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PAPER

Endotoxin and β-(1 → 3)-glucan exposure in poultry and ruminant clinics

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Background: Exposure to organic dust is a well-known hazard for farm animal workers leading to respiratory diseases. Organic dust exposure has not been adequately evaluated in environmental settings in relation to veterinarians. *Objective*: To investigate inhalable dust, endotoxin, and β-(1 → 3)-glucan exposure among caretakers, veterinarians, and veterinary students. Task-based determinants of exposure were studied. *Methods*: This study investigated the exposure during veterinary education in the ruminant and poultry clinics. Dust measurements were performed using the conical inhalable samplers (CIS). Endotoxin and β-(1 → 3)-glucan were determined by the kinetic *limulus* amebocyte lysate (LAL) assay and inhibition enzyme immunoassay (EIA), respectively. Determinants of exposure were identified by multiple linear regression analysis. *Results*: Personal exposure levels of dust, endotoxin, and β-(1 → 3)-glucan were higher for poultry [geometric mean (GM): dust, 1.32 mg m^{-3} (below the lower limit of detection (<LOD) to 20.9); endotoxin, 1498 EU m^{-3} (115–49 846); and β-(1 → 3)-glucan, 3.10 μg m^{-3} (<LOD–46.1)] than for ruminant settings [GM: dust, 0.60 mg m^{-3} (<LOD–20.8), endotoxin, 520 EU m^{-3} (60–7492), and β-(1 → 3)-glucan, 3.39 μg m^{-3} (<LOD–111)]. Dust and endotoxin levels correlated significantly when stratified by work-sites and job-titles, except for caretakers in the ruminant clinic. Modeling of task-based determinants revealed some activities to be associated with higher exposure, but tasks were dependent on the job-title. *Conclusion*: This study showed substantial endotoxin exposure in modern animal clinics. Exposure occurred not only in animal houses, but also in practical teaching rooms. β-(1 → 3)-Glucan was substantial as well. Observed exposure levels might present an occupational respiratory health risk for veterinary populations.

Introduction

Working in farm animal facilities involves exposure to airborne dust produced by various activities[1–6] such as feeding, application of bedding materials, sweeping, cleaning, and manure handling. Dust released due to these activities is largely organic (so-called "*bio-aerosol*") and can result in high levels of endotoxin and β-(1 → 3)-glucan components. These components are recognized to be powerful inflammatory agents and are known to play a role in development of respiratory diseases.[7,8]

Most studies involving measurements of organic dust and its components considered exposure in pig farms. Exposure to dust

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Environmental impact

Bio-aerosols are well-known powerful inflammatory agents and are recognized to play a role in development of respiratory diseases among farm animal workers. Veterinary populations can be potentially exposed to bio-aerosol agents. Nonetheless, exposure to these agents has not been adequately investigated through veterinary practice. This study provides evidence of substantial endotoxin exposure for caretakers, veterinarians, and veterinary students. β-(1 → 3)-Glucan was occasionally high as well. Exposure levels were strongly influenced by animal species, job titles, and sampling sites. Observed exposure levels likely present an occupational respiratory health risk for veterinary populations.

and endotoxin in pig farms is well-known to be associated with respiratory diseases.[9–14] For other animal farm types, less data are available. Dust levels in poultry houses were reported to be high,[2,6,15,16] accompanied by high levels of endotoxin.[6,16] Lower but still considerable levels of dust[1,17] and endotoxin[1] have been reported in dairy barns. Only few epidemiological studies have been conducted in the context of poultry houses and dairy barns,[4,18–23] which showed that exposure to bio-aerosol particularly endotoxin in these settings is responsible for an elevated risk of respiratory symptoms and decline in lung function. Veterinary populations are a group of workers who regularly visit poultry houses and dairy barns as well. Nevertheless, there is a lack of data on endotoxin and β-(1 → 3)-glucan exposure among veterinary populations involving in poultry and ruminant settings, although two recent studies demonstrated substantial exposure to bio-aerosol components such as endotoxin, β-(1 → 3)-glucan and animal allergens among veterinary populations exposed to horse and companion animals,[5,24] confirming the presence of bio-aerosol components during veterinary practice. A recent study also indicated that respiratory symptoms associated with animal husbandry specialization are common among the veterinary medicine students.[25] These findings provoked us to hypothesize that what exposure levels would be encountered by caretakers, veterinarians, and veterinary students during their practical activities in the veterinary medicine training.

The purpose of the present study was to explore inhalable dust, endotoxin, and β-(1 → 3)-glucan exposure among caretakers, veterinarians (teachers), and veterinary students in two different animal clinics as well as 5 farm animal houses visited for practical teaching activities. Task-based determinants of exposure were evaluated.

Methods

Study design and population

This study was carried out in the clinic for (small) ruminants and the poultry clinic at the Department of Farm Animal Health at the Faculty of Veterinary Medicine of Utrecht University, The Netherlands. Both clinics are subservient to education and research. In addition, one ruminant farm stable and four poultry farm houses outside the clinics were included where practical teaching activities also took place. Active personal and stationary inhalable dust sampling and passive settling dust collection were used to capture dust samples.

