Journal of Exposure Science and Environmental Epidemiology (2012) 22, 361 – 368
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www.nature.com/jes

ORIGINAL ARTICLE

The influence of bedding materials on bio-aerosol exposure in dairy barns

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Bio-aerosol is a well-known cause of respiratory diseases. Exposure to bio-aerosols has been reported previously in dairy barns, but little is known about the sources of bio-aerosol. Bedding materials might be a significant source or substrate for bio-aerosol exposure. The aim of this study was to explore bio-aerosol exposure levels and its determinants in dairy barns with various bedding materials. Dust samples were collected at dairy barns using various bedding materials. Samples were analyzed for endotoxin and $\beta(1\rightarrow3)$-glucan contents. Culturable bacteria and fungi were sampled by the Anderson N6 impactor. Exposure models were constructed using linear mixed models. The personal exposure levels to dust, endotoxin, and $\beta(1\rightarrow3)$-glucan differed significantly between the barns utilizing diverse main bedding types ($P<0.05$), with the highest levels (GM: dust, 1.38 mg/m^3; endotoxin, 895 EU/m^3; $\beta(1\rightarrow3)$-glucan, 7.84 μg/m^3) in barns with compost bedding *vs* the lowest in barns with sawdust bedding (GM: dust, 0.51 mg/m^3; endotoxin, 183 EU/m^3; $\beta(1\rightarrow3)$-glucan, 1.11 μg/m^3). The exposure levels were also highly variable, depending on various extra bedding materials applied. Plant materials, particularly straw, utilized for bedding appeared to be a significant source for $\beta(1\rightarrow3)$-glucan. Compost was significantly associated with elevated exposure levels. Between-worker variances of exposure were highly explained by determinants of exposure like type of bedding materials and milking by robot, whereas determinants could explain to lesser extent the within-worker variances. Exposure levels to endotoxin, $\beta(1\rightarrow3)$-glucan, bacteria, and fungi in dairy barns were substantial and differed depending on bedding materials, suggesting bedding material types as a significant predictor of bio-aerosol exposure.

Journal of Exposure Science and Environmental Epidemiology (2012) **22,** 361–368; doi:10.1038/jes.2012.25; published online 2 May 2012

Keywords: inhalation exposure; exposure modeling; personal exposure

INTRODUCTION

The air in animal barns can contain a great amount of organic material with microbial, animal, and plant origins.[1,2] It is known that workers involving in such animal settings are exposed to considerable levels of bio-aerosols, especially immune-active components like endotoxin and $\beta(1\rightarrow3)$-glucan.[1–9] In general, bio-aerosol exposure levels are associated with the microbial contamination of the source materials and to what extent these materials can become airborne. In animal barns, applied bedding materials might be a significant source of bio-aerosol exposure.[3,7] Different types of bedding materials are applied, largely determined by the type of animal species which is housed, for example, pigs are mostly housed on bare concrete and slatted floors, whereas dairy cows are housed on either deep litter straw yards or on concrete, often slatted, floors in combination with cubicles that are bedded with deep litter bedding such as sawdust, rubber mattresses, or rubber mats. Compost, produced from municipal green and vegetable waste, is recently introduced as a new bedding material for cows. Straw and sawdust are the most common materials used as main or extra bedding (on top of mattresses or mats), but they are known to be very dusty.[10] Chalk powder (lime) is sometimes applied in combination with the bedding to make the bedding drier. These diverse bedding materials could differ broadly in their physical and chemical properties, which might affect their inherent ability to promote microbial growth or their ability to generate aerosols.

Studies investigating the association between bio-aerosol exposure levels and use of different bedding materials in farm animal buildings are completely absent, despite the application of various bedding materials. Studies in laboratory animal facilities showed that type of bedding materials applied can influence allergen exposure levels.[11,12] This prompted us to hypothesize that bedding materials might be a significant determinant for bio-aerosol exposure in cow barns as well.

The main goal of this study was to provide a detailed exposure assessment of airborne inhalable dust, endotoxin, $\beta(1\rightarrow3)$-glucan, and viable microorganisms with the application of different bedding materials in dairy barns. This study was designed to determine (1) the association between exposure levels with different bedding materials and (2) the effect of different potential determinants on exposure levels and exposure variability.

METHODS

Study Design

Dust samples were collected during the period of July to November 2010. A minimum of five dairy barns per bedding type was included. Dairy barns applying the following bedding materials were selected: deep litter

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Received 18 August 2011; accepted 29 March 2012; published online 2 May 2012

bedding with compost ($n = 6$) and sawdust ($n = 5$); and rubber-filled mattress ($n = 5$), and rubber mats ($n = 7$). The rubber-filled mattresses and rubber mats were topped with a thin (2–5 mm) layer of sawdust. In each barn, active airborne inhalable dust sampling was performed to determine the exposure levels to endotoxin and $\beta(1 \rightarrow 3)$-glucan. Moreover, stationary airborne dust sampling was performed to explore the culturable bacteria and fungal levels. Each dairy barn employed one or two workers, who were mostly the owners. All workers were included in personal dust sampling and minimally three inhalable dust samples per worker were collected on different consecutive days. A structured inventory of farm characteristics was obtained when visiting the barns.

Dairy Barn Description

Dairy barns were confinement buildings with dimensions ranging from 12×4 m to 70×35 m. Most of the barns had two doors, of which one in front of the building was the main entrance and the other at the end of the building was for removing manure during cleaning and taking cows in and out. Both doors were generally open during dust sampling. The buildings in which the animals were housed were naturally ventilated through an open ridge, openings in the sidewalls, and the doors. Tractors were used to distribute silage for feeding. The number of cows accommodated in each barn ranged from 55 to 185, with a surface area of 3–18 m^2 for each cow. The cows stayed in the barn all day during the sampling. Eight barns used an automatic milking system, the other 16 barns milked with a manually operated milking system.

