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ORIGINAL ARTICLE

Evaluation of working conditions of workers engaged in tending horses

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Abstract

Introduction. A growing interest in the horse business has resulted in the increased engagement of many people in this area, and the health problems occurring among workers create the need to search for prophylactic measures.
Objective. The objective of the study was evaluation of the level of exposure to air pollution in a stable, and estimation of the degree of work load among workers engaged in tending horses.
Material and methods. The study was conducted twice, during the winter season, in a stable maintaining race horses, and in a social room. In order to evaluate workers' exposure, air samples were collected by the aspiration method. After the incubation of material, the total number of bacteria and fungi in the air was determined, as well as the number of aerobic mesophilic and thermophilic bacteria, expressed as the number of colony forming units per cubic meter of air (CFU/m^3). The measurement of total dust concentration in the air was also performed, simultaneously with the measurement of microclimatic parameters. The study of work load also covered the measurement of energy expenditure, evaluation of static physical load, and monotony of movements performed.
Conclusions. The stable may be considered as a workplace with considerable risk of the occurrence of unfavourable health effects.

Key words

air pollution, workers, exposure, stable, physical effort

INTRODUCTION

Farmers are the most numerous occupational group, a considerable part of whom are engaged in animal production. According to the data by the Main Statistical Office, in 2010, on the territory of Poland there were 173,600 horses. An increasing worldwide interest in the horse business is manifested in Poland by a change in the use of horses and an increase in their status, which has resulted in the growing engagement of many people in its functioning. There occurred a change in the proportions between breeding working and riding horses, and those maintained for recreational purposes, which was followed by attention being paid to the type and quality of buildings for horses, as well as the quality of the indoor air. Considering the recurrent inflammatory state of the airways in horses, their conventional keeping has changed on behalf of their grazing on pastures. A stable is a limited area where bedding material, fodder, and the animals themselves are the sources of many contaminants. In the respiration process, dust is introduced into the organism from the air containing microorganisms, endotoxins and glucans. The presence in the air of filamentous fungi is the main source of risk for humans exposed to organic dust. Considerable risk is created by metabolites of these fungi present in the air – mycotoxins. In many sectors of animal production, cases of illness were observed among workers exposed to mycotoxins, the presence of which in the air decreases the activity of alveolar macrophages. Inhalation of filamentous fungi with dust may be the cause not only of allergy, but also the cause of immunotoxic diseases [1, 2, 3, 4, 5, 6].

To-date, more than 600 biological factors have been identified, which may, together with other particles, be the cause of the respiratory system diseases: asthma, allergy, infectious and invasive diseases, or may even have a carcinogenic effect [3, 6, 7, 8].

Work tasks related with to animal production are of a dynamic character. Additionally performed work activities are associated with the carrying and transporting of heavy loads, assuming uncomfortable body positions at work, repeated movements of some parts of the body, which contribute to the work load among farmers [9, 10]. At workplaces in horse breeding there occurs additional risk related with the tending of the animals (being kicked by an animal, crushing, falls, etc.), which may cause stress and mental load in the workers, chemical risk related with the use of washing and cleaning agents, and care articles, also risk resulting from the effect of factors of the occupational environment, such as vibration, noise or changeable microclimatic conditions [11].

The objective of the presented study was evaluation of the working conditions of workers engaged in tending horses.

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MATERIALS AND METHODS

Investigations of the air in and around a stable maintaining 40 race horses in the Lublin Region were conducted twice in the winter season. The biological fraction of inhaled aerosol was measured using a de Ville Microbio impactor with programmed air flow (F.W. Parrett Ltd., UK). TSA (tryptone-soya-agar) medium was applied with the addition of 5% of defibrinated ram blood, and MEA – Malt Extract Agar (Emapol AG, Gdańsk, Poland). During the study, air samples were collected at 4 sites in the rooms marked: Stable 1, Stable 2, Stable 3, social room and background – outdoors. In order to determine the total number of the bacteria, samples were incubated: 24 at 37°C, 72 at 25°C and 48 at 5°C. Thermophilic bacteria were determined after incubation for 120 h at 55°C. The total number of fungi was determined after 5–7 days of incubation at 25°C. After the incubation of samples, the total number of bacteria and fungi in the air was determined, and the number of aerobic mesophilic and thermophilic bacteria expressed as the number of colony-forming units per cubic meter of air (CFU/m^3) [12]. In order to identify bacterial colonies, microscopic observations, staining by the Gram's method, and biochemical API bioMerieux tests, were performed. Filamentous fungi were identified by creating micro-cultures and using keys [13]. Total dust in the air of the fraction inhaled at the specified sites was carried out by the filtration-weighing method, according to the standard [14]. Whatman filters (No. 1820–025) were used in the study. Simultaneously, microclimatic parameters were measured: temperature, humidity, air flow.