Samples have been collected with caretakers, veterinarians, and veterinary students. In principal, 5 repeated dust samples were collected per individual to investigate variability over time. In addition, 5 randomly selected individuals per job titles, shifts and worksites were included. Caretakers at the clinics of Farm Animal Health are involved in a variety of activities: feeding, sweeping, cleaning with high pressure water, bedding, milking, and taking samples from animals. Caretakers at the ruminant clinic work in three shift periods: daytime (8:00–16:00), morning (6:00–14:00), and afternoon (14:00–22:00). During all shifts samples have been collected (daytime, $n = 5$ samples for one caretaker; morning shift, $n = 17$ samples for 4 caretakers; afternoon shift, $n = 10$ samples for 2 caretakers). Also 5 samples were collected for one caretaker at the poultry clinic.

Veterinary students can be subdivided according to the study phase in *pre-clinical students* (1–4th years), students involved in *uniform internships* (5th year), and students involved in *differential internships* (6th year). Five *pre-clinical students*, as well as 5 and 3 students in the *uniform* and *differential internships*, respectively, took part in the study within the small ruminant clinic. In the poultry clinic and poultry farm houses, all students and teachers involved in a practical teaching course part of the pre-clinical phase of the veterinary medicine study participated. The nature of the study was described and collaboration of participants was elicited. Daily or course related job tasks performed and additional related information were recorded by the participants on a prepared form.

Buildings description

The ruminant clinic is a confined and modern building which was completely renewed in 2009. This clinic contains stables with different housing systems, examination rooms, and a canteen. The building is equipped with an automatic heating system and mechanical ventilation in the stables. The floor system included: (1) tie stalls, covered with rubber mattresses and sawdust bedding; (2) pens with slatted floors, covered with rubber mattresses and straw bedding. Cows were manually fed with silage, hay and compounds.

The poultry clinic, consisting of poultry houses and teaching rooms, was a confined and modern building which was fully reconstructed in 2006 and contains an automatic heating system. Each poultry house was divided into 4 pens supplied with automatic watering system, mechanical ventilation, and sawdust on the floor.

Poultry farm houses outside the university differed in size between 1600 and 12 000 m^2. The floor surfaces of two poultry farm houses were covered with bedding of wood shavings, one with sand bedding, and the other with slatted floor bedding. Buildings were equipped with automatic feeding, watering, and heating systems.

Exposure measurements

Personal inhalable dust sampling was performed using Gil-Air5 portable constant-flow pumps (Gillan, Sensidyne, Clearwater, FL, USA) and plastic conical inhalable samplers (CIS) at a flow rate of 3.5 l min^{-1}.[26] The CIS sampler is a commercial equivalent of the 'gesamt staub probenahme' (GSP) sampling head (Germany). The CIS/GSP samplers were equipped with 37 mm glass fiber filters (Whatman International Ltd Maidstone England, GFA) mounted in a reloadable cassette. The CIS samplers were clipped to the subject's lapel close to the breathing zone with the inlet facing forward. All measurements for students were performed throughout practical teaching work. Measurements with caretakers were collected during normal shift-work. Stationary inhalable dust sampling was conducted utilizing the same equipment as for personal sampling but then mounted at a tripod at 1.5 m above the floor level. The average sampling times for personal and stationary dust measurements were, respectively, 2 hours and 50 minutes and 6 hours and 40 minutes.

Samplers were prepared under pyrogen-free conditions: the CIS sampling head was cleaned and washed with water and soap, and then immersed in ethanol. Samplers were loaded with glass fiber filters (Whatmann, GF/A) out of the package and then packed in aluminium foil until usage. Numerous field blank samples were taken without drawing air through the filters to control any primary or secondary contamination of filters. Blank filters resulted in non-detectable endotoxin levels.

Dust samples were quantified by gravimetric analysis applying an analytical balance (AX 105, Mettler Toledo Inc., Columbus, OH, USA) in a preconditioned room. The lower limit of detection (LOD) for dust weight was estimated using the average weight change of blank filters plus three times the standard deviation of blank filters weight changes.

A plastic manufactured electrostatic dust-fall collector (EDC) was used along with electrostatic cloths (Zeeman, The Netherlands) to capture settling dust as previously described.[27] Each plastic manufactured EDC contains two cloths (sampling area per cloth 0.0209 m^2). EDCs were placed on the top of prepared supporting holders hanging from ceiling (roughly 170 cm above the floor level) in the middle of locations. Sampling was performed for 14 consecutive days. Afterwards cloths were taken from the sampler and stored in 50 ml tubes (Greiner) at −20 °C until extraction. Because of logistic constraints, EDC samples were only collected in the ruminant and poultry clinics.