Barns in the current study made use of bedding materials in two main subcategories: A — deep litter applying either compost or sawdust, and B — mats being either rubber-filled mattresses or rubber mats. Cows in the barns were allowed to move freely on slatted concrete floors and could lie down in free-stalls (cubicles). These consisted of a concrete base covered with a deep layer of either compost or sawdust as main bedding materials, or of a rubber mat or a mattress filled with grinded rubber car tires, mostly covered with an extra top layer of bedding materials (2–5 mm) such as chopped straw, sawdust or chopped straw together with grinded lime, this in order to keep cows clean and dry.

Exposure Measurements

Personal and Stationary Dust Collection. Inhalable dust samples (defined as the mass fraction of total airborne particles that can be inhaled through the nose and the mouth) were collected using Gil-Air5 portable sampling pumps (Gillan, Sensidyne, Clearwater, FL, USA) in combination with GSP sampling heads equipped with 37 mm glass fiber filters (Whatman GF/A, SKC, Maidstone, England). A calibrated rotameter was used to adjust the flow rate at 3.5 l/min. To obtain average daily personal exposure, the sampler was clipped to the worker's collar, allowing it to collect dust samples throughout a full work-shift, in most cases from 600 hours when morning activities were started till 1400 hours. At each barn, full work-shift stationary samples were collected in parallel to personal samples by placing the sampler in the center of each barn, 150 cm above ground level. Field blanks were included for each sampling day. Following dust sampling, filters were returned to the laboratory and stored at −20 °C until post-weighing and extraction. The levels of dust on filters were calculated gravimetrically using an analytical balance (AX105, Mettler Toledo Columbus, OH, USA). Filters were acclimatized before weighing for 24 h in a temperature and humidity-controlled room. The lower limit of detection (LOD) of dust was 0.12 mg per filter. Only 3.2% of samples had dust levels below this LOD, which were assigned a value of two-thirds of the detection limit.

Dust extraction, Endotoxin and $\beta(1 \rightarrow 3)$-Glucan Detection. Following post-weighing of filters, samples were extracted for endotoxin and $\beta(1 \rightarrow 3)$-glucan in the same way as described previously.[13] Endotoxin was determined using the kinetic Limulus Amebocyte Lysate (LAL) assay (Lonza, 50-650U; Lysate lot no. KL046N) as described in details elsewhere.[13] Samples were analyzed at a dilution of 1:50 with a 12-point calibration curve (Cambrex Bio Whittaker, standard *E coli*, lot no. 145394) with a concentration range of 0.01–25 EU/ml. $\beta(1 \rightarrow 3)$-glucan was assayed with a specific inhibition enzyme immunoassay (EIA) as described by Douwes et al.[14] but modified by increasing the sample volume and decreasing the antibody amount for improved sensitivity. The $\beta(1 \rightarrow 3)$-glucan was quantified applying four times serial sample dilution (1:2, 1:6, 1:18, 1:54) using a 8-point standard curve with concentration ranging from 9.8 μg/ml to 1250 μg/ml. The levels for endotoxin and $\beta(1 \rightarrow 3)$-glucan were expressed as endotoxin units per cubic meter (EU/m^3) and $\mu g/m^3$, respectively. The average lower LOD for endotoxin was 3.13 EU per filter or 2.77 EU/m^3. None of the samples had endotoxin levels below this LOD. The average LOD of the $\beta(1 \rightarrow 3)$-glucan assay was 0.83 μg per filter or 0.74 $\mu g/m^3$. In all, 52 out of 191 samples (27.2%) remained undetectable. Thus, a concentration of two-thirds of the LOD was assigned to these samples.

Culturable Bacteria and Fungal Aerosol. Airborne dust sampling for culturable bacteria and fungi were collected roughly 150 cm above ground level in the center of each barn using an Anderson N6 single-stage impactor. Tryptone soy agar (TSA) (Oxoid Deutschland, lot no. 927526, PO5012A) was used for bacteria and dichloran-glycerol agar 18 (DG18) (Oxoid Deutschland, lot no. 927573, PO5088A) for fungi. The airflow rate was set at 28.3 l/min, and sampling duration was 30 sec. All samples were collected in duplicate per type of agar in the morning between 800 hours and 1000 hours. Following sample collection, plates were kept in cool box until they were transferred to an incubator on the same day. Bacterial samples were incubated for 18–24 h at 37 °C and fungal samples for 3–7 days at 24 °C. Formed colonies on each plate were counted twice and corrected for counts on field blanks to control for cross-contamination that occurred sporadically during preparation in the field. Additionally, counted colonies were corrected using the positive hole correction factor.[15] The number of bacteria and fungi were expressed as colony-forming units (CFU) per cubic meter of air (m^3).

Statistical Analysis

Linear mixed models (random intercept) with restricted maximum likelihood estimation were used to determine the association between levels of exposure and potential exposure determinants such as main bedding materials, extra bedding materials, and other stable characteristics, for example, milking by robot and available surface area per each cow, and to determine the within- and between-worker exposure variability. The exposure measurements of dust, endotoxin and $\beta(1 \rightarrow 3)$-glucan were nested within workers and modeled as log-transformed. Models were fitted for each type of exposure separately, using SAS (SAS 9.2, SAS institute, Cary, NC, USA). A forward stepwise modeling procedure was applied to select the influential exposure determinants.

