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Exposure to Inhalable Dust, Endotoxins, β(1→3)-Glucans, and Airborne Microorganisms in Horse Stables

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Objectives: Workers in horse stables are likely exposed to high levels of organic dust. Organic dusts play a role in increased risk of inflammatory reactions and are associated with respiratory diseases. The aim of this study was to investigate dust, endotoxin, β(1→3)-glucan, and culturable microorganisms exposure levels in horse stables.

Methods: Ambient ($n = 38$) and personal ($n = 42$) inhalable dust samples were collected using PAS-6 sampling heads. As a special measurement, we included sampling near the horses' heads. Samples were analyzed for endotoxin and β(1→3)-glucan by Limulus amebocyte lysate assay and an inhibition enzyme immunoassay, respectively. Culturable bacteria and fungi were collected with an Anderson impactor.

Results: Geometric means (GMs) of personal exposure to dust, endotoxin, and β(1→3)-glucan were 1.4 mg m^{-3} (range 0.2–9.5), 608 EU m^{-3} (20–9846), and 9.5 μg m^{-3} (0.4–631 μg m^{-3}), respectively. Exposure levels in the morning shift were higher compared to other shifts. The GMs (ranges) of culturable bacteria and fungi were 3.1×10^3 colony-forming unit (CFU) m^{-3} (6.7×10 to 1.9×10^4) and 1.9×10^3 CFU m^{-3} (7.4×10 to 2.4×10^4), respectively. Variance components for endotoxin and β(1→3)-glucan were considerably higher than for dust. Based on dummy variable in a mixed regression analysis, the predominant task explaining exposure levels of dust, endotoxin, and β(1→3)-glucan was sweeping the floor. For β(1→3)-glucan, feeding the horse was also an important determinant.

Conclusion: Dust, endotoxin, and β(1→3)-glucan exposure are considerable in horse stables. Bacterial and fungal exposure levels were moderate. Endotoxin exposures were above the Dutch proposed standard limits, suggesting workers in horse stables to be at risk of adverse health effects.

Keywords: dust; endotoxin; glucan; microorganism; stables; variance components

INTRODUCTION

Organic dust usually has a heterogeneous composition of materials from microbial, plant, and animal sources. Organic dust may contain pathogenic or non-pathogenic living or dead bacteria and fungi, high molecular weight allergens, bacterial endotoxins, β(1→3)-glucans, pollen, and plant fibers (Douwes *et al.*, 2003). These components may penetrate into the lungs of exposed workers. Two identified major pro-inflammatory components of organic dusts are bacterial endotoxin (Liebers *et al.*, 2008) and mould β(1→3)-glucan (Douwes, 2005). Endotoxin is a component of the cell wall of Gram-negative bacteria and a ubiquitous component of organic dusts (Douwes *et al.*, 2003). Endotoxin has been proven to be a powerful inflammatory agent

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and much of its toxicity is associated with the lipid A component. Several occupational studies have demonstrated that workers who come into contact with high levels of endotoxin have an increased risk of inflammatory reactions that are associated with respiratory diseases such as asthma, chronic bronchitis, and organic dust toxic syndrome (Liebers *et al.*, 2008). β(1→3)-glucan is an important cell wall constituent of most fungi, some bacteria, and numerous plants (Douwes *et al.*, 2003) and can cause various adverse health effects. It has been suggested that β(1→3)-glucan might be of importance in bioaerosol-induced inflammatory responses as well, although the health effects of glucans are not yet conclusive (Douwes, 2005). Most evidence comes from epidemiological studies in households in which β(1→3)-glucan levels were associated with respiratory symptoms (Douwes *et al.*, 2003; Schram-Bijkerk *et al.*, 2005).

Recent studies demonstrated that working with horses was associated with an increased risk of respiratory symptoms and/or organic dust toxic syndrome (Gallagher *et al.*, 2007; Mazan *et al.*, 2009). Some small studies found that horses were exposed to high levels of endotoxin in stables (McGorum *et al.*, 1998; Berndt *et al.*, 2008), confirming the presence of endotoxin in horse stables. These findings prompted us to hypothesize that workers in horse stables might be exposed to high levels of endotoxin-contaminated organic dust as well. However, to date, comprehensive and systematic studies on organic dust exposure have not been conducted in horse stables. Therefore, the purpose of the current study was to quantify the exposure levels of dust, endotoxin, and β(1→3)-glucan in horse stables. In addition, we explored levels of culturable microbial exposure in horse stables. Exposure variability and determinants of exposure levels were investigated.