Physical load among workers in the stable was evaluated considering the investigation of energy expenditure, physical static load, and monotony of movements performed. Energy expenditure was examined using a microprocessor meter of energy expenditure WE-4, while static load and monotony of movements were examined by the tabular method [15, 16].

RESULTS

Table 1 presents the results of evaluation of the load among workers in the stable. The workers tending horses perform their activities throughout the entire year, using 90% of the daily working time as an effective period of work. Work with tending horses is very arduous, which is evidenced by the total energy expenditure of 2,649 kcal/8h (11,099.3 kJ/8h), low static load and mediocre monotony of the movements performed.

Table 1. Evaluation of physical load

Activity	Duration of activity (min/h)	Energy expen-diture (kcal/min)	Total energy expen-diture (kcal)	Static load	Monotony of working movements
Preparation Provision of fodder Transport of fodder	60 60 30	6.3 6 4.6	378 360 138	low	mediocre
Cleaning (removal of impurities)	120	8.5	1.020	low	mediocre
Cleaning horses	120	4.2	504	mediocre	mediocre
Assistance with veterinary procedures	30	3.6	108	low	low
Other activities – preparation and taking horses outdoors	30	4.7	141	low	low
Final evaluation	450	----	2.649	low	mediocre

Table 2 presents the values of miocroclimatic parameters in the stable, which not always remained within the optimum limits specified for this type of room while tending horses. Based on the tests performed – Wilk and F-Snedecor, at the level of confidence of 0.01, it was found that at least one of the characteristics examined varied significantly (Tab. 3). Analysis of the measurements of the total dustiness showed that the social room for workers and outdoor conditions (background) did not differ from the aspect of the dust content (Fig.1), while for workers, this was an environment with low temperatures (Tab. 2).

Table 2. Microclimatic parameters of air in the stable

Sampling point	Temperature (°C)	Air flow (m/s)	Relative humidity (%)
Stable	3.83	0.16	65.2
Social room	5.6	0.10	58.3
Background / Outside	-2.8	1.22	54.1

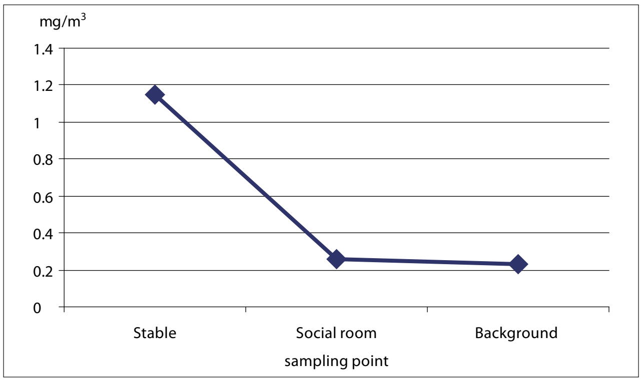

Figure 1. Mean values of dustiness at sampling points examined

Table 3. One-way analysis of variance tests for microclimatic conditions

Effect	Test					
	Air flow		Temperature		Absolute humidity	
	F	p	F	P	F	p
Intercept	412.74	0.00	316.76	0.00	35.114	0.00
Sampling point	208.55	0.00	393.43	0.00	114.75	0.00

The mean concentration of the total number of bacteria in the air in the stable exceeded the allowable level of microbiological contamination in animal rooms and the safe value for workers – 3.2×10^5 cfu/m^3 in the stable and 1.1×10^5 cfu/m^3 in the social room (Tab. 4) [17]. The concentration of aerobic mesophilic bacteria in the air in the stable was within the range 4.4×10^4–3.2×10^5 cfu/m^3; mean value in the social room – 1.6×10^5 cfu/m^3. At the majority of sampling points these values exceeded the proposals for allowable concentrations of mesophilic bacteria (1.0×10^5 cfu/m^3) for rooms contaminated with organic dust [3].