RESULTS

Dust, Endotoxin, and $\beta(1 \rightarrow 3)$-Glucan Exposure Levels

Table 1 presents personal exposure levels stratified by different bedding materials. Overall exposure levels ranged from <LOD–6.86 mg/m^3 (GM 0.89) for dust, 21–8292 EU/m^3 (GM 392) for endotoxin and 0.15–232 $\mu g/m^3$ (GM 2.44) for $\beta(1 \rightarrow 3)$-glucan. The exposure levels of dust, endotoxin, and $\beta(1 \rightarrow 3)$-glucan varied significantly between barns applying different main bedding materials ($P < 0.05$). Highest levels of dust (GM 1.38 mg/m^3), endotoxin (GM 895 EU/m^3), and $\beta(1 \rightarrow 3)$-glucan (GM 7.84 $\mu g/m^3$) were found in barns with compost bedding, whereas samples from barns with sawdust bedding had the lowest levels. Dust (GM ratio of 0.87) and endotoxin (GM ratio of 1.12) exposure levels were comparable for barns with bedding of rubber filled mattress and rubber mats, while $\beta(1 \rightarrow 3)$-glucan levels at barns with rubber-filled mattress (GM ratio 1.61) were slightly higher than at barns with rubber mats.

Personal dust exposure levels (GM 2.50 mg/m^3) appeared to be highest in cases where a mixture of chopped straw with chalk was added on the bedding of rubber-filled mattress, consequently resulting in higher levels of endotoxin (GM 1803 EU/m^3) and $\beta(1 \rightarrow 3)$-glucan (48 $\mu g/m^3$). In barns with only compost utilized as

Table 1. Personal inhalable dust and endotoxin levels stratified by the type of bedding materials.

Bedding types	*n*	*Dust (mg/m^3)*					*Endotoxin (EU/m^3)*					*$\beta(1\rightarrow3)$-Glucan ($\mu g/m^3$)*					
		ND	*AM*	*GM*	*GSD*	*Range*	*ND*	*AM*	*GM*	*GSD*	*Range*	*n*	*ND*	*AM*	*GM*	*GSD*	*Range*
Deep litter																	
Compost																	
Compost	9	—	1.84	1.59	1.9	0.46–3.06	—	1228	1006	2.0	277–3188	9	—	10.9	6.98	3.0	1.12–29.8
Compost+chopped straw	1	—	0.97	—	—	—	—	467	—	—	—	1	—	7.62	—	—	—
Compost+chopped straw+chalk	3	—	1.65	1.63	1.2	1.46–1.96	—	2303	1268	3.8	439–5648	3	—	34.7	13.6	5.5	3.38–92.6
Compost+sawdust	2	—	0.79	0.70	2.1	0.41–1.16	—	473	456	1.8	290–655	2	—	7.18	5.82	2.6	2.97–11.4
Total	15	—	1.60	1.38	1.8	0.41–3.06	—	1291	895	2.9	277–5648	15	—	14.9	7.84	3.1	1.130–92.6
Sawdust																	
Sawdust	12	2	0.51	0.40	2.4	<LOD–1.15	—	191	137	2.6	21–429	12	4	1.13	0.62	3.2	0.15–4.86
Sawdust+chopped straw	3	—	1.57	1.45	1.6	1.06–2.51	—	574	574	1.0	556–596	3	—	14.0	11.2	2.4	4.19–23.2
Total	15	2	0.72	0.51	2.6	<LOD–2.51	—	268	183	2.8	21–596	15	4	3.71	1.11	5.0	0.15–23.2
Total	30	2	1.16	0.84	2.5	<LOD–3.06	—	780	404	3.4	21–5648	30	4	9.35	2.95	5.4	0.15–92.6
Mats																	
Rubber-filled mattress																	
Chopped straw	3	—	0.60	0.53	1.8	0.32–1.01	—	229	190	2.2	85–400	3	1	1.26	0.93	3.2	0.31–3.26
Chopped straw+chalk	2	—	3.87	2.50	4.3	0.88–6.86	—	4342	1803	8.6	393–8292	2	—	121	48.4	9.1	10.0–232
Sawdust	4	—	0.87	0.80	1.8	0.44–1.59	—	447	401	1.7	281–846	3	2	1.45	0.76	3.9	0.33–3.64
Sawdust+chalk	2	—	0.79	0.75	1.5	0.55–1.02	—	352	308	2.1	182–522	2	—	8.51	6.01	3.5	2.48–14.5
Total	11	—	1.33	0.86	2.3	0.32–6.86	—	1079	410	3.2	85–8292	10	3	26.8	2.82	7.9	0.31–232
Rubber mats																	
Sawdust	21	—	1.35	0.99	2.2	0.28–5.70	—	636	366	3.2	41–2672	21	3	3.78	1.74	3.6	0.34–15.2
Total	32	—	1.34	0.94	2.2	0.28–6.86	—	788	380	3.1	41–8292	31	6	11.2	2.04	4.8	0.31–232
Overall	62	2	1.26	0.89	2.3	<LOD–6.86	—	784	392	3.2	21–8292	61	10	10.3	2.44	5.1	0.15–232

Abbreviations: AM, arithmetic mean; GM, Geometric mean; GSD, geometric standard deviation; <LOD, below the lower limit of detection; *n*, number of measurements; ND, number of measurements <LOD; range, min–max.

bedding, exposure levels to dust and endotoxin were roughly two times higher when compared with the levels collected from barns with adding of either straw or sawdust on compost bedding, while the levels of $\beta(1\rightarrow3)$-glucan were more or less similar for these bedding materials. In barns where chopped straw is used on the sawdust bedding, the exposure levels of dust and endotoxin were roughly three times as high as in barns where sawdust without chopped straw was used. Stationary samples showed a similar exposure pattern as the personal samples, but at considerably lower levels (Table 2).

