ORIGINAL ARTICLE

Annals of Agricultural and Environmental Medicine 2015, Vol 22, No 3, 473–477
www.aaem.pl

Relationship between concentrations of microbiological agents in the air of agricultural settings and occurrence of work-related symptoms in exposed persons

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Mackiewicz B, Skórska Cz, Dutkiewicz J. Relationship between concentrations of microbiological agents in the air of agricultural settings and occurrence of work-related symptoms in exposed persons. Ann Agric Environ Med. 2015; 22(3): 473–477. doi: 10.5604/12321966.1167717

Abstract

For assessment of the dose-response relationship between concentrations of microbial agents in the air of various agricultural settings and occurrence of work-related symptoms in exposed workers, a meta-analysis of the results obtained in 1994–2007 on the territory of eastern Poland was performed. The studies on the airborne concentrations of total culturable microorganisms, mesophilic bacteria, Gram-negative bacteria, thermophilic actinomycetes, fungi, and bacterial endotoxins, as well as on the frequency of work-related respiratory and general symptoms in the exposed workers, were carried out at grain, thyme, valerian, flax, and hop handling on farms, in cow barns, piggeries, horse stables and in a modern hatchery. The airborne concentrations of the total microorganisms were in the range of 9.2–1236.5 × 10^3 CFU/m^3, of the total mesophilic bacteria 3.5–1225.8 × 10^3 CFU/m^3, of Gram-negative bacteria 0.0- 46.2 × 10^3 CFU/m^3, of thermophilic actinomycetes 0.0–7.1 × 10^3 CFU/m^3, of fungi 2.1–77.9 × 10^3 CFU/m^3, and of bacterial endotoxin 0.00925–429.55 µg/m^3. The frequency of work-related symptoms ranged between 21.7–63.8%. In a meta-analysis for assessment of the correlations between the log-transformed concentrations of airborne microbial agents and the occurrence of work-related symptoms, the multiple regression test was applied. Statistically significant correlations were found between the occurrence of work-related symptoms and the concentration of total airborne microorganisms (R=0.748555; P=0.020317), mesophilic bacteria (R=0.7573; P=0.029548), Gram-negative bacteria (R=0.835938; P=0.019129), and endotoxins (R=0.705356; P=0.03378). The correlations between the concentrations of thermophilic actinomycetes and fungi, on one side, and frequency of work-related symptoms on the other, did not attain the threshold of significance (P=0.087049 and P=0.062963, respectively). Results of the meta-analysis confirm harmful health effects of the total airborne microorganisms, total airborne mesophilic bacteria, airborne Gram-negative bacteria, and airborne bacterial endotoxin on the occupationally-exposed agricultural workers, and indicate a need for the establishment of internationally recognized occupational exposure limits for these microbial agents.

Key words

agricultural settings, bioaerosol, work-related symptoms, relationship, total microorganisms, mesophilic bacteria, Gram-negative bacteria, endotoxins

INTRODUCTION

In the past 50 years, it has been well documented that microorganisms present in airborne organic dusts produce various harmful substances which may cause work-related specific or unspecific diseases (such as hypersensitivity pneumonitis or organic dust toxic syndrome) in people occupationally-exposed to the inhalation of such dusts. Allergens and endotoxins produced by Gram-negative bacteria, allergens produced by thermophilic actinomycetes and allergens, mycotoxins and glucans produced by fungi are among the best known agents evoking the disorders [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11]. Nevertheless, adverse substances may also be produced by other microorganisms, and hence the total microbiota of the air is regarded by many scientists as a potent source of allergens and toxins, and a good indicator of occupational risk [12, 13, 14, 15].