Extraction and detection of endotoxin and β-(1 → 3)-glucan

Sequential extraction of endotoxin and β-(1 → 3)-glucan was carried out as described elsewhere.[28] Briefly, filters and EDCs were transferred to 50 ml Greinter tubes, and 5 ml pyrogen-free water (Aqua B. Braun Melsungen AG) containing 0.05% (v/v) Tween-20 (Merck Schuchardt OHG 85662 Hohenbrunn, Germany) was added to each personal and stationary filter sample and 20 ml to each EDC sample. After mixing in an end-over-end roller for 1 h and centrifuging for 15 min at 1000*g*, 10% of the extraction solution was removed and five aliquots of 100 µl were stored at −20 °C until endotoxin analysis. The removed volume was replaced with the same volume of 10× concentrated phosphate-buffered saline for allergen extraction which is not of interest for this paper, followed by heat incubation for glucan extraction.

Endotoxin was determined using the kinetic *limulus* amebocyte lysate (LAL) assay (Lonza, 50-650U; Lysate lot no. GL155U and FL147M) as described previously.[29] A calibration curve (Cambrex Bio Whittaker, Inc, standard *E coli*, lot no. GL1157 and GL0006) was included on each plate ranging from 0.01–25 EU ml^{-1}. Personal and stationary dust samples were diluted in 1 : 50 to 1 : 200, while EDC samples were diluted 1 : 500 to 1 : 1000 on the basis of preliminary analyses such that outcomes fall within the range of the standard curve. The endotoxin levels of personal and stationary samples were expressed as Endotoxin Units (EU) per cubic metre (m^{-3}) of air, and for EDC samples as Endotoxin Units per squared metre of surface (EU m^{-2}).

β-(1 → 3)-Glucan was assayed with a specific inhibition enzyme immunoassay (EIA) which has been described by Douwes *et al.* (1996).[30] β-(1 → 3)-Glucan levels related to personal and stationary samples were presented as µg m^{-3} and for EDC samples as µg m^{-2}.

More than 10% of all samples were analyzed in duplicate to calculate the coefficient of variation (CV %) as a measure of reproducibility, being 21.6% for endotoxin and 31.5% for β-(1 → 3)-glucan analysis. The estimated LOD of dust depending on blank filters was 0.13 mg per filter corresponding to 0.16 mg m^{-3}. The LOD of endotoxin for personal and stationary samples was 4.30 EU per filter corresponding to 5.64 EU m^{-3}. The LOD of endotoxin for EDC samples was 226 EU per cloth corresponding to 9855 EU m^{-2}. The LOD of β-(1 → 3)-glucan for personal and stationary samples was 0.56 µg per filter corresponding to 0.65 µg m^{-3}. The LOD of β-(1 → 3)-glucan for EDC samples was 1.03 µg per cloth corresponding to 44.70 µg m^{-2}. Samples below LOD were assigned a value of two-thirds of the respective LOD.[31]

Statistical analysis. Analysis was performed using the Statistical Analysis System (SAS, version 9.2, Institute Inc., Cary, NC, USA). Exposure levels were resembled by log-normally distribution, subsequently; further analysis was carried out based on log-transformed data. Exposure levels [geometric mean (GM), geometric standard deviation (GSD) and range] were calculated stratified per type of animal exposure for different job titles and work sites. Correlations between exposures were estimated using *Pearson* correlation. Multiple linear regression analysis (PROC REG) was performed to explore the impact of potential task-based determinants on exposure levels. The exposure concentration of dust, endotoxin, and β-(1 → 3)-glucan was considered as dependent variables, and the time spent on the tasks performed (continuous variables) was included as independent variables. Potential determinants to be included in the models were selected with backward stepwise selection methods. In the initial stage, models were constructed by introducing the entire set of determinants and in the next stage determinants with a P value of <0.2 were presented in the models. The exponent of the β-coefficient ($P < 0.05$) multiplied with the median time spent on each task was used to estimate the proportion of increase in exposure levels associated with the determinant.

Results

Two hundred and ten personal inhalable dust samples were collected after excluding 10 samples (3.7%) because of pump failures or damaged filters. Fifty two stationary inhalable dust samples and 15 EDC settling dust samples were collected as well.

Personal exposure levels are presented in Table 1. Exposure levels of dust, endotoxin, and β-(1 → 3)-glucan differed largely between the two clinics. In general, personal exposure levels in the ruminant clinic including the ruminant farm visits (overall GM: dust, 0.60 mg m^{-3}; endotoxin, 520 EU m^{-3}; and β-(1 → 3)-glucan, 3.39 µg m^{-3}) were lower than in the poultry clinic including the poultry farm visits (overall GM: dust, 1.32 mg m^{-3}; endotoxin, 1498 EU m^{-3}; and β-(1 → 3)-glucan, 3.10 µg m^{-3}). Lowest levels of exposure were seen for students at the clinic of ruminants (overall GM: dust, 0.37 mg m^{-3}; endotoxin, 368 EU m^{-3}; and β-(1 → 3)-glucan, 2.13 µg m^{-3}), while highest exposure levels were observed for students during the poultry farm visits (overall GM: dust, 4.89 mg m^{-3}; endotoxin, 4376 EU m^{-3}; and β-(1 → 3)-glucan, 11.25 µg m^{-3}). Endotoxin levels for students differed by a factor of 4 between poultry clinic together with farm visits (1485 EU m^{-3}) and clinic of ruminants together with farm