Eighty nine percent of all personal dust samples had endotoxin levels higher than the Dutch 8-hr time-weighted average endotoxin exposure limit of 90 EU/m^3,[16] with 43.5% of samples exceeding five times this exposure limit. Probabilities of non-compliance with the exposure limit of 90 EU/m^3 in different beddings were 100% for compost, 80% for sawdust, 91% for rubber filled mattress, and 86% in rubber mats. Also, 62.6% of stationary dust samples had endotoxin levels higher than this Dutch exposure limit.

Bacterial and Fungal Exposure Levels

Significant differences in bacterial and fungal levels were observed between barns with the different bedding materials ($P<0.05$; Table 3). Bacterial levels were highest (GM 5.22×10^4 CFU/m^3) in barns with compost bedding, roughly six times as high as in barns using other bedding types. Bacterial levels were comparable between barns with bedding of rubber filled mattress (GM 7.80×10^3 CFU/m^3) and rubber mats (GM 6.11×10^3 CFU/m^3). In contrast to the results for bacteria, levels of culturable fungi were comparable between barns with compost and rubber-filled mattress ($P>0.05$), but these levels were approximately 4 and 16 times as high as in barns using sawdust and rubber mats, respectively.

Correlations between Exposure Estimates

Personal inhalable dust levels correlated strongly with endotoxin and $\beta(1\rightarrow3)$-glucan levels (dust *vs* endotoxin, $r=0.80$, $P<0.0001$; dust *vs* $\beta(1\rightarrow3)$-glucan $r=0.75$, $P<0.0001$; Figure 1a and b). The same was found for stationary samples (dust *vs* endotoxin, $r=0.75$, $P<0.0001$; dust *vs* $\beta(1\rightarrow3)$-glucan $r=0.62$, $P<0.0001$, Figure 1c and d). Levels of culturable bacteria were significantly correlated with stationary levels of endotoxin and dust (bacteria *vs* endotoxin, $r=0.42$, $P=0.0002$; bacteria *vs* dust, $r=0.44$, $P<0.0001$; Figure 2a and b). Similarly, significant correlations were seen between culturable fungi with $\beta(1\rightarrow3)$-glucan and dust (fungi *vs* $\beta(1\rightarrow3)$-glucan $r=0.34$, $P=0.003$; fungi *vs* dust, $r=0.38$, $P=0.0008$; Figure 2c and d) for stationary samples.

Determinants of Exposure Levels

Several dairy barn characteristics were found to determine dust, endotoxin and $\beta(1\rightarrow3)$-glucan personal exposure levels (Table 4). After adjustment for the effect of milking by robot and surface area per cow, compost bedding was found to be related with higher exposure levels compared with the other types of bedding. Milking by robot showed higher dust and $\beta(1\rightarrow3)$-glucan exposure than milking in a traditional parlor.

Between- and within-workers variability of exposure were bigger for $\beta(1\rightarrow3)$-glucan and endotoxin than dust exposure (Table 5). Between-worker variance decreased considerably when potential determinants of exposure were included as fixed effects: a maximum reduction of 75% (from 0.32 to 0.08) for dust,

Table 2. Stationary inhalable dust and endotoxin levels stratified by the type of bedding materials.

Bedding types	*n*	*Dust (mg/m^3)*					*Endotoxin (EU/m^3)*					*$\beta(1 \rightarrow 3)$-Glucan (μg/m^3)*					
		ND	*AM*	*GM*	*GSD*	*Range*	*ND*	*AM*	*GM*	*GSD*	*Range*	*n*	*ND*	*AM*	*GM*	*GSD*	*Range*
Deep litter																	
Compost																	
Compost	23	—	0.58	0.46	2.0	0.15–1.42	—	488	289	2.8	52–2309	23	1	1.46	0.86	2.9	0.06–6.00
Compost+chopped straw	5	—	0.53	0.45	1.9	0.20–0.97	—	271	176	3.3	36–528	5	—	1.23	0.65	3.1	0.14–2.32
Compost+chopped straw+chalk	8	—	0.78	0.72	1.5	0.49–1.43	—	1334	975	2.3	362–3066	8	1	1.75	0.94	3.8	0.13–4.67
Compost+sawdust	9	—	0.56	0.33	3.1	0.08–1.59	—	159	117	2.3	48–425	8	—	3.40	2.52	2.3	0.99–7.22
Total	45	—	0.61	0.47	2.2	0.08–1.59	—	544	283	3.2	37–3066	44	2	1.88	1.09	3.8	0.06–7.22
Sawdust																	
Sawdust	16	4	0.36	0.21	2.7	<LOD–2.35	—	258	111	2.9	24–2169	16	10	0.90	0.30	3.2	0.15–8.74
Sawdust+chopped straw	9	1	0.30	0.24	2.1	<LOD–0.56	—	192	146	2.4	28–392	9	1	5.47	1.62	9.1	0.04–15.5
Total	25	5	0.34	0.22	2.5	<LOD–2.35	—	234	123	2.7	24–2169	25	11	2.25	0.56	5.8	0.04–15.5
Total	70	5	0.51	0.36	2.4	<LOD–2.35	—	443	210	3.2	24–3066	69	13	2.12	0.85	4.1	0.04–15.5
Mats																	
Rubber-filled mattress																	
Chopped straw	8	1	0.72	0.44	3.0	<LOD–2.72	—	494	233	3.2	86–2374	8	2	1.75	0.63	5.0	0.08–7.35
Chopped straw+chalk	6	—	0.57	0.46	2.1	0.20–1.09	—	279	236	1.9	116–544	6	—	3.36	2.15	2.7	0.82–11.2
Sawdust	14	1	0.20	0.18	1.6	<LOD–0.37	—	448	143	4.8	10–3247	14	12	0.17	0.17	1.2	0.12–0.23
Sawdust+chalk	6	1	0.19	0.16	1.8	<LOD–0.36	—	107	95	1.7	49–204	6	3	0.50	0.40	2.0	0.22–1.15
Total	34	3	0.39	0.26	2.3	<LOD–2.73	—	369	163	3.4	10–3247	34	17	1.16	0.42	3.6	0.08–11.2
Rubber mats																	
Sawdust	27	7	0.28	0.18	2.4	0.05–1.27	—	154	90	2.7	19–972	27	12	0.55	0.77	2.9	0.04–3.04
Total	61	10	0.34	0.22	2.4	<LOD–2.73	—	274	125	3.2	10–3247	61	29	0.89	0.35	3.3	0.04–11.2
Overall	131	15	0.43	0.28	2.5	<LOD–2.7	—	359	165	3.3	10–3247	130	42	1.55	0.56	4.0	0.04–15.5