Similarly, at many sampling points the concentration of fungi exceeded the recommended values, while the numbers of thermophilic bacteria was in accordance

Table 4. Concentration of microorganisms in air (cfu/m^3)

Sampling point		Total No. of bacteria	Total No. of aerobic mesophilic bacteria	Total No. of thermophilic bacteria	Total No. of fungi
Stable	I	2.8×10^5	4.4×10^4	8.3×10^2	6.2×10^3
	II	3.7×10^5	3.2×10^5	1.3×10^4	1.3×10^4
	III	3.3×10^5	2.6×10^5	2.0×10^3	1.6×10^4
	arithmetic mean	3.2×10^5	2.1×10^5	5.6×10^3	1.2×10^4
Social room		1.6×10^5	1.1×10^5	2.9×10^2	6.3×10^3
Background / Outdoor		3.1×10^2	3.2×10^1	1.5×10^1	5.8×10^2

with recommendations, despite their high levels (Tab. 4). The highest values of total dust were noted in the stable (1.15 mg/m^3) (Fig. 1). This value was lower than the maximum concentration of particulate organic plant and animal origin in the working environment at the defined level of 4 mg/m^3.

Table 5. Filamentous fungi and bacteria identified

Sampling point	Identified fungi	Intensity of occurrence
Stable	*Acremonium sp.*	+
	Aspergillus flavus	++
	Aspergillus niger	++
	Aspergillus sp.	++
	Aspergillus versicolor	+
	Cladosporium herbarum	+
	Cladosporium sp.	+
	Fusarium sp.	+++
	Mucor sp.	+
	Penicilliomyces	+++
	Penicillium expansum	+
	Penicillium sp.	++
	Rhizopus oryzae	++
	Trichoderma viridae	+
	Trichoderma oryzae	+
	Ulocladium botrytis	+++
	Ulocladium chartarum	+
	Ulocladium sp.	+++
Social room	*Acremonium sp.*	+
	Aspergillus flavus	+
	Aspergillus Niger	++
	Aspergillus sp.	+
	Penicillium verrucosum	++
	Penicillium sp.	+
	Penicilliomyces	+
	Trichophyton rubrum	+
	Ulocladium botrytis	+
	Ulocladium chartarum	+
	Ulocladium spp.	+
Background / Outdoors	*Aspergillus flavus*	+
	Cladosporium macrocarpum	+
	Cladosporium sp.	+
	Penicillium sp.	+
	Penicillium expansum	++
	Rhizopus oryzae	++

Table 5. Filamentous fungi and bacteria identified (Continuation)

Bacteria identified during the study			
Identified bacteria	Intensity of occurrence	Identified bacteria	Intensity of occurrence
Staphylococcus aureus	+	*Microbacterium spp.*	+++
Staphylococcus lentus	+	*Propionibacterium avidum*	++
Stenotrophomonas malthophilia	++	*Acinetobacter spp.*	+
Arcanobacterium pyogenes (Actinomyces pyogenes)	++	*Escherichia coli*	+
Listeria grayi	+		
Brevibacterium spp.	+++		
Cellulomonas cellulans	+++		
Cellulomonas spp.	++		
Streptococcus spp.	+		

+ Few cfu, + + increase in the number of cfu, + + + abundant growth of cfu

Table 6. Matrix of correlation of variables describing microbiological contamination and dustiness

Variable	Total No. of bacteria	Total No. of aerobic mesophilic bacteria	Total No. of fungi	Total No. of thermo-philic bacteria	Total dust
Total No. of bacteria	1.00	0.62 *	0.78 *	0.28	0.45
Total No. of aerobic mesophilic bacteria	0.62 *	1.00	0.76 *	0.63 *	0.51
Total No. of fungi	0.78 *	0.76 *	1.00	0.65 *	0.65 *
Total No. of thermophilic bacteria	0.28	0.63 *	0.65 *	1.00	0.62 *
Total dust	0.45	0.51	0.65 *	0.62 *	1.00

– statistically significant correlation (p< 0.05).

The majority of microorganisms identified in the air in the stable were saprophytes; however, in this group there were also biological factors classified into Group 2 of hazards (Tabs. 5 and 6). Contact with these factors is not only via the aerogenic route, but also through the skin and mucous membranes.