METHODS

Study design

All sampling took place during spring. Ambient inhalable dust and culturable airborne microbial samples were collected in barns on four separate premises, numbered 1–4, which all included at least one barn. The number of pens per barn varied between 4 and 50 pens, and pens consisted of solid wall panels of ∼2.5 m in height with open tops. Pen floor covering (bedding) in premise 1 consisted of wood chips, while other premises used straw. In all stables, the majority of pens in a barn were occupied with horses, containing one horse per pen. All premises used hay for feeding.

In addition to the ambient samples, repeated personal inhalable dust samples were collected in two stables (Stables 1 and 2) and in a farriery which was a part of the first stable during 1 week.

Workers in the stables worked during workshifts and samples were collected for all shifts. Stable 1 had three workshifts: a morning shift, 6 a.m. to 2 p.m.; an afternoon shift, 2 p.m. to 10 p.m.; and a night shift, 10 p.m. to 6 a.m. The farriery of stable 1 had only one shift, a morning shift. stables 2, 3, and 4 had two workshifts: a morning shift, 6 a.m. to 2 p.m., and an afternoon shift, 2 p.m. to 10 p.m. During a workshift, several tasks were carried out: cleaning out the stable, working with horses, feeding the horses, sweeping the floor, horseshoe repair, and administration. Most of these tasks were performed during the morning shift and fewer tasks were performed in the afternoon shift, while the night shift tasks were mostly observational. Daily activities and other information were documented on field forms.

Exposure measurements

Personal and stationary inhalable dust samples were collected on 25-mm glass fiber filters (Whatman International Ltd, Maidstone, UK) using a PAS-6 inhalable dust sampler (Kenny *et al.*, 1997) and a Gil-Air 5 pump (Gillan, Sensidyne, Clearwater, FL, USA). The pump flow rate (2 l min^{-1}) was calibrated using a rotameter at the beginning of each sampling period, and it was again checked at the end of sampling. The volume of air sampled was calculated based on the flow rate and duration of sampling. Dust samples were stored at −20°C immediately after collection until further processing. In addition, one field blank based on each shift per day was collected to control for cross-contamination.

Personal inhalable dust samples were collected from the breathing zone of workers during complete workshifts. Stationary inhalable dust samples were collected during an entire shift period by positioning the sampler in the middle of the barn near the pens, 150 cm above the floor level, as well as in the middle of the farriery.

In addition, we collected one to two dust samples near a horse head as a 'special measurement' of general environmental exposure in each barn at all stables. The same equipment for personal inhalable dust sampling was used. The PAS-6 inhalable dust sampler was mounted at the holster in the breathing zone of horses at a distance of ∼10 cm from the nostrils.

The concentration of dust on filters was estimated gravimetrically by pre- and post-weighing of filters on a Mettler AT261 analytical balance (Mettler-Toledo Ltd, Greifensee, Switzerland) with 0.01 mg readability which was used in an acclimatized room (temperature 22.6°C, relative humidity 35.2%, air pressure 1019 mbar) where all filters were conditioned for 24 h prior to weighing.

Extraction and analysis of endotoxin were performed as previously described by Spaan *et al.* (2008). Briefly, filters were immersed in 5 ml of pyrogen-free water plus Tween 20 (0.05% v/v). After shaking for 60 min on a horizontal shaker, the tubes were centrifuged for 15 min at 1000 g (2094 r.p.m.). Supernatants were harvested and stored in 0.1 ml aliquots at −20°C until analysis. The endotoxin concentration was determined in supernatant using a quantitative kinetic Limulus amebocyte lysate method (lot no. lysate 1L6756, lot no. 2L0090; Bio Whittaker). For each assay, a 12-point standard curve of an *Escherichia coli* standard obtained by the supplier was created over the concentration range 0.01–25 EU ml^{-1}. The results were estimated as endotoxin units per cubic meter (EU m^{-3}).