Abbreviations: AM, arithmetic mean; GM, Geometric mean; GSD, geometric standard deviation; <LOD, below the lower limit of detection; *n*, number of measurement; ND, number of measurements <LOD; range, min–max.

Table 3. Stationary culturable bacteria and fungi levels stratified by the type of bedding materials.

	n	*Bacteria samples (TSA) (CFU/m^3)*				*Fungi samples (DG18) (CFU/m^3)*			
		AM	*GM*	*GSD*	*Range*	*AM*	*GM*	*GSD*	*Range*
Deep litter									
Compost	5	6.71×10^4	5.22×10^4	2.1	2.83×10^4 to 1.58×10^5	7.74×10^3	7.25×10^3	1.5	4.74×10^3 to 1.26×10^4
Sawdust	8	1.24×10^4	9.13×10^3	2.3	2.78×10^3 to 3.19×10^4	5.16×10^3	2.25×10^3	3.9	3.02×10^2 to 2.18×10^4
Total	13	3.34×10^4	1.79×10^4	3.2	2.78×10^3 to 1.58×10^5	6.15×10^3	3.53×10^3	3.4	3.02×10^2 to 2.18×10^4
Mats									
Rubber filled Mattress	5	9.36×10^3	6.11×10^3	3.7	6.43×10^2 to 1.72×10^4	9.26×10^3	8.74×10^3	1.5	4.23×10^3 to 1.15×10^4
Rubber mats	9	9.47×10^3	7.80×10^3	1.9	4.02×10^3 to 2.38×10^4	7.89×10^2	6.45×10^2	2.0	2.42×10^2 to 1.96×10^3
Total	14	9.43×10^3	7.15×10^3	2.4	6.43×10^2 to 2.38×10^4	3.82×10^2	1.64×10^3	2.0	2.42×10^2 to 1.15×10^4
Overall	27	2.10×10^4	1.11×10^4	3.0	6.43×10^2 to 1.58×10^5	4.94×10^3	2.37×10^3	3.9	2.42×10^2 to 2.18×10^4

Abbreviations: AM, arithmetic mean; GM, Geometric mean; GSD, geometric standard deviation; *n*, number of measurement; range, min–max.

58% (from 0.71 to 0.30) for endotoxin, and 70% (from 1.45 to 0.44) for $\beta(1 \rightarrow 3)$-glucan. Milking by robot explained the most exposure variability in dust and endotoxin, followed by main bedding types and extra bedding materials (both explained 19%). Extra bedding materials explained between-worker variance of $\beta(1 \rightarrow 3)$-glucan exposure the most, followed by main bedding types. Within-worker exposure variability could only marginally be explained: maximum reduction of 11% (from 0.37 to 0.33) for dust, 6% (from 0.68 to 0.64) for endotoxin, and 3% (from 1.29 to 1.25) for $\beta(1 \rightarrow 3)$-glucan.

DISCUSSION

This study is the first to assess bio-aerosol exposure levels in dairy barns in which different bedding materials are applied. Results showed that high exposure levels to inhalable dust, endotoxin, $\beta(1 \rightarrow 3)$-glucan, and microorganisms could occur in dairy barns, which are largely dependent on main bedding types applied, although extra bedding materials used also affected bio-aerosol exposure levels.

The overall GM of personal endotoxin concentrations (392 EU/m^3) measured in this study was slightly lower than those levels

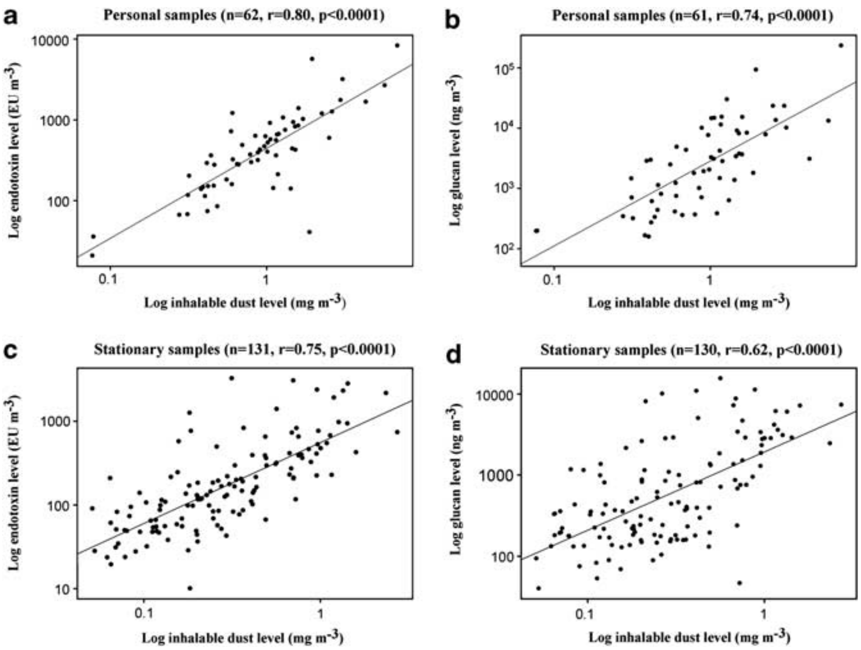

Figure 1. Scatterplot of personal and stationary inhalable dust levels *vs* endotoxin and $\beta(1\rightarrow3)$-glucan levels. Dust *vs* endotoxin: (**a**) personal and (**c**) stationary; dust *vs* $\beta(1\rightarrow3)$-glucan, (**b**) personal and (**d**) stationary.

