We have previously studied associations between microbial components in school dust (LPS; MuA; Erg) and asthmatic symptoms in school children in a cross-sectional study in Taiyuan City, China, where we found mainly negative associations for bacterial components (LPS; MuA) and positive associations for Erg (Zhao et al., 2008). With these findings in mind, we performed a two-year follow-up study on SBS in school children in the same schools.

In this study, our aim was to measure selected microbial components in school dust, selected fungal DNA, and furry pet allergens in schools and to study associations between this exposure and two-year incidence and two-year remission of SBS symptoms. The study was performed in school pupils in Taiyuan, a city in north China. To our knowledge, this is the first longitudinal study on associations between microbial exposure and SBS in China.

2. Materials and methods

2.1. Study population

This study is a two-year follow-up of a cohort in a random sample of Chinese pupils, with microbial components measurement at baseline.

Ten junior high schools in urban areas of Taiyuan City were randomly selected. There were no reports of health complaints or environmental problems from any of the schools before the investigation. At baseline (2004) in each of the 10 schools, five first-year classes were randomly selected. If there were fewer than five first-year classes, all were selected. The study population consisted of 2209 pupils (11–15 years of age) in 46 classes, of which 1993 (90.2%) completed a questionnaire. Since school started in the beginning of September, so they had been 4–5 months in the classroom in 2004. At follow-up (2006), two schools that had been selected at baseline chose not to participate in the follow-up, so it was pupils in 37 classes in the remaining 8 schools who answered the same questionnaire again. Settled dust for analysis of allergen and microbial components were collected in 33 classrooms (N = 1062), and 4 classrooms (N = 81) could not be sampled due to practical reason (lack of electric outlet). A total of 1143 pupils participated in both the initial study and the follow-up. All had the same classroom during the two-year study period, since each class had a fixed classroom for all lessons, except for sports, during all three years in junior high school. The study was performed from December to January both times.

2.2. Information on SBS

The pupils answered a self-administered questionnaire with questions on age, sex, symptoms compatible with SBS, parental asthma or allergy and own pollen or pet allergy and asthma. The same questionnaire was used in 2004 and 2006. The questionnaire was based on previous SBS studies (Bjornsson et al., 1998) and school environmental studies (Smedje and Norback, 2001; Zhao et al., 2008). SBS-type symptoms occurring "yes everyday or days 1–4 times/week" in the last 3 months and improving away from school were defined as SBS positive symptoms (Zhang et al., 2011). There were 16 questions on symptoms: facial and hand rash or itching; eczema; eye irritation; swollen eyelids; nasal catarrh and obstruction; dryness in the throat; sore throat; irritating cough; headache; nausea; sensation of getting a cold; and tiredness. The recall period was three months. Each question had four alternative answers: "Yes, everyday"; "Yes, 1–4 times per week"; "Yes, 1–3 times per month"; and "No, never". In the statistical calculations, for mucosal (eye irritation, swollen eyelids, nasal catarrh, nasal obstruction, dryness in throat, sore throat or irritating cough), general (headache, nausea, sensation of getting a cold or tiredness) and dermal symptoms (facial and hand rash or itching, eczema), weekly symptom (yes everyday or days 1–4 times/week) was coded 1 and 1–3 times/month or never was coded 0. In addition, a question about whether any of the SBS symptoms improved when they stayed away from school was included. For symptoms improved when away from school, any symptom improved coded 1, no symptoms coded 0. Questions on allergy and respiratory health included "yes/no" questions on doctor-diagnosed asthma, current asthma, and allergies to furry pets or pollen. The survey was performed one week before the classroom measurements. The questionnaire was distributed in the school by the class teachers, and answered at home in cooperation with parents. The study was approved by the ethical committee of the Institute of High School Student Health Care in Taiyuan and performed with informed consent from pupils and parents. All personal information from the questionnaires was kept confidential. All data analyses were done at Uppsala University, Sweden.

2.3. Building inspection and climate measurements

Details on building construction, materials, and type of ventilation heating system were noted, as were any sign of building dampness, such as damp spots, water leakage or indoor mold growth in the classrooms. Temperature (°C), relative humidity (RH%), and CO_2 concentration (ppm) were measured during normal activities in the classrooms for 50–70 min using a Q-track IAQ monitor (TSI Incorporated, St. Paul, MN, USA) by logging average values per minute (Zhao et al., 2008). Corresponding Q-track measurements were done outside each school.

2.4. Dust collection

Dust collection was performed one week before the questions study in 2004. Settled dust in the classrooms was collected from desks, chairs, and floors by vacuuming with a 400 W vacuum cleaner equipped with a special dust collector fitted with a Millipore filter (pore size 6 μm) (ALK Abello, Copenhagen, Denmark). Two samples were collected in each classroom by dividing the room into roughly an entrance-side half and a window-side half. Vacuum cleaning was performed for 4 min. per sample, equally divided between floor and desks/chairs (Kim et al., 2007; Smedje et al., 1997). The filters were stored at −20 °C until extraction.

Airborne settling dust was collected in three Petri dishes in each classroom, placed on the top of the blackboard (about 2 m height) and kept open for seven days (Cai et al., 2011; Karlsson et al., 2002). Two Petri dishes were extracted for cat and dog allergens analysis. After buffer extraction with 3 ml of phosphate buffered saline (PBS) with 1% bovine serum albumin (BSA), the liquid was transferred into an Eppendorf tube and centrifuged (Zhao et al., 2006), after which the supernatants were stored at −20 °C until analysis. The third Petri dish was extracted for fungal DNA analysis.

2.5. Measurement of LPS, MuA, and Erg

The settled dust samples were sieved (particle diameter < 400 μm) to obtain the fine dust (Zhao et al., 2008). The amount of fine dust was weighed and stored at −20 °C until extraction. Portions of 1–5 mg of the fine dust fraction were used for analyses of MuA, Erg, and 3-hydroxy fatty acids (3-OH FA) with 10, 12, 14, 16, and 18 carbon chain lengths by GC–MS/MS (Sebastian and Larsson, 2003). A hydrolysate of ^{13}C-labeled cyanobacterial cells was used as internal standard for the analysis of MuA and 3-OH FA, and dehydrocholesterol was used for the analysis of Erg. Briefly, MuA was analyzed as methyl ester o-methyl acetate derivative after acid methanolysis of samples,