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Received: 10 February 2015; accepted: 20 May 2015

To-date, there are no internationally recognized occupational exposure limit values defining the allowable concentrations of total microbiota or its specific constituents in the air [15, 16, 17, 18]. Various authors have published proposals for such values, concerning the allowable concentrations of total microorganisms in the air (in CFU/m^3 or cells/m^3) and/or the concentrations of particular microorganisms (such as total mesophilic bacteria, Gram-negative bacteria, thermophilic actinomycetes or fungi) and/or the substances of microbial origin, such as bacterial endotoxins [14, 19]. One of the main obstacles in establishing of widely accepted allowable concentrations of microorganisms and their products in the air is the scarcity of observations on the dose-response relationship between quantitative levels of airborne microbial agents, and the occurrence of work-related symptoms and disorders in the exposed occupational populations. Previously, most of the research on this subject has concerned the effects of airborne endotoxin on the respiratory system of occupationally-exposed individuals, whereas much less attention has been paid to the effects of airborne microorganisms, with regard either to the total count or to particular groups, such as

Gram-negative bacteria, thermophilic actinomycetes or fungi [8, 19, 20, 21, 22].

Taking into consideration the need for better understanding of the relationships between the concentrations of airborne microbial agents in occupational facilities and the occurrence of work-related disorders in exposed persons, as well as the importance of such knowledge for establishing of the reliable occupational exposure limit values, the appropriate meta-analysis for various agricultural settings was undertaken, based on the results of studies performed in 1994–2007 by our group from the Department of Pneumonology, Oncology and Allergology of the Medical University and the Institute of Rural Health in Lublin, Poland.

MATERIALS AND METHODS

During 1994–2007, measurements of the total concentration of airborne culturable microorganisms, mesophilic bacteria, Gram-negative bacteria, thermophilic actinomycetes, fungi, and the concentration of airborne bacterial endotoxins were carried out on grain farms during grain threshing [23], on thyme farms during cleaning thyme herb [24], on valerian farms during the processing of valerian roots [25], on flax farms during scutching (threshing) flax [26], on hop farms during the handling of hops (picking and sorting of hop cones, drying and packing cones) [27], in cow barns [28], piggeries [28], horse stables [28], and in a modern hatchery [29]. Following the aerobiological measurements, the workers of the investigated settings were asked about the occurrence of work-related symptoms when handling grain [30], thyme [31], valerian [32], flax [33], and hops [34], or working in cow barns [35], piggeries [36], horse stables [37], and a modern hatchery [38].

The methods used in afore-mentioned studies have been described in the above-cited publications. Briefly, microorganisms were isolated from the air by the impaction method with a custom-designed particle-sizing slit sampler [39] enabling estimation of both total and respirable fractions of the bioaerosol [23, 24, 26, 28], or by the filtration method, collecting air samples on preweighed glass fibre or polypropylene filters, with the use of commercial one-stage samplers: AP-2A personal sampler [25, 27] or AS-50 stationary sampler [29] (TWOMET, Zgierz, Poland). Mesophilic bacteria were isolated on blood agar, Gram-negative bacteria on eosin methylene blue (EMB) agar, thermophilic actinomycetes on half-strength tryptic soya agar and fungi on malt agar. After incubation, colonies were counted, taxonomically identified and the data reported as colony forming units (CFU) per 1 m^3 of air. The concentration of total airborne culturable microorganisms was calculated as a sum of the counts of mesophilic bacteria, thermophilic actinomycetes and fungi. Air samples for the determination of bacterial endotoxins were collected on preweighed polyvinyl chloride filters using an one-stage sampler. Concentration of endotoxins in the airborne dust and in the air ($\mu g/m^3$) was determined by the *Limulus* amoebocyte lysate (LAL) test.

People working in the agricultural settings examined with aerobiological methods were interviewed by the American Thoracic Society Standard Questionnaire (ATS Questionnaire), compiled by Ferris [40] and by the questionnaire developed in the Institute of Rural Health in Lublin for the examination of work-related symptoms caused by organic dusts [41].

Statistical analysis was performed by the multiple regression test for assessment of the correlations between the airborne concentrations of microbial agents and occurrence of work-related symptoms, using the STATISTICA™ ver. 5.0 package (Statsoft©, Inc., Tulsa, Oklahoma, USA). Log-transformed values of the concentrations of airborne microbial agents were used for analysis. The $P<0.05$ level was considered significant.