The linear relationship between the examined characteristics of microbiological contamination and dustiness was investigated using analysis of variance (Tab. 7). It was found that all characteristics were significantly correlated, the strongest correlation being obtained between the total number of fungi and the total number of bacteria (0.78), and between the total number of fungi and the total number of aerobic mesophilic bacteria (0.76) (Tab. 7). Considering

Table 7. One-way analysis of variance tests for microbiological contamination and dustiness

Effect	**Test**									
	Total No. of bacteria		Total No. of aerobic mesophilic bacteria		Total No. of fungi		Total No. of thermo-philic bacteria		Total dust	
	F	p	F	P	F	p	F	P	F	p
Intercept	18.08	0.00	5.37	0.04	38.30	0.00	2.27	0.16	46.15	0.00
Sampling point	7.12	0.01	1.76	0.22	11.57	0.00	2.57	0.12	19.39	0.00

the correlations between characteristics, multi-dimensional analysis of variance was performed. With the Wilk's and F-Snedecor's tests on the level of significance 0.01, it was confirmed that at least one of the examined characteristics describing microbiological contamination and dustiness significantly differed according to the sampling point (Tab. 7). In order to test this phenomenon, one-way analyses of variance were performed. On the level of significance 0.01, it was found that each of the investigated characteristics of bioaerosol and dustiness significantly differed according to the sampling point (Tab. 7).

DISCUSSION

Animal production requires adequate organization of work by farmers while performing a number of work activities related with tending animals and the maintenance of farm rooms. This work is performed throughout the entire year, during the week and at weekends, and engages a worker from the early morning hours until late afternoon. Work with breeding animals, as confirmed by studies conducted at the Institute of Rural Health, is classified as heavy [10, 18]; similarly, the presented study confirms that work with tending animals is very heavy, which is evidenced by the energy expenditure. The noxiousness of the work is exacerbated by the conditions in which it is performed.

The air in animal rooms is a heterogenous mixture containing – together with organic dust – microorganisms and their toxins. The American Industrial Hygiene Association (AIHA), while describing the components of bioaerosol, devoted special attention to dust, especially the respirable fraction, which penetrates the area of gas exchange in the lungs. This fraction has been considered as the indicator of health risk in horses. The AIHA recommends measurement of dustiness for evaluation of the quality of the air in a stable. Keeping horses in the contaminated air of the stable leads to recurrent obstruction of the airways [6]. It has been estimated that in 33–80% of stables there occurs in horses non-septic inflammation of the airways, with a lower tolerance of physical effort, which disqualifies many animals from participation in training or competitions.

Similar relationships were sought for in humans in order to determine biomarkers in the respiratory system of workers tending animals (8, 19). Elfman et al. [8], report that in Sweden it is very difficult to specify the exposure of workers during the winter season, because the majority of processes and reactions taking place in the air are lowered. The value of dustiness in these buildings did not exceed allowable values, which is in accordance with the presented study. Samadi et al. [6] obtained higher concentrations of dust, endotoxins and β(1–3) glucans on the level of horses' heads. High correlations were observed between the levels of organic dust and endotoxins. According to the research technique used, Pomorska et al. [5] showed differences in concentrations of endotoxins in the air in stables (LAL test -1.14 μg/m^3, GC-MSMS – 1.42 μg/m^3). Considerably lower levels were noted in rooms where hay was stored (0.09 g/m^3 and 0.03 μg/m^3, respectively. The application of GC-MSMS analysis for the determination of endotoxins seems important because it simultaneously allows determination of the type of bacteria producing LPS. Studies conducted among blacksmiths at work, despite a high level of dust, showed low levels of endotoxins and glucans. A high changeability of exposure to dust on subsequent workdays and in the daily cycle was indicated. Intensification of work activities in the stable during morning hours (including: cleaning of the boxes and animals, preparation and provision of fodder) correlated with a higher dustiness in the air. In some rooms, mechanical ventilation systems were installed in order to decrease workers' exposure to contaminations. An increase in air exchange reduced the concentration of chemical contaminants in the air, while the levels of total and respirable dust remained unchanged [20].