Following extraction for endotoxin, heat extraction was performed for β(1→3)-glucan determination. β(1→3)-glucan was assayed with a specific inhibition enzyme immunoassay which was developed and described by Douwes *et al.* (1996). All dust samples were analyzed in duplicate. β(1→3)-glucan levels were expressed as μg m^{-3}.

At each of the worksites, multiple samples of culturable bacteria and fungi were collected with an Andersen one-stage impactor. Samples were collected during the morning shift between 9 and 11 a.m. and during the afternoon shift between 3 and 5 p.m. Tryptone soy agar (TSA) plates were used for bacterial samples, and dichloran-glycerol agar 18 (DG18) plates for fungal samples. Samples were collected ∼150 cm above the ground to correspond with the height of the personal breathing zone. Before sampling, Andersen impactors were cleaned and washed with water and soap, then autoclaved, allowed to cool, and packed in aluminum foil. Andersen samples were collected at a flow rate of 28.3 l min^{-1} for 30 s up to 1 min. In between sampling, Andersen impactors were cleaned by wiping with ethanol. TSA plates were incubated for 24 h at 37°C. Dichloran-glycerol agar plates were incubated for 4 days at 24°C. Colonies were counted twice using the positive hole correction factor (Andersen, 1958). The average of two counts was divided by the volume of air sampled to express the bioaerosol concentrations as colony-forming units per cubic meter of air (CFU m^{-3}). The numbers of counted colonies were corrected for blank values.

Based on blank filters, the lower limit of detection (LOD) for dust weight was 0.08 mg, which corresponds to 0.10 mg m^{-3}. The average LOD for endotoxin was 16.25 EU per filter (range 15–17.5) which corresponds to 22.19 EU m^{-3}; four samples (4.4%) were below this LOD. The average LOD for β(1→3)-glucan was 0.56 μg per filter (range 0.16–0.85), which corresponds to 0.75 μg m^{-3}; nine samples (10%) were below this LOD. Samples below the LOD were replaced by a value of 2/3 of the LOD.

Computational and statistical analysis

Exposure levels were log-normally distributed (Kolmogorov–Smirnov test, $P < 0.05$) and so further analyses were carried out on log-transformed data. Descriptive statistics were estimated in term of arithmetic means (AMs), geometric means (GMs), as well as the corresponding geometric standard deviations (GSDs). The analysis of variance test was used to compare the exposure levels between shifts, followed by the Tuckey–Kramer test as a *post hoc* test to examine the differences between groups. Pearson correlation coefficients were calculated to assess relationships between dust, endotoxin, and β(1→3)-glucan concentrations. Variance components of exposure concentrations were estimated employing mixed-effects models (PROC MIXED). In these models, the effects of potential exposure determinants including 'date of sampling' and 'shift' on between- and within-worker exposure variances were investigated as fixed effects (Spaan *et al.*, 2008), while 'worker identity' was included as a random effect. Between-worker (${}_{bw}\sigma^2$) and within-worker (${}_{ww}\sigma^2$) variance components were estimated using a restricted maximum likelihood method. Additionally, the effect of tasks on exposure levels was identified in a mixed regression analysis. Workers with only one measurement were excluded from the analysis to get more robust estimates. Variables were entered in the model when in a univariate regression they met a statistical significance level of $P < 0.20$. Variables that had a statistical significance level of $P < 0.05$ were retained in the final mixed regression model. Data analyses were performed using SAS software version 9.1 (SAS Institute Inc.).

RESULTS

A total of 95 samples were analyzed for dust levels, 91 samples for endotoxin, and 90 samples for β(1→3)-glucan after excluding samples in which equipment failed during sampling or extraction (dust 12 samples, 11.2%; endotoxin 4 samples, 4.2%; and β(1→3)-glucan 5 samples, 5.3%). The average sampling times for personal, stationary, and near the horse head samples were 6.5, 6.2, and 3.5 h, respectively. The reproducibility expressed as a coefficient of variation (CV %) for endotoxin and β(1→3)-glucan analysis were 17 and 28%, respectively.