RESULTS

The results of the meta-analysis are presented in Tables 1–2 and in Figures 1–4. As confirmed by the Shapiro-Wilk test ($P<0.01$), the values showing levels of microbial contaminants of the air were not normally distributed, and therefore the log-transformed median values were used for the presentation and for statistical assessment of relationships between the concentrations of particular contamination and the occurrence of work-related symptoms. The concentrations of the total airborne culturable microorganisms were in the range $9.2–1236.5 \times 10^3$ CFU/m^3, the total airborne culturable mesophilic bacteria in the range of $3.5–1225.8 \times 10^3$ CFU/m^3, airborne culturable Gram-negative bacteria in the range of $0.0- 46.2 \times 10^3$ CFU/m^3, airborne culturable thermophilic actinomycetes in the range of $0.0–7.1 \times 10^3$ CFU/m^3, airborne

Table 1. Comparison of concentrations of airborne microbial agents in agricultural settings with occurrence of work-related symptoms in exposed workers – Part 1

Setting, working process	Total microorganisms		Gram-negative bacteria		Endotoxins		Frequency of work-related symptoms (%)
	Conc. (x) CFU/m³´ 10³	Log_{10} x	Conc. (x) CFU/m³´ 10³	Log_{10} x	Conc. (x) µg/m³	Log_{10} x	
Grain farms, threshing grain	351.5	2.546	Nd	Nd	50.0	1.699	44.7
Thyme farms, cleaning thyme herb	372.2	2.571	Nd	Nd	429.55	2.633	63.8
Valerian farms, processing valerian roots	10.75	1.031	0.7	- 0.155	33.43	1.524	30.7
Flax farms, scutching flax	358.2	2.554	46.2	1.665	30.0	1.477	62.7
Hop farms, handling hop cones	9.2	0.964	0.0	- 1.000	0.0522	- 2.718	21.7
Cow barns	216.0	2.334	3.9	0.591	0.00925	- 3.966	22.2
Piggeries	1236.5	3.092	15.5	1.190	31.25	1.495	58.5
Horse stables	109.95	2.041	3.8	0.580	0.02611	- 2.417	51.6
Modern hatchery*	16.83	1.226	0.58	- 0.237	0.36425	- 1.561	25.0

Conc. – median concentration; Nd – not determined; *fungi not determined

Table 2. Comparison of concentrations of airborne microbial agents in agricultural settings with occurrence of work-related symptoms in exposed workers – Part 2

Setting, working process	Total mesophilic bacteria		Thermophilic actinomycetes		Fungi		Frequency of work-related symptoms (%)
	Conc. (x) CFU/m^3 ´ 10^3	Log_{10} x	Conc. (x) CFU/m^3 ´ 10^3	Log_{10} x	Conc. (x) CFU/m^3 ´ 10^3	Log_{10} x	
Grain farms, threshing grain	Nd	Nd	Nd	Nd	Nd	Nd	44.7
Thyme farms, cleaning thyme herb	281.5	2.449	7.1	0.851	77.9	1.892	63.8
Valerian farms, processing valerian roots	3.5	0.544	0.2	- 0.699	3.4	0.532	30.7
Flax farms scutching flax	255.8	2.408	0.6	-0.222	41.6	1.619	62.7
Hop farms, handling hop cones	5.0	0.699	0.4	- 0.398	2.1	0.322	21.7
Cow barns	125.1	2.097	1.2	0.090	4.5	0.653	22.2
Piggeries	1225.8	3.088	1.4	0.146	3.6	0.556	58.5
Horse stables	94.6	1.976	4.6	0.663	5.4	0.732	51.6
Modern hatcher.	16.8	1.225	0.0	- 1.000	0.36425	N. d.	25.0