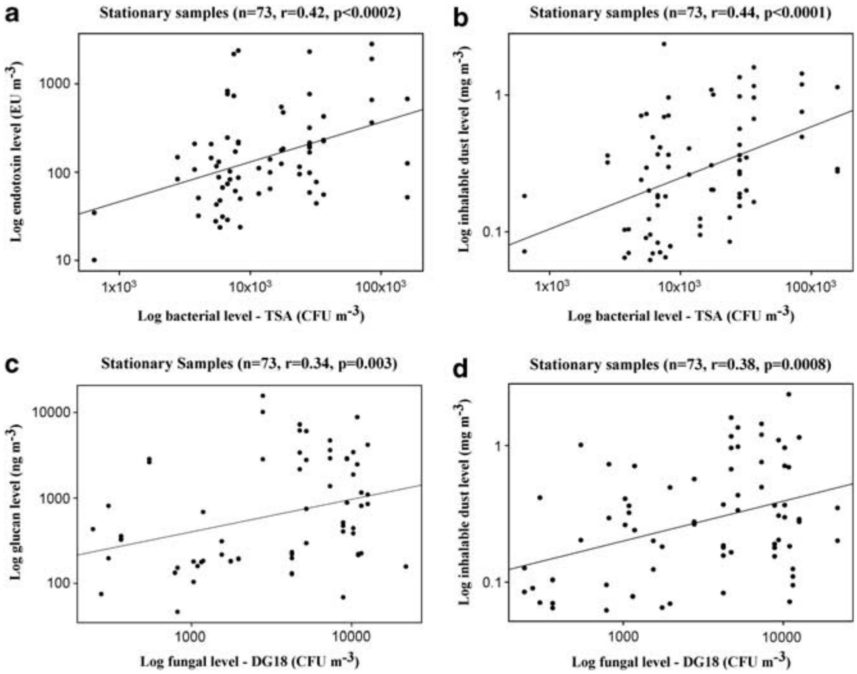

Figure 2. Scatterplot between stationary bacterial or fungal exposure levels with endotoxin, glucan, and dust levels. (**a**) Bacteria *vs* endotoxin, (**b**) bacteria *vs* dust, (**c**) fungi *vs* $\beta(1\rightarrow3)$-glucan and (**d**) fungi *vs* dust.

reported in Dutch dairy farms (560 EU/m^3)[7] and Wisconsin dairy barns (647 EU/m^3).[2] The personal GM Levels of $\beta(1\rightarrow3)$-glucan (2.44 μg/m^3) were lower than the levels (9.50 μg/m^3) we reported earlier in horse stables,[4] as well as in ruminant (8.55 μg/m^3) and poultry (9.68 μg/m^3) clinics.[17] Levels were also substantially lower than those levels reported in grain farming (120 μg/m^3),[18] but higher than the levels obtained from household green waste-composting plants (1.22 μg/m^3),[19] the source of the compost bedding.

We found that exposure levels were strongly associated with various bedding materials applied. Detailed comparisons with other studies are not possible owing to the absence of similar data. The highest personal levels of endotoxin were observed in barns utilizing compost bedding. Moreover, the pattern of endotoxin and dust do not change in a similar way based on the various bedding materials applied: dust particles collected from barns with using only compost carried higher endotoxin (dust 1.59 mg/m^3 and endotoxin 1006 EU/m^3) than dust particles

Table 4. Parameter estimates of the final mixed effects multivariate model of the log-transformed personal exposure to inhalable dust and endotoxin.

Determinant of exposure	*Dust*			*Endotoxin*			*β(1→3)-Glucan*		
	β	*SE*	*P value*	*β*	*SE*	*P value*	*β*	*SE*	*P value*
Intercept	0.57	0.35	0.125	6.925	0.518	0.0001	7.14	0.47	<0.0001
Type of beddings									
Compost	0.72	0.30	0.022	1.335	0.453	0.005	1.61	0.62	0.013
Sawdust	−0.48	0.31	0.127	−0.763	0.456	0.103	−0.13	0.70	0.853
Rubber mattress	−0.28	0.31	0.370	0.190	0.462	0.682	−0.16	0.70	0.818
Rubber mats	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Milking by robot (yes *vs* no)	0.80	0.27	0.004	—	—	—	1.13	0.59	0.064
Surface size per each cow	−0.07	0.03	0.009	−0.102	0.042	0.022	—	—	—

Variables were kept in the models if they were significantly associated with exposure.

Table 5. Variance components and confidence intervals (95% CI) of the log-transformed personal exposure to inhalable dust, endotoxin, and *β*(1→3)-Glucan.