Table 1 gives a summary of observed exposure levels. The highest dust, endotoxin, and β(1→3)-glucan levels were found for near the horse head samples, followed by personal samples. Personal samples collected in the farriery showed high dust levels associated with relatively low endotoxin and β(1→3)-glucan levels. Overall, significant correlations were observed between dust and endotoxin ($R = 0.75$, $P < 0.0001$), dust and β(1→3)-glucan ($R = 0.69$,

Table 1. Distribution of dust, endotoxin, and β(1→3)-glucan exposure levels in horse stables

	Worksite	Dust (mg m^{-3})						Endotoxin (EU m^{-3})						β(1→3)-glucan (μg m^{-3})					
		n	ND	AM	GM	GSD	Min–max	*n*	ND	AM	GM	GSD	Min–max	*n*	ND	AM	GM	GSD	Min–max
Personal	Stable 1	35	—	1.9	1.2	2.5	0.2–9.5	35	—	1478	742	3.0	92–9846	34	2	24	8.4	4.2	<LOD–177
	Farriery	4	—	2.9	2.3	2.3	1.0–5.3	4	1	30	28	1.5	<LOD–47	4	—	3.6	2.4	3.3	0.4–6.2
	Stable 2	3	—	3.7	2.8	2.5	1.2–7.4	3	—	4270	3429	2.2	1587–8320	3	—	297	211	2.7	86–631
	Total	42	—	2.1	1.4	2.6	0.2–9.5	42	1	1539	608	4.5	<LOD–9846	41	2	42	9.5	5.3	<LOD–631
Stationary	Stable 1	25	1	0.5	0.4	2.0	<LOD–1.1	23	2	271	178	2.6	<LOD–1385	23	5	3.8	2.2	2.8	<LOD–28
	Farriery	1	—	0.4	—	—	—	1	1	—	—	—	—	1	1	—	—	—	—
	Stable 2	3	—	0.4	0.37	2.6	0.2–0.7	1	—	86	—	—	—	1	—	7.1	—	—	—
	Stable 3	1	—	0.2	—	—	—	1	—	136	—	—	—	1	—	2.7	—	—	—
	Stable 4	2	—	0.3	0.3	1.6	0.2–0.4	2	—	413	412	1.1	382–444	2	—	28	25	1.8	17–39
	Total	32	1	0.5	0.4	1.9	<LOD–1.1	28	3	260	167	2.8	<LOD–1385	28	6	5.8	2.6	3.3	<LOD–39
On the horse head	Stable 1	12	—	7.3	5.1	2.3	1.6–25	12	—	3756	1826	3.4	262–2512	12	1	50	16.6	5.1	<LOD–213
	Stable 2	3	—	2.4	2.1	1.9	1.0–3.5	3	—	1720	1703	1.6	1542–1859	3	—	423	228	5.3	34–799
	Stable 3	3	—	3.1	3.0	1.5	2.3–4.9	3	—	2253	1939	1.9	1332–4051	3	—	88	76	2.0	39–149
	Stable 4	3	—	6.2	3.1	5.8	0.4–12.7	3	—	19955	8691	5.9	1395–48486	3	—	565	308	5.2	47–1041
	Total	21	—	5.9	3.9	2.6	0.4–25	21	—	5559	2296	3.4	262–48485	21	1	183	46	6.7	<LOD–1041
Overall		95	1	2.4	1.2	3.3	<LOD–25	91	4	2073	555	5.0	<LOD–48484	90	9	63	9.2	6.7	<LOD–1041

n, number of samples; ND, number of non-detectable samples; AM, arithmetic mean; GM, geometric mean; GSD, geometric standard deviation; Min–max, minimum and maximum; <LOD, below the lower limit of detection.

$P < 0.0001$), and endotoxin and β(1→3)-glucan ($R = 0.86$, $P < 0.0001$).