Table 1 Exposure levels of inhalable dust, endotoxin, and β-(1 → 3)-glucan according to work sites and jobs based on personal sampling[a]

	Dust/mg m^{-3}							Endotoxin/EU m^{-3}						β-(1 → 3)-Glucan/μg m^{-3}					
	K	*N*	ND	AM	GM	GSD	Range	*N*	ND	AM	GM	GSD	Range	*N*	ND	AM	GM	GSD	Range
Ruminant clinic and farm visits																			
Students																			
Preclinical (clinic)	5	25	7	0.52	0.43	1.8	<LOD–1.48	25	—	391	324	1.9	88–1279	25	10	1.07	0.83	2.0	<LOD–3.50
Uniform																			
Clinic	5	20	11	0.31	0.28	1.6	<LOD–0.69	20	—	316	229	2.2	68–1459	20	2	3.20	2.40	2.2	<LOD–9.40
Farm visits	5	5	—	0.66	0.60	1.7	0.23–0.90	5	—	1784	1592	1.7	689–3047	5	—	19.96	18.54	1.5	11.11–32.80
Total	10	25	11	0.38	0.33	1.9	<LOD–0.90	25	—	610	338	2.9	68–3047	25	2	6.55	3.63	3.0	<LOD–32.80
Differentiated (clinic)	3	14	2	0.48	0.38	2.0	<LOD–1.04	14	—	847	538	2.9	67–2383	14	—	7.72	4.38	2.8	0.66–40.99
Total	18	64	20	0.46	0.37	1.9	<LOD–1.48	64	—	576	368	2.5	67–3047	64	12	4.67	2.13	3.3	<LOD–40.99
Caretakers (clinic)	7	32	—	3.67	1.56	3.6	0.14–20.75	32	—	1827	1042	3.1	60–7492	32	1	22.11	8.55	4.8	<LOD–111.54
Overall	20	96	20	1.53	0.60	3.0	<LOD–20.75	96	—	993	520	3.1	60–7492	96	13	10.50	3.39	4.4	<LOD–111.54
Poultry clinic and farm visits																			
Students																			
Preclinical (clinic)	12	81	6	1.70	0.95	2.6	<LOD–19.00	79	—	2412	1177	2.8	115–49 846	79	20	3.89	2.29	2.7	<LOD–46.14
Uniform (farm visits)	5	17	—	6.16	4.89	2.0	1.52–20.90	17	—	5886	4376	2.1	1524–25 139	17	—	15	11.25	2.4	1.89–32.2
Total	17	98	6	2.47	1.27	3.0	<LOD–20.90	96	—	3032	1485	3.0	115–49 846	96	20	5.93	3.03	3.2	<LOD–46.14
Teachers																			
Clinic	2	10	3	1.01	0.79	2.1	<LOD–2.68	10	—	1402	938	2.7	237–4489	10	1	2.07	1.71	2.1	<LOD–3.93
Farm visits	1	1	—	12.4	—	—	—	1	—	16 927	—	—	—	1	—	21.7	—	—	
Total	3	11	3	2.05	1.01	3.0	<LOD–12.39	11	—	2813	1221	3.6	237–16 927	11	1	3.85	2.18	2.8	<LOD–21.66
Caretakers (clinic)	1	5	—	8.37	5.72	3.0	1.62–14.66	5	—	4934	2749	3.7	454–10 820	5	—	13.9	9.68	2.9	2.45–26.57
Overall	21	114	9	2.69	1.32	3.1	<LOD–20.90	112	—	3082	1498	3.1	115–49 846	112	21	6.08	3.10	3.2	<LOD–46.14

[a] *K*, Number of workers sampled in each group; *N*, number of samples; ND, number of samples < LOD; AM, arithmetic mean; GM, geometric mean; GSD, geometric standard deviation; and <LOD, below the lower limit of detection.

visits (368 EU m^{-3}). The same trend was found for caretakers (a factor of 2.5).

For the ruminant clinic, highest levels were observed for caretakers. Dust levels in the morning (GM 1.50 mg m^{-3}) and afternoon (GM 1.35 mg m^{-3}) shifts were similar, but slightly although not significantly increased during the daytime shift (GM 2.40 mg m^{-3}) ($p > 0.05$). Endotoxin levels were borderline significantly different between shifts ($p = 0.07$), with a tendency towards higher levels in the morning (GM ratio 2.01, 95% confidence interval (CI) 0.82–4.92, $p > 0.05$) and daytime shifts (GM ratio 4.3, 95% CI 1.24–14.6, $p = 0.02$) *versus* the afternoon shift. There are no differences in dust exposure between students in the ruminant clinic for different job titles ($p > 0.05$), while the GM levels of endotoxin and β-(1 → 3)-glucan for students during farm visits were increased 7-fold compared to working within the clinic.