Exposure variable determinants	*BW variance*[a]	*95% CI*	*WW variance*[b]	*95% CI*	*Reduction in BW variance*[c]	*Reduction in WW variance*[d]
Dust						
Random effect model only	0.32	0.17–0.94	0.37	0.25–0.63		
Main bedding types	0.26	0.11–0.91	0.37	0.25–0.63	19	0
Extra bedding materials	0.26	0.12–1.92	0.37	0.24–0.63	19	0
Milking by robot	0.23	0.10–0.88	0.38	0.25–0.64	28	−3
Surface size per each cow	0.37	0.19–1.06	0.36	0.23–0.61	−16	3
Number of cows per each house	0.34	0.17–0.99	0.38	0.25–0.64	−6	−3
Main bedding type+extra bedding materials	0.18	0.07–1.07	0.37	0.25–0.64	44	0
Main bedding type+extra bedding materials+milking by robot	0.08	0.02–3.39	0.37	0.26–0.62	75	0
Main bedding type+extra bedding materials+milking by robot+surface size for each cow	0.09	0.02–2.13	0.33	0.21–0.56	72	11
Endotoxin						
Random effect model only	0.71	0.36–1.96	0.68	0.45–1.15		
Main bedding types	0.48	0.22–1.71	0.67	0.44–1.12	32	1
Extra bedding materials	0.46	0.21–1.66	0.67	0.44–1.09	35	1
Milking by robot	0.66	0.33–1.98	0.68	0.45–1.16	7	0
Surface size per each cow	0.75	0.39–2.01	0.67	0.44–1.14	−6	1
Number of cows per each house	0.71	0.36–2.02	0.67	0.44–1.14	0	1
Main bedding type+extra bedding materials	0.39	0.16–1.87	0.65	0.44–1.10	45	4
Main bedding type+extra bedding materials+milking by robot	0.36	0.15–1.97	0.66	0.45–1.10	49	3
Main bedding type+extra bedding materials+milking by robot+surface size for each cow	0.30	0.11–1.16	0.64	0.43–1.08	58	6
Glucan						
Random effect model only	1.45	0.73–4.06	1.29	0.84–2.22		
Main bedding types	1.05	0.49–3.75	1.28	0.84–2.19	28	1
Extra bedding materials	0.57	0.21–4.09	1.28	0.83–2.19	61	1
Milking by robot	1.26	0.62–3.88	1.29	0.84–2.22	13	0
Surface size per each cow	1.42	0.71–4.14	1.30	0.85–2.26	2	−1
Number of cows per each house	1.51	0.77–4.30	1.28	0.84–2.23	−4	1
Main bedding type+extra bedding materials	0.51	0.17–5.19	1.28	0.85–2.19	65	1
Main bedding type+extra bedding materials+milking by robot	0.44	0.16–5.59	1.25	0.82–2.11	70	3
Main bedding type+extra bedding materials+milking by robot+surface size for each cow	0.48	0.14–5.97	1.26	0.83–2.14	67	2

[a] Between-worker variance.
[b] Within-worker variance.
[c] Reduction in BW variance = $[(S^2_{bw}$ empty model $- S^2_{bw}$ full model$)/S^2_{bw}$ empty model$] \times 100$.
[d] Reduction in WW variance = $[(S^2_{ww}$ empty model $- S^2_{ww}$ full model$)/S^2_{ww}$ empty model$] \times 100$.

collected from barns with only using sawdust (dust 0.40 mg/m^3 and endotoxin 137 EU/m^3) or straw (dust 0.53 mg/m^3 and endotoxin 190 EU/m^3) beddings. These findings suggesting compost being a significant source for endotoxin exposure, likely due to the nature of compost which favors bacterial growth in combination with the fact that specific bacteria are seeded when applying compost as bedding material to circumvent mastitis. Previously Wouters et al.[19] indicated preparation of compost as a potential source for endotoxin exposure. The lowest endotoxin exposure (137 EU/m^3) were seen in barns with only sawdust bedding. This might be explained by antibacterial characteristics of wood due to the hygroscopic properties of wood and the effect of wood extractives.[20] Samples collected from barns with a mixture of chopped straw and sawdust yielded three times higher levels of endotoxin (574 EU/m^3) compared with those barns utilizing only sawdust bedding (137 EU/m^3). This finding is consistent with a previous study reporting higher bacteria counts in straw than in sawdust.[21] Chopped straw together with chalk used on compost bedding resulted in much higher dust levels and subsequently higher endotoxin levels. Similar exposure levels were also observed in barns with rubber-filled mattress that included chopped straw together with chalk. This observation indicates that chalk besides chopped straw is likely attributing as a potential source for dust and endotoxin exposure. Chalk as fine particles is more prone to be released and remain airborne for longer duration which possibly led to high dust exposure. The straw top layer is usually quite wet and thus "sticky". Chalk is keeping the bedding dryer and thus more prone to releasing dust particles from straw top layer. While chalk has a bactericidal properties,[22] it has been reported that the load of bacteria in bedding materials treated with chalk were elevated compared with untreated bedding materials.[22,23] This is suggested to be related to pH changes of bedding materials as adding chalk to bedding initially (in the first two days) will raise the bedding PH, resulting in lower bacterial growth, and then after 2 days PH will steeply reduce from alkaline towards acidity, promoting bacterial growth.[22,23]

Similar to endotoxin, we found significantly higher levels of $\beta(1\rightarrow3)$-glucan in barns with compost bedding compared with other bedding materials. No data are available for comparison, but results from recent studies by Cyprowski et al.[24] and Wouters et al.[19] exploring $\beta(1\rightarrow3)$-glucan exposure in compost plants suggested compost as a potential source for $\beta(1\rightarrow3)$-glucan exposure. Chopped straw together with chalk applied on compost bedding was associated with substantial higher levels of $\beta(1\rightarrow3)$-glucan (2 times higher) when compared with only compost bedding. The same trend but with a much larger increase (18 times) of $\beta(1\rightarrow3)$-glucan was observed in barns utilizing straw together with sawdust compared with barns with only sawdust bedding. This observation is plausible as plant materials utilized for bedding are likely contribute as a source for $\beta(1\rightarrow3)$-glucan, besides airborne fungi.