The personal exposure levels were used to explore the chance of exceeding the Dutch proposed occupational exposure limit of nuisance dust (10 mg m^{-3}) and endotoxin (recommended health-based exposure limit of 50 EU m^{-3} and proposed standard limit of 200 EU m^{-3}) (Douwes *et al.*, 2003). All personal dust exposure levels were lower than the occupational exposure limit of nuisance dust of 10 mg m^{-3}, while 85.7% of endotoxin samples exceeded the proposed standard limit of 200 EU m^{-3} and 90.5% were higher than the recommended health-based exposure limit of 50 EU m^{-3}. No current standards are available for β(1→3)-glucan exposure levels.

The range of exposures was large, especially for β(1→3)-glucan in near the horse head samples (GSD 6.7) and personal samples (GSD 5.3) (Table 1). The personal exposure levels of β(1→3)-glucan and endotoxin ranged from 0.4 to 631 μg m^{-3} (GSD 5.3) and 20 to 9846 EU m^{-3} (GSD 4.5), respectively. The overall GM concentrations for inhalable dust, endotoxin, and β(1→3)-glucan were 1.2 mg m^{-3} (GSD 3.3), 555 EU m^{-3} (GSD 5.0), and 9.2 μg m^{-3} (GSD 6.7), respectively.

Significant differences were found between shifts for personal exposure levels of dust, endotoxin, and β(1→3)-glucan samples ($P < 0.05$), with the highest exposure levels during the morning shift (Table 2). A similar trend was observed for stationary exposure levels of dust, endotoxin, and β(1→3)-glucan, although this did not reach statistical significance.

Fungal and bacterial exposures

Bacterial levels in the air were moderately elevated and ranged from 6.7×10 to 1.9×10^4 CFU m^{-3} (Table 3). The highest GM concentration (6.6×10^3 CFU m^{-3}) was found in Stable 2 compared to the other stables ($P < 0.05$). The levels of airborne bacteria did not differ between shifts, although a tendency for higher values in the morning shift compared to the afternoon shift was observed [GM ratio 1.6, 95% confidence interval (CI) 0.8–3.0]. The mould concentration in the air ranged from 7.4×10 to 2.4×10^4 CFU m^{-3} (Table 3). The levels of airborne moulds differed significantly between stables ($P < 0.05$). The GM concentration of moulds was ~3.5 times greater in the morning versus the afternoon shift (95% CI 0.9–14.2), but this difference was not significant ($P > 0.05$).

Variance components

The results of the mixed-effect models for dust, endotoxin, and β(1→3)-glucan exposure levels with only workers showed a between-worker variance of 0.29, 1.36, and 1.14, respectively (Table 4). The within- and between-worker variances for endotoxin and β(1→3)-glucan were higher than dust, and between-worker variances were considerably higher than within-worker variances. In contrast, the within-worker variances for dust were higher than between-worker variances. Introducing shift as fixed effect explained 48% of the between-worker variance for dust, while the model that only included date of sampling explained 34% of the between-worker variance. The variance ratio (λ) for different variability measurements is presented in Table 4.

Modeling exposure for tasks

We recorded the tasks (activities) and duration of tasks that were performed during personal inhalable dust sampling. The median time spent at each task was determined, being 125 min for cleaning out the stable, 75 min for working with the horses, 60 min for feeding the horses, 50 min for sweeping the floor, and 30 min for administration. Using mixed regression analyses, we determined if tasks affect the personal exposure levels, excluding samples taken in the farriery. This was done based on whether the tasks was performed or not (dummy variables) or based on the time spent at tasks (Table 5). Only those tasks that remained significant are presented in Table 5.

Based on dummy variables, sweeping the floor was the predominant task that explained exposure levels of dust, endotoxin, and β(1→3)-glucan. For β(1→3)-glucan, feeding the horse was also an important task. Reported tasks explained 57, 80, and