A significant difference was found between caretakers, teachers, and students in the poultry clinic in dust and β-(1 → 3)-glucan exposure ($p < 0.05$), but not for endotoxin exposure ($p > 0.05$). Furthermore, *post-hoc* comparisons showed similar dust and β-(1 → 3)-glucan exposure for students and teachers ($p > 0.05$).

The distribution of stationary exposure levels is presented in Table 2. The levels of dust, endotoxin, and β-(1 → 3)-glucan were much higher in poultry houses compared to the practical teaching room (GM ratio: dust 11.47, 95% CI 6.37–20.66; endotoxin 6.05, 95% CI 1.86–19.59; β-(1 → 3)-glucan 16.10, 95% CI 3.95–68.51; $p < 0.05$). A similar trend but with lower GM ratios was observed at the ruminant clinic (GM ratio: dust 1.66, 95% CI 1.22–2.25, $p < 0.05$; endotoxin 2.48, 95% CI 1.49–4.13, $p < 0.05$; β-(1 → 3)-glucan 1.81, 95% CI 0.83–3.90, $p > 0.05$).

Positive significant correlations were observed between dust and endotoxin levels for all personal samples collected across diverse work sites and job-titles (overall: poultry clinic, $R = 0.87$ and $P < 0.0001$; ruminant clinic, $R = 0.57$ and $P < 0.0001$), except for caretakers at the ruminant clinic ($R = 0.30$ and $P = 0.09$). Similarly, significant correlations were obtained between dust and endotoxin levels for all stationary samples (overall: poultry clinic, $R = 0.68$ and $P = 0.004$; ruminant clinic, $R = 0.53$ and $P = 0.0009$).

The endotoxin levels of 97.1% of personal samples and 82.7% of stationary samples exceeded the exposure limit of 90 EU m^{-3} proposed by the Health Council of The Netherlands.[32] In addition, the dust levels of few samples exceeded the acceptable limit of 4 mg m^{-3} suggested for organic dust exposure in the animal feed industries.[33] Up to now, no limit has yet been established for β-(1 → 3)-glucan.

EDC settled dust samples

Endotoxin and β-(1 → 3)-glucan levels on the EDC samples collected in animal houses within ruminant or poultry clinics were measurable and significantly higher than in the examination rooms utilized for teaching (Table 3). Interestingly, endotoxin in the canteen within the ruminant clinic was measurable but markedly lower than in the examination rooms. β-(1 → 3)-Glucan in the canteen was measurable as well. The levels of endotoxin measured by the EDC samples at the clinic of

Table 2 Exposure levels of inhalable dust, endotoxin, and β-(1 → 3)-glucan according to work sites based on stationary sampling[a]

	Dust/mg m^{-3}						Endotoxin/EU m^{-3}						β-(1 → 3)-glucan/μg m^{-3}					
	N	ND	AM	GM	GSD	Range	*N*	ND	AM	GM	GSD	Range	*N*	ND	AM	GM	GSD	Range
Ruminant clinic																		
Stables	25	1	0.21	0.19	1.6	<LOD–0.49	25	—	306	228	2.1	59–1475	25	—	2.92	1.71	3.0	0.20–11.94
Examination rooms	11	6	0.12	0.11	1.4	<LOD–0.22	11	—	108	92	1.8	27–196	11	—	1.51	0.95	3.0	0.17–4.55
Total	36	7	0.18	0.15	1.6	<LOD–0.49	36	—	245	173	2.3	27–1475	36	—	2.49	1.43	3.1	0.17–11.94
Poultry clinic																		
Poultry houses	12	—	2.71	2.33	1.8	0.68–5.37	12	—	2530	1470	3.2	188–10 655	11	—	5.78	3.06	3.6	0.24–19.86
Teaching room	4	—	0.20	0.20	1.1	0.18–0.22	4	—	264	243	1.6	140–435	4	2	0.27	0.19	2.9	<LOD–0.54
Total	16	—	2.01	1.25	3.2	0.18–5.37	16	—	1964	938	3.6	140–10 655	15	2	4.30	1.46	5.7	<LOD–19.86

[a] *N*, Number of samples; ND, number of samples < LOD; AM, arithmetic mean; GM, geometric mean; GSD, geometric standard deviation; and <LOD, below the lower limit of detection.

ruminants correlated very well with the endotoxin levels of personal or stationary samples (EDC *versus* personal samples, $R = 0.95$ and $P = 0.04$; EDC *versus* stationary samples, $R = 0.86$ and $P = 0.02$).