During the winter season, the quality of indoor air in a stable considerably differs from that of the ambient air. This results from both the specificity of managing this environment, and the hermetization of the rooms [8]. Samadi et al [6] observed a higher concentration of bioaerosol in a stable during the morning hours, which ranged within 6.70×10 -1.92×10^4 for bacteria, and 7.40×10 – 2.42×10^4 for fungi. Also, smears from the walls contained an elevated content of microorganisms. However, the researchers found slightly lower numbers of bacteria in the air, compared to the presented study. The concentrations of fungi in the air in these stables were similar. The dominant fungi species were *Alternaria* and *Cladosporium*. In own studies, the following species were most abundant in the stable: *Penicilliomyces, Fusarium sp, Ulocladium botritis,* and *Ulocladium sp.* In the social room for workers, *Trichophyton rubrum* was identified, classified in Group 2 of hazards. Filamentous fungi belong to allergens which are very common. Most frequently, allergy is identified for *Alternaria, Cladosporium, Aspergillus,* or *Penicillium*. Other filamentous fungi may be also responsible for dermal fungal changes, often manifested in farmers after many years, and may also be the cause of bronchial asthma. In patients with decreased immunity, fungi considered as saprophytes (*Mucor, Absidia, Rhizopus*) may cause infections [21]. Nardoni et al. [22] compared concentrations of fungi in stables in Italy in individual seasons of the year. The highest concentrations were observed in summer, winter and spring. These values ranged from 1,750 – 3,000 cfu/m^3. Statistically significant values were noted for *A. niger* in winter and spring. Fungi of the genus *Aspergillus* were reported as causative agents of allergic diseases. Inhalation of *A. fumigatus* may cause lung diseases in horses affected by Chronic Obstructive Pulmonary Disease (COPD). This species is also an etiologic factor of fungal changes in the horse cornea, whereas *A. flavus* – of an infection of the airways. A humid environment (optimum absolute humidity of over 70%) and abundance of easily absorbed sources of nutrients is conducive to the development of microflora in animal rooms. Elfman et al. [8], in February and March, i.e. within a period of limited air exchange, noted the growth of microorganisms, of which the dominant agent was *Streptomyces spp.* and correlated with respiratory system problems in humans. In the presented study, *Brevibacterium spp., Arcanobacterium,* and *Microbacterium spp.,* were most frequently identified, as well as agents classified into Group 2 of hazards, such as: *Staphylococcus aureus, Actinomyces pyogenes,* and *Streptococcus spp.* [23], while investigating air in stables in eastern Poland, found that concentrations of mesophilic bacteria were within the range 26.2–150.1×10^3 CFU/m^3. The concentration of these microorganisms in the presented study was on a similar level; however, lower concentrations of thermophilic bacteria were noted. Among thermophilic actinomycetes, the researchers found the largest numbers of *Saccharopolyspora rectivirgula, Saccharomonospora viridis* and *Thermomonospora fusca*. A considerable diversity of microflora was observed. In all stables, *Aspergillus spp., Penicillium spp., Eurotium spp., Monilia spp.* and *Alternaria alternate* were identified. The concentration of dust in the rooms examined corresponded to the results of the presented study.

Into the composition of identified bioaerosols in animal rooms enter Gram-negative and Gram-positive bacteria, actinomycetes, fungi and their toxins causing non-specific inflammatory reactions in the lungs of occupationally-exposed individuals [3, 23]. There are reports concerning the necessity to carry out monitoring of the quality of the air in stables due to the frequent occurrence of upper airways disorders. This problem especially concerns closed rooms where animals are kept, horse riding is practiced, or hay of poor quality is provided. The consequence of such exposure may be chronic bronchitis (CB). In horses, a clear decrease in efficiency is observed. The disease affects even as much as 50% of animals (Nardoni et al., 2005). Therefore, biomarkers of exposure of workers engaged in tending horses should be sought [6, 8]. Methods for the measurement of eosinophilic cationic protein (ECP) seem to be very useful diagnostically as a marker of inflammation and bronchi hypersensitivity. These studies should be supplemented by the measurement of the peak expiratory flow (PEF).

An increase in the recreational use of horses brings about the risk of occurrence of respiratory system problems in workers engaged in tending the animals. The conducted studies indicate that it is necessary to search for new methods of evaluation of exposure in order to effectively prevent these problems.

CONCLUSIONS

1. The air in the stable was considerably contaminated with microorganisms.
2. Among the microorganisms identified there occurred agents from Group 2 of biological hazards.
3. A stable may be considered as a workplace with a considerable risk of occurrence of unfavourable health effects.
4. Work with tending horses requires from the workers a good physical and health conditions, as well as proper predispositions.

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