Table 2. Concentration of dust, endotoxin, and β(1→3)-glucan regarding shift

	Shift	Dust (mg m^{-3})					Endotoxin (EU m^{-3})					β(1→3)-glucan (μg m^{-3})				
		n	AM	GM	GSD	Min–max	*n*	AM	GM	GSD	Min–max	*n*	AM	GM	GSD	Min–max
Personal	Morning	18	3.0	2.1	2.3	0.7–9.5	18	2497	1503	2.8	252–9846	18	41	21	3.4	3.3–177
	Afternoon	7	0.9	0.9	1.5	0.4–1.6	7	505	491	1.3	349–762	7	6.0	5.5	1.6	3–11.4
	Night	10	0.6	0.5	1.9	0.2–1.2	10	326	282	1.8	92–605	9	3.2	2.0	2.7	<LOD–12
Stationary	Morning	11	0.6	0.4	2.3	<LOD–1.1	10	353	227	3.6	<LOD–1385	10	6.1	3.7	2.8	<LOD–28
	Afternoon	9	0.5	0.4	1.8	0.2–1.0	8	187	159	1.9	74–329	8	2.0	1.6	2.2	<LOD–4.3
	Night	5	0.4	0.3	1.7	0.2–0.6	5	161	130	2.1	41–319	5	2.1	1.3	3.0	<LOD–5.3

n, number of samples; AM, arithmetic mean; GM, geometric mean; GSD, geometric standard deviation; Min—max, minimum and maximum; <LOD, below the lower limit of detection.

Table 3. Summary of culturable bacteria and fungal concentrations in horse stables collected by means of an Anderson impactor

	Bacteria samples (TSA)					Fungi samples (DG18)				
	n	AM	GM	GSD	Min–max	*n*	AM	GM	GSD	Min–max
Stable 1	20	6.26×10^3	4.70×10^3	2.6	2.87×10^2 to 1.83×10^4	18	1.54×10^3	9.00×10^2	2.8	7.40×10 to 7.79×10^3
Stable 2	8	7.82×10^3	6.62×10^3	1.8	3.54×10^3 to 1.92×10^4	6	5.21×10^3	4.89×10^3	1.5	3.17×10^3 to 8.65×10^3
Stable 3	12	1.22×10^3	8.30×10^2	3.0	6.70×10 to 2.66×10^3	8	1.00×10^4	5.13×10^3	4.5	3.84×10^2 to 2.42×10^4
Stable 4	10	4.22×10^3	3.76×10^3	1.7	1.97×10^3 to 7.41×10^3	—	—	—	—	—
Morning	30	5.95×10^3	3.74×10^3	3.5	6.70×10 to 1.92×10^4	26	5.13×10^3	2.33×10^3	4.2	7.40×10 to 2.42×10^4
Afternoon	20	3.31×10^3	2.40×10^3	2.5	2.87×10^2 to 7.45×10^3	6	9.30×10^2	8.20×10^2	1.8	3.07×10^2 to 1.54×10^3
Overall	50	4.89×10^3	3.13×10^3	3.1	6.70×10 to 1.92×10^4	32	4.35×10^3	1.91×10^3	3.9	7.40×10 to 2.42×10^4

TSA, Tryptone soy agar; DG18, dichloran-glycerol agar 18; *n*, number of samples; AM, arithmetic mean; GM, geometric mean; GSD, geometric standard deviation; Min–max, minimum and maximum; <LOD, below the lower limit of detection.

57% of the variation in dust, endotoxin, and β(1→3)-glucan exposure levels, respectively. The estimated effect of sweeping the floor was the highest for endotoxin and β(1→3)-glucan exposure level (factor 4.4 and 4.9, respectively). This means that spending time on sweeping the floor results in an exposure level which is increased by a factor of 4.4 or 4.9 compared to when no time is spent on sweeping the floor. Including the time spent on tasks resulted in the same activities explaining the exposure, except feeding the horses for β(1→3)-glucan levels was no longer significant. Factors depicted in Table 5 express the change in exposure levels that were associated with the median time spent on that task, e.g. sweeping the floor for 50 min (the median time) will result in a factor 2.3 ($e^{0.017 \times 50\ min}$) increased endotoxin level.

DISCUSSION

To our knowledge, this is the first study to comprehensively assess inhalable dust, endotoxin, and β(1→3)-glucan exposures in horse stables. Thus far, levels on personal exposure of dust, endotoxin, and β(1→3)-glucan have not been previously reported, and only a few studies have published stationary dust levels in different horse barns (Woods *et al.*, 1993; McGorum *et al.*, 1998; Rosenthal *et al.*, 2006; Clements and Pirie, 2007).