Task based exposure determinants

Table 4 shows the impact of potential task-based determinants on exposure levels. Effects of tasks have been investigated stratified by job titles, only for those job-title groups that at least included 3 individuals and a minimum of 4 repeated measurements per individual. For caretakers in the ruminant clinic, feeding was associated with a significant increase of dust exposure (8.0 times, 95% CI 1.85–34.56) explaining 17% of the variation of exposure, also applying bedding material was related to a significant increase in endotoxin exposure (1.83 times, 95% CI 1.14–2.93) explaining 16% of the exposure variability. For preclinical students in the ruminant clinic, physical examination of animals and listening to lectures were predominant tasks with an increase of dust exposure explaining 36% of exposure variation, while physical examination of animals was the only task with a significant increase of endotoxin exposure explaining 13% of exposure variation. Modeling for students within the poultry clinic demonstrated that most tasks were significantly associated with an increase of dust, endotoxin, and β-(1 → 3)-glucan levels. Exposure levels increased most with the tasks restraining chickens, checking neck and beak, collecting feces and physical examination.

Discussion

This study provides comprehensive information on exposure to inhalable dust, endotoxin, and β-(1 → 3)-glucan among individuals involved in veterinary medicine in different animal clinics and during farm visits. Findings in our study demonstrated that elevated levels of endotoxin exposure would likely arise in modern animal facilities; not only in poultry houses and ruminant barns, but also in examination and teaching rooms during practical teaching work. In addition, β-(1 → 3)-glucan in most samples was detectable (83%) with occasionally high levels. This is the first study to report on endotoxin and β-(1 → 3)-glucan exposure levels of students during their education in veterinary medicine, comparisons with other studies are thus not possible. Exposure levels of veterinary students are roughly half that of care-takers in the same clinic.

Dust levels for caretakers at the ruminant clinic (GM 1.56 mg m^{-3}; range 0.14–20.8) were comparable with those reported by others for Wisconsin dairy barns (GM 1.78 mg m^{-3}, range 0.007–53.6),[3] Dutch dairy farms and cattle breeding (GM 1.5 mg m^{-3}, range 0.7–2.7) and Dutch dairy farms (GM 1.3 mg m^{-3}, range 0.4–2.3),[34] but the exposure range in our study was markedly greater. Dust levels in pig barns have been reported frequently to be much higher, with a GM or median from 1.11 to 5.78 mg m^{-3}.[34–36] Dust levels for caretakers at the poultry clinic (GM 5.72 mg m^{-3}, range 1.62–14.66) were similar to those previously found in the Dutch broiler poultry farm (GM 4.2 mg m^{-3}, range 4–4.4),[34] but somewhat lower than those reported in the UK

Table 3 Exposure levels of endotoxin and β-(1 → 3)-glucan according to work sites based on settling dust (EDC) sampling[a]

	Endotoxin/EU m^{-2}						β-(1 → 3)-glucan/μg m^{-2}				
	N	ND	AM	GM	GSD	Range	ND	AM	GM	GSD	Range
Ruminant clinic											
Stables	6	—	2.70×10^6	2.15×10^6	2.1	7.42×10^5 to 6.58×10^6	—	1151	837	3.6	145–4490
Examination rooms	3	—	6.36×10^4	5.61×10^4	1.8	3.32×10^4 to 1.08×10^5	—	24	23	1.3	18.56–31
Canteen	2	—	1.70×10^4	1.69×10^4	1.1	1.54×10^4 to 1.86×10^4	—	10	8.3	2.4	4.59–15
Total	11	—	1.49×10^6	3.30×10^5	9.7	1.54×10^4 to 6.58×10^6	—	833	137	10	4.59–4490
Poultry clinic											
Poultry houses	3	—	6.01×10^6	3.99×10^6	3.4	1.08×10^6 to 1.20×10^7	—	2892	2565	1.8	1543–4889
Teaching room	1	—	7.23×10^5	—	—	—	—	133	—	—	—
Total	4	—	4.68×10^6	2.59×10^6	3.7	7.23×10^5 to 1.20×10^7	—	2202	1224	4.7	133–4889

[a] *N*, Number of samples; ND, number of samples < LOD; AM, arithmetic mean; GM, geometric mean; and GSD, geometric standard deviation.

Table 4 Multiple linear regression analysis of tasks performed related to personal dust, endotoxin, and β-(1 → 3)-glucan exposure[a]