The observation that personal exposure levels are usually higher than stationary exposure levels is consistent with results of earlier studies in dairy barns,[2,9,25] and is likely explained by proximity to the source of the dust.

Overall culturable bacteria levels (GM 1.11×10^4 CFU/m^3) in the current study were 3 (3.13×10^3 CFU/m^3) and 1.5 (GM 1.91×10^3 CFU/m^3) times higher than those levels reported respectively in horse stables[4] and in swine farms,[26] but were considerably lower than those levels reported in Danish pig farms (5.8×10^6 CFU/m^3).[1] Comparison with these studies should be considered with caution owing to diverse bedding materials utilized, which might affect overall exposure levels. Significantly higher levels of culturable bacteria were observed in dairy barns with compost bedding than other bedding materials, probably owing to the nutritional property of compost for bacterial growth, as suggested by the observed positive correlation between culturable bacteria and endotoxin levels, which is consistent with previous studies.[26,27]

The culturable fungal levels (GM 2.37×10^3 CFU/m^3) in this study were comparable with those levels previously reported from Dutch horse barns (GM 1.91×10^3 CFU/m^3),[4] but higher than those levels reported in Carolinian swine farms (GM 4.56×10^2 CFU/m^3)[26] and lower than those levels in Danish pig farms (GM 3.8×10^5 CFU/m^3).[1] The absence of a significant correlation between $\beta(1\rightarrow3)$-glucan and culturable fungal levels for personal samples was in agreement with earlier findings reported by Halstensen et al.[18] In contrast to personal samples, we found a significant correlation for stationary samples which was in accordance with those reported by Adhikari et al.[27] in greenhouses. $\beta(1\rightarrow3)$-Glucan is a component of fungal cell walls and it has often been considered as an indicator for fungal exposure,[28] but plant materials utilized for bedding are possibly additional sources for $\beta(1\rightarrow3)$-glucan exposure besides fungi.[29] This result was supported by higher $\beta(1\rightarrow3)$-glucan levels in barns utilizing straw as bedding material.

The Dutch health council recommended an occupational exposure limit of 90 EU/m^3 to protect workers for development of respiratory outcomes.[16] Eighty nine percent of personal endotoxin levels clearly exceeded this limit; and the overall GM level (392 EU/m^3) and the highest level (8292 EU/m^3) were about 4 and 92 times higher than this limit. Moreover, the lower GM endotoxin level (183 EU/m^3) measured in barns with sawdust bedding was still twice as high. Selection of appropriate bedding materials could be of importance when reducing endotoxin exposure levels, but the application of other exposure control measures like better management practices (e.g., more cleaning and better ventilation) are required as well.

This study has a limitation that needs to be considered. In addition to bedding materials, exposure levels in this study to some extent might likely be influenced by other potential determinants that we did not include, such as climatic conditions on the measuring days, type of ventilation and ventilation rate, methods and intervals on cleaning, and sampling season as suggested elsewhere.[3,6,30]

The variability of exposure levels between workers and within workers over time were high in our study, consistent with findings from previous studies.[1,4,7,19] Final exposure models in this study explained a significant proportion of between-worker variability for inhalable dust, endotoxin, and $\beta(1\rightarrow3)$-glucan levels. Milking by robot appeared to be the predominant determinant explaining the between-workers exposure variability to dust. Since workers working in a barn with automatic milking robot will not perform milking activities, they will spend more time on other tasks in the barn, with presumably higher dust exposure. In contrast to dust, main bedding types as well as extra bedding materials were the predominant determinants explaining between-worker variability of endotoxin exposure, probably owing to different load of endotoxin (EU per mg of dust) based on bedding types. Nonetheless, overall endotoxin variance was less well explained than dust variance (58% *vs* 72%), which most likely can be attributed to other unmeasured determinants that favor bacterial growth such as humidity and temperature.[26] The application of extra bedding material explained between-worker variability for $\beta(1\rightarrow3)$-glucan very well (61%) much more than for dust (19%) or endotoxin (35%). This may be explained in part by the fact that bedding materials with plant origin might serve as a main source for $\beta(1\rightarrow3)$-glucan. This was supported by the low levels of $\beta(1\rightarrow3)$-glucan in sawdust bedding compared with straw and compost beddings.

Possible determinants of exposure in this study could only marginally explain within-worker exposure variability because these determinants did not change over time, resulting in similar exposure levels. The main explanation for within-worker exposure variability could be task rotations over time as previous studies demonstrated some associations between specific tasks (e.g., feeding and sweeping) performed and elevated exposure levels to dust and endotoxin.[3,4]

CONCLUSIONS

This study is the first to investigate the effect of different bedding materials in dairy barns on bio-aerosol exposure levels. Type of bedding materials appeared to be a predominant determinant of exposure to dust, endotoxin, bacteria, and fungi. Workers in barns with compost bedding had the highest exposure *vs* the lowest in sawdust bedding. Additionally, exposure levels based on extra bedding materials showed large variability. The between-worker variability of exposure levels was substantially explained by determinants of exposure, while these determinants to less extent explained the within-worker variability. The endotoxin levels of most personal samples exceeded the Dutch proposed standard limit, suggesting that workers in dairy barns are at risk for developing adverse respiratory outcomes.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank the management and workers of the dairy farms for the collection of dust samples. We also acknowledge Jack Spithoven and Siegfried de Wind for endotoxin and $\beta(1\rightarrow3)$-glucan analysis.

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