The present study demonstrates that workers are exposed to high concentrations of inhalable dust. Inhalable personal dust concentrations in the current study are comparable to those found for workers in cowsheds and the animal feed industry (Virtanen *et al.*, 1988; Simpson *et al.*, 1999;). As it has been reported by others (Smid *et al.*, 1992; Virtanen *et al.*, 1988), considerably higher dust levels were found in personal samples compared with stationary samples. Lower levels of dust, endotoxin, and β(1→3)-glucan in stationary samples versus personal samples is likely due to the greater distance from the dust sources, as stationary sampling took place in the middle of the corridor near the pens. The observed high levels of dust, endotoxin, and β(1→3)-glucan near the horse head samples are probably due to the breathing patterns of horses during feeding of potentially dusty hay.

In the present study, dust exposure levels of near the horse head samples were relatively low (AM 5.8 mg m^{-3}) compared to those obtained (AM 17.5 mg m^{-3}) by Woods *et al.* (1993), while levels were higher than those found (AM 2.05 mg m^{-3}) by Bartz and Hartung (1993). Comparisons need to be considered with caution though, since other types of dust samplers were used.

The levels of endotoxin and β(1→3)-glucan were associated with the level of dust exposure and the contamination of the dust. Lowest dust exposures

were found in stationary samples, where the contaminations of the dust with endotoxin and β(1→3)-glucan were also low. In contrast, endotoxin and β(1→3)-glucan exposure levels in the compartment of the farriery were low or not detectable, whereas the dust exposure levels were high. Farriers usually carry out a variety of tasks, including removal of worn shoes, cleaning the hooves, cutting the metal, heating and hammering shoes, and nailing the shoes to the hooves. Most of the dust exposure associated with these activities likely included other components than organic dust, e.g. metal dust. This could be the reason why we found high dust exposures with low concentrations of endotoxin and β(1→3)-glucan.

Overall, our measurements demonstrated that personal endotoxin exposure levels were moderately high based on Dutch proposed standard limits, which is supported by a moderate high level of culturable bacteria as well. The personal endotoxin exposure levels in the current study are comparable to those levels previously reported in German pig houses (average 585 EU m^{-3}, range 43–7469) (Schierl *et al.*, 2007), as well as similar to levels found at diary farms (GM 560 EU m^{-3}), but somewhat lower than the levels in pig farms (GM 1510 EU m^{-3}) (Spaan *et al.*, 2006). This also shows that endotoxin is ubiquitous in horse stables, but control measures can reduce exposures. To reduce exposures to within exposure standard limit of 50 EU m^{-3}, a decrease in exposure of 2–200 fold is needed. Since sweeping the floor was the predominant task associated with higher exposure, control measures must be tailored to this working process, e.g. pre-wetting the surfaces before sweeping.

There is a limitation in our study regarding the effect estimates of tasks on exposure levels. Since the time spent on each task in horse stables was short, we assumed that the exposure concentrations were simply proportional to task being performed or spent time at tasks during the shift.

The measured concentrations of β(1→3)-glucan were found to be high, which have been supported by the high levels of culturable fungi as well. Only a few studies have described β(1→3)-glucan in occupational environments. The personal β(1→3)-glucan exposures in horse stables were higher than levels previously reported in the grain farmers (Halstensen *et al.*, 2007), waste management chain (Wouters *et al.*, 2006), and waste composting facility (Douwes *et al.*, 1996). This might be explained

Table 4. Estimated variance components for personal exposure models

Exposure models	G[a]	$_{ww}\sigma^{2b}$	$_{bw}\sigma^{2b}$	$_{ww}R^{c}_{0\cdot95}$	$_{bw}R^{c}_{0\cdot95}$	λ^{d}
Dust (mg m^{-3})						
Worker only	—	0.33	0.29	9.5	8.2	1.14
Date	8	0.40	0.19	11.9	5.5	2.10
Shift	3	0.28	0.15	7.9	4.6	1.90
Endotoxin (EU m^{-3})						
Worker only	—	0.42	1.36	12.7	97.7	0.31
Date of sampling	8	0.38	1.19	11.2	72.0	0.32
Shift	3	0.39	1.36	11.5	96.7	0.27
β(1→3)-glucan (μg m^{-3})						
Worker only	—	0.54	1.14	17.5	65.7	0.47
Date of sampling	7	0.57	1.01	18.9	51.4	0.56
Shift	3	0.48	0.59	14.9	20.3	0.81