			Dust/mg m^{-3}					Endotoxin/EU m^{-3}					β-(1 → 3)-Glucan				
	Determinants	Median (time-min)	*β*	SE	*P*-Value	Adj R-Sq	FEC (95% CI)	*B*	SE	*P*-Value	Adj R-Sq	FEC (95% CI)	*β*	SE	*P* value	Adj R-Sq	FEC (95% CI)
Ruminant clinic and farm visits																	
Preclinical	Intercept	—	−2.663	—	<0.0001	0.36	—	3.902	—	<0.0001	0.13	—	—	—	—	—	—
students	Physical examination	125	0.011	0.003	0.001		2.16 (1.44–3.24)	0.008	0.004	0.03		2.74 (1.14–6.60)	—	—	—		—
	Listening lectures	35	0.017	0.007	0.009		1.78 (1.20–2.65)	—	—	—		—	—	—	—	—	—
Uniform	Intercept		−1.418	—	<0.0001	0.48	—	3.925	—	<0.0001	0.57	—	−0.704	—	0.2		—
students	Physical examination	97.5	0.008	0.005	0.002		2.08 (2.08–3.61)	0.029	0.005	<0.0001		15.3 (5.94–39.2)	0.031	0.010	0.005	0.28	21 (3.04–139)
	Listening lecture	30	−0.071	0.018	0.01		0.12 (0.04–0.33)	−0.172	0.030	<0.0001		0.01 (0.00–0.03)	0.195	0.061	0.005		0.00 (0.00–0.10)
	Anesthetic injection	150	—	—	—		—	0.012	0.003	0.002		5.88 (2.13–16.3)	0.011	0.004	0.02		5.21 (1.61–17)
Differentiated	Intercept	—	−2.831	—	0.0006	0.46	—	4.004	—	<0.0001	0.25		—	—	—		
students	Stall round in clinic	95	0.016	0.006	0.01		4.68 (4.68–14.1)	0.025	0.010	0.04		8.97 (1.31–61.6)	—	—	—		—
	Visiting animals	47.5	0.012	0.006	0.05		1.77 (1.05–2.97)	—	—	—		—	—	—	—		—
Caretakers	Intercept	—	−0.904	—	<0.0001	0.17	—	6.362	—	<0.0001	0.16	—	2.325	—	<0.0001	0.41	—
	Feeding	45	0.024	0.009	0.009		8.0 (1.85–35)		—	—		—	—	—	—		—
	Bedding	85	—	—	—		—	0.013	0.005	0.01		1.83 (1.14–2.93)	0.017	0.006	0.01		2.15 (1.27–3.65)
	Cleaning with water	45	—	—	—		—	—	—	—		—	−0.010	0.004	0.02		0.64 (0.45–0.91)
Poultry clinic and farm visits																	
Students	Intercept	—	−3.531	—	<0.0001	0.50	—	3.915	—	<0.0001	0.43	—	−4.945	—	<0.0001	0.69	—
	Restraining chickens	20	0.084	0.024	0.0005		5.37 (2.13–13.6)	0.082	0.025	0.001		5.16 (1.95–13.7)	0.176	0.023	<0.0001		34 (14–83)
	Checking of nutrition	10	−0.146	0.027	<0.0001		0.23 (0.14–0.39)	−0.149	0.028	<0.0001		0.22 (0.13–0.39)	−0.25	0.026	<0.0001		0.08 (0.14–0.05)
	Checking of neck and beak	10	0.295	0.052	<0.0001		19.06 (6.94–52)	0.301	0.051	<0.0001		20.25 (7.44–55)	0.415	0.042	<0.0001		63 (28-144)
	Listening lecture	15	0.062	0.023	<0.0001		2.52 (1.27–5.00)	0.142	0.041	0.0009		8.37 (2.48–28)	—	—	—		—
	Consulting with owners	15	−0.192	0.039	<0.0001		0.06 (0.02–0.18)	−0.226	0.061	0.0004		0.03 (0.01–0.20)	−0.245	0.033	<0.0001		0.03 (0.01–0.07)
	Taking blood	60	—	—	—		—	−0.058	0.029	0.04		0.03 (0.00–0.90)	—	—	—		—
	Collecting feces	30	0.059	0.010	<0.0001		5.91 (3.30–10.6)	0.082	0.021	0.0002		11.62 (3.41–40)	0.092	0.008	<0.0001		15.8 (9.87–25)
	Physical examination	45	0.075	0.013	<0.0001		29.67 (9.34–941)	0.047	0.015	0.003		8.23 (2.14–32)	0.107	0.01	<0.0001		123 (51–298)
	Evaluating feathers	10	—	—	—		—	—	—	—		—	0.101	0.016	<0.0001		2.75 (2.01–3.76)

[a] *β*, Regression coefficient; SE, standard error; FEC, factor of exposure change ($\exp^{(\beta \times \text{median time})}$); and CI, confidence interval.

broiler poultry farm (GM 10.58 mg m^{-3}, range 8.38–13.34)[36] and the Dutch layer poultry farm (GM 9.5 mg m^{-3}, range 6.6–14).[34]

It is not surprising that personal dust measurements in both poultry and ruminant clinics yield much higher dust levels than stationary measurements because this has been observed in several other occupational studies in animal houses.[1,3,17]