Conc. – median concentration; Nd – not determined

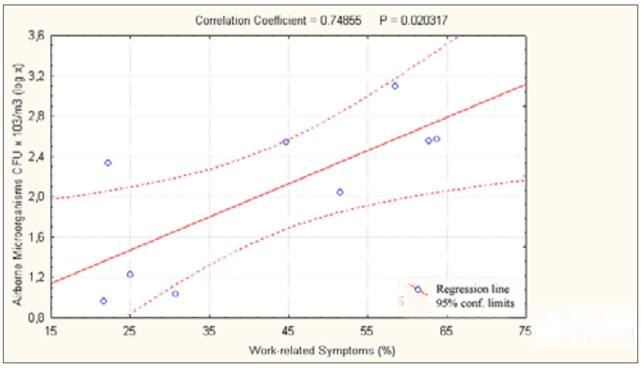

Figure 1. Correlation between concentration of total airborne culturable microorganisms (CFU × 10^3/m^3, log_{10}x) and frequency of work-related symptoms in exposed agricultural workers

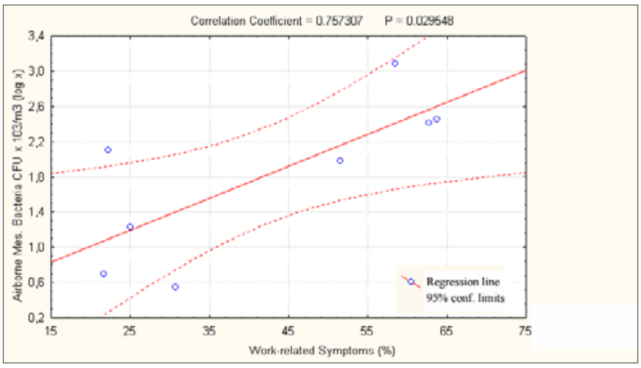

Figure 2. Correlation between concentration of total airborne culturable mesophilic bacteria (CFU × 10^3/m^3, log_{10}x) and frequency of work-related symptoms in exposed agricultural workers

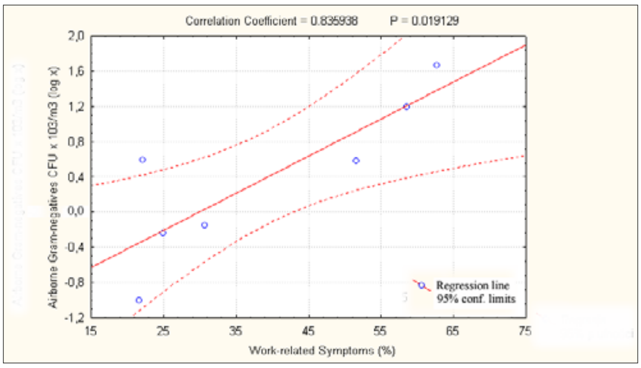

Figure 3. Correlation between concentration of total airborne culturable Gram-negative bacteria (CFU × 10^3/m^3, log_{10}x) and frequency of work-related symptoms in exposed agricultural workers

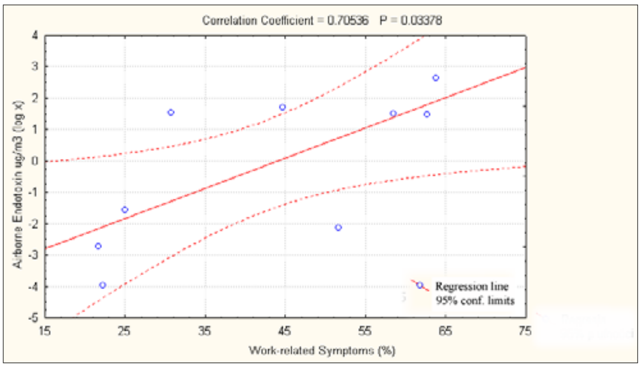

Figure 4. Correlation between concentration of total airborne endotoxins (µg/m^3, log_{10}x) and frequency of work-related symptoms in exposed agricultural workers

culturable fungi in the range of 2.1–77.9 × 10^3 CFU/m^3, and the airborne bacterial endotoxins in the range of 0.00925–429.55 µg/m^3. The frequency of work-related symptoms ranged between 21.7–63.8%. Statistically significant correlations were found between the occurrence of work-related symptoms and the concentrations of total airborne microorganisms (R=0.748555; P=0.020317) (Fig. 1), mesophilic bacteria (R=0.7573; P=0.029548) (Fig. 2), Gram-negative bacteria (R=0.835938; P=0.019129) (Fig. 3), and endotoxins (R=0.705356; P=0.03378) (Fig. 4). The correlations between the concentrations of thermophilic actinomycetes and fungi on one side and frequency of work-related symptoms on the other side did not attain the threshold of significance (P=0.087049 and P=0.062963, respectively).