[a]The number of fixed-effects levels (determinants of exposure).
[b]$_{ww}\sigma^2$ and $_{bw}\sigma^2$: the estimating of the within- and the between-worker variance components, respectively.
[c]$_{ww}R_{0\cdot95}$ and $_{bw}R_{0\cdot95}$: estimated ratio of the 2.5th and 97.5th percentile of the log-normally distributed mean exposures.
[d]λ: variance ratio of within- and between-worker variance.

Table 5. Activities associated with personal inhalable dust, endotoxin, and β(1→3)-glucan exposure and estimated factor for exposure change (present versus not present or median values)

	Units (1/0)[a]		Time (median)	
	Regression coefficient (β)	Factor for exposure change (95% CI)	Regression coefficient (β)	Factor for exposure change (95% CI)
Dust (mg m^{-3})				
Intercept	−0.58	—	0.216	—
Sweeping the floor	1.29	3.6 (2.4–5.6)	0.023	3.2 (1.7–5.8)
Endotoxin (EU m^{-3})				
Intercept	5.73	—	6.249	—
Sweeping the floor	1.47	4.4 (2.5–7.6)	0.017	2.3 (1.4–3.9)
β(1→3)-glucan (μg m^{-3})				
Intercept	0.86	—	1.491	—
Feeding the horse	1.06	2.9 (1.3–6.3)	—	—
Sweeping the floor	1.58	4.9 (2.3–10.5)	0.018	2.5 (1.2–5.2)

CI, confidence interval.
[a](1/0) dummy variable: present versus not present.

by differences in dust composition, like a relatively large promotion of plant material. At present, no exposure standards exist for β(1→3)-glucan.

Higher dust and microbial exposures were found during the morning shift. The lower exposure levels to dust, endotoxin, and β(1→3)-glucan for the night shift is probably due to the reduced workload and mostly observational nature of the tasks; whereas during the morning shift, horses were fed and stalls were cleaned.

In the present study, the concentrations of fungi varied between stables which are in agreement with findings reported by Nardoni *et al.* (2005). Given the limited number of samples, determinants of exposure levels were not explored.

In general, our results showed a large exposure variability, with the largest variation in β(1→3)-glucan levels (GSD = 6.7 for β(1→3)-glucan versus GSD = 5.0 for endotoxin and GSD = 3.3 for dust). The large dust exposure variability in the current study is further supported by the large variation in dust concentrations collected from horse barns (Rosenthal *et al.*, 2006). Similarly, large variations for dust and endotoxin levels were reported in agricultural industries (Spaan *et al.*, 2006). The within-worker variance for dust exposures was higher than the between-worker variance, indicating that day to day differences in dust exposure were more considerable than between-worker. This result is similar to that reported by Mamuya *et al.* (2006). Interestingly, for endotoxin and β(1→3)-glucan, the between-worker variances were higher than the within-worker variances. The within-worker variances could not be explained by exposure determinants and this is in agreement with the results of Wouters *et al.* (2006), most likely due to the fact that exposure determinants do not vary over time. The between-worker variances could be explained by some determinants, although the remaining unexplained variance was still markedly high. However, the high day to day variance for dust is presumably caused by rotation tasks in highly and less dust exposure. Also the high between-worker variances for endotoxin and β(1→3)-glucan can be presumably explained by different jobs, since job and task patterns were not available.

CONCLUSIONS

The present study clearly shows that, despite large exposure variability, workers in horse stables were moderately highly exposed to endotoxin and β(1→3)-glucan. Endotoxin exposures were higher than Dutch proposed standard limits, indicating that workers are at risk of developing adverse health effects. Horse stables should be placed among the working environments characterized as having high dust, endotoxin, and β(1→3)-glucan levels.

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