The endotoxin levels for caretakers at the ruminant clinic (GM 1042 EU m^{-3}, range 60–7492) were somewhat higher than those reported in the Dutch dairy farms (GM 560 EU m^{-3}, range 62–22 330),[34] Wisconsin dairy barns (GM 647 EU m^{-3}, 25–348 000),[3] the Dutch horse stables (GM 698 EU m^{-3}, <LOD–9846),[5] but slightly lower than those reported in the Dutch dairy farming and cattle breeding (GM 1570 EU m^{-3}).[34] Since dust levels in the present study were in agreement with the mentioned studies, endotoxin load (concentration of endotoxin per mg of dust) might be dependent on the farming characteristics and country of origin where the study was performed (*e.g.* due to climatologically differences). Additionally, the major fraction of the airborne dust in the current study is likely to consist of fecal material as earlier studies suggested that fecal particles contaminated with bacteria are the predominant supplier to endotoxin present in animal houses.[34] Endotoxin levels can be highly influenced by other determinants such as characteristics of ventilation, seasonal variation, and temperature.[37–39]

Higher exposure levels in daytime and morning shifts for caretakers in ruminant clinic compared to afternoon shift are likely due to performing more dusty activities during daytime and morning shifts, while activities in the afternoon shift were more observational resulting in lower exposure levels.

Levels of endotoxin exposure for poultry caretakers (GM 2749 EU m^{-3}) were consistent with those found in layer poultry farms (GM 2090 EU m^{-3}),[34] markedly lower than those reported in the UK broiler poultry farm (GM 8341 ng m^{-3}),[36] and higher than those findings in the Dutch broiler poultry farm (GM 880 EU m^{-3}).[34] Comparison with these studies needs to be made with care since probably other activities have been performed in the poultry clinic, resulting in different exposure levels. The same explanation could also apply for exposure in the ruminant clinic.

The levels of β-(1 → 3)-glucan for caretakers in the clinics of poultry and ruminants were higher than levels in a study conducted previously in poultry houses,[18] although different sampling methods were used. Levels compared very well with those findings that we reported earlier in horse stables.[5] In fact, these comparable results were expected since ruminant and horse clinics used similar bedding materials and hay for feeding, as well as similar tasks and management systems are applied to all these clinics.

Levels of dust and endotoxin exposure in association with the veterinary jobs in companion animal treatment were much lower.[24,40] When comparing the exposure levels stratified by job titles, within the ruminant and poultry clinics, the trends of exposure levels for students and veterinarians were substantially lower than the levels measured for caretakers. Elevated levels for caretakers probably reflect the greater time spent in animal houses and performance of more dusty activities, while students and veterinarians spent most of their time in teaching rooms and are less often involved in dusty activities. It is important to note that exposure for preclinical students was only measured during practical teaching work and we assumed that exposure through theoretical study is negligible. However, exposure may differ when performing other activities besides practical teaching work.

As expected, higher dust and endotoxin levels were observed in poultry clinic *versus* ruminant clinic, which is in accordance with earlier studies.[6,41] A plausible explanation for these higher exposure levels in the poultry clinic is likely related to a higher animal density in poultry houses, the nature of the animals, and also a lower cleaning frequency of the animal houses especially in privately owned poultry farm houses.

The Expert Committee on Occupational Standards of the Health council in The Netherlands[32] recently proposed a health based occupational exposure limit for endotoxin of 90 EU m^{-3} as an eight-hour-time-weighted-average. Findings of endotoxin exposure in the current study suggest that adverse health effects might occur since 97.1% of personal samples clearly exceeded the limit of 90 EU m^{-3}, thus, lowering of exposure levels is warranted, as well as usage of personal protective equipment.

A similar pattern in endotoxin exposure levels over sampling locations was observed for EDC settled dust samples as for personal or stationary dust samples collected in the same room. This is in agreement with what we found previously in the companion animal clinic,[24] supporting the idea that EDC settled dust samples can be applied as a surrogate measure of personal endotoxin exposure like stationary sampling. Nevertheless, this should be applied with caution, as these EDC measurements might underestimate or overestimate the personal exposure, and conclusions are up-to-now based on a limited number of EDC samples.

Exposure measurements in the present study were conducted for different groups of workers who performed diverse tasks. Tasks performed explained 0–69% of the exposure variation in endotoxin or β-(1 → 3)-glucan in the ruminant clinic. This is consistent with our previous findings in horse stables.[5] Feeding and applying bedding materials were the predominant predictors of elevated exposure levels in caretakers. This finding is in line with earlier studies in dairy farms showing associations between these activities and increased dust exposure.[3,42] Such an inventory has not yet been studied in poultry farms, but our findings show that for veterinary students restraining chickens, checking neck and beak, collecting feces, and physical examinations are important tasks leading to exposure.

Conclusion

This study shows that substantial levels of endotoxin and β-(1 → 3)-glucan exposure are present in modern animal clinics. Exposure levels were strongly influenced by animal species, sampling sites, and job titles. In general, sampling associated with the poultry clinic had higher dust and endotoxin levels compared to the ruminant clinic. Endotoxin levels of most personal samples obviously exceeded the Dutch proposed standard limits of 90 EU m^{-3}, and this presents a concern for adverse health effects. Further work is needed to reduce exposure and use of personal protective equipment during some tasks is advised.

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