DISCUSSION

Until recently, bacterial endotoxin has been the best characterized microbial contaminant of the air in the agricultural working environment. This lipopolysaccharide occurring in the outermost layer of the cell membrane of Gram-negative bacteria is released in huge amounts into the air and dust in the form of vesicular nanoparticles measuring on average 50 nm [42]. Endotoxin nanoparticles are massively inhaled by exposed workers and penetrate into the deep parts of the lungs, causing chest tightness, fever, and other symptoms typical for organic dust toxic syndrome. *Pantoea agglomerans* (synonyms: *Enterobacter agglomerans*, *Erwinia herbicola*), a common Gram-negative bacterium of plant origin, has been identified as a main source of extremely potent endotoxin and allergens which are present in grain dust, cotton dust and other dusts occurring in the agricultural and industrial working environments [2, 3, 43, 44, 45]. A significant relationship between the concentration of endotoxin in the air and occurrence of work-related symptoms and/or decline in lung function was found in various occupational environments, such as swine production [46], poultry industry [47], animal feed industry [48], grain handling [48, 49, 50], textile industry [51], household waste recycling [52], and wastewater treatment [53]. Interestingly, occupational endotoxin exposure increases the risk for non-allergic diseases, such as organic dust toxic syndrome or chronic bronchitis, but may have a protective effect on allergic sensitization [54].

Nevertheless, bioaerosol that occurs in the various occupational environments represents a rich mosaic of bacteria and fungi, and its adverse effects are not limited to endotoxins, having been largely due to bacterial and fungal allergens, and non-specific harmful substances such as peptidoglycans, (1→3)-β-D-glucans, and mycotoxins [21]. Accordingly, some researchers paid attention also to the bioaerosol components other than endotoxin. Thus, Erman et al. [12] proved a significant relationship between the concentrations of total microorganisms in the air of animal houses and the occurrence of respiratory disorders in the exposed breeders. Eduard et al. [55] found in Norwegian farmers, an exposure-response associations between the concentrations of airborne fungal spores and endotoxin and acute work-related symptoms. Melbostad et al. [56] recorded among sewage workers a significant association between levels of airborne bacteria and such symptoms, but not between levels of airborne endotoxin and symptoms. Similarly, Sprince et al. [57] found in machine operators an exposure-response relationship between the concentrations of airborne bacteria and fungi and occurrence of respiratory symptoms. The relationship between airborne endotoxin and such symptoms was also not confirmed.

The studies on bioaerosol and its effects on exposed agricultural workers subjected to meta-analysis in the present work cover a wide spectrum of farm facilities typical for Polish agriculture. They revealed a high degree of exposure to the total airborne microorganisms, mesophilic bacteria and endotoxins which at the most facilities exceeded the proposed allowable concentrations, and lower degrees of exposure to airborne Gram-negative bacteria, airborne thermophilic actinomycetes and airborne fungi which mostly did not exceed the proposed allowable concentrations [12, 14]. The significant exposure-response relationship found in the presented study between concentration of airborne endotoxins in a wide variety of agricultural settings and occurrence of work-related symptoms in exposed workers confirms the role of endotoxin as an important adverse factor affecting health of the farmers. Nevertheless, similar significant relationships were found also in the case of total airborne microorganisms, total airborne mesophilic bacteria and airborne Gram-negative bacteria, indicating the harmful effects of these factors on the exposed population, and the need for their measurement for the most comprehensive assessment possible of the occupational risk.

In conclusion, the results of the presented study confirm the harmful health effects of the total airborne microorganisms, mesophilic bacteria, Gram-negative bacteria, and bacterial endotoxins on the occupationally-exposed agricultural workers, and indicate the need for the establishment of internationally recognized occupational exposure limits for these microbial agents.

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