Occupational bio-aerosol exposure in veterinary medicine

a comprehensive assessment of exposure and exploration of bio-aerosol related health effects

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Beroepsmatige blootstelling aan bio-aerosolen in de diergeneeskunde

een uitgebreide beoordeling van blootstelling en verkenning van bio-aerosol gerelateerde gezondheidseffecten

(*met een samenvatting in het Nederlands*)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof. dr. G.J. van der Zwaan, in gevolge het besluit van het college voor promoties in het openbaar te verdedigen op maandag 28 november 2011 des middags te 2.30 uur

door

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geboren op 22 mei 1969 te Shazand, Iran

Promotor: Prof.dr.ir. D.J.J. Heederik

Co-promotor: Dr.ir. I.M. Wouters

Contents

Chapter 1

General introduction

Background

Workers in agricultural occupations are often exposed to bio-aerosols with microbial, animal, and plant origins. This exposure occurs especially during activities involving breeding, raising, and taking care of animals. For example, workers in livestock farms (*e.g*. pig, cow, and poultry) are likely exposed to high levels of bio-aerosols [1-3], which has been shown to affect their health [4-8]. Little is known about exposure to bio-aerosols and related health risks of veterinarians. Practicing veterinary medicine has great similarities with other occupations exposed to livestock or companion animals because most veterinarians and co-workers spent a considerable amount of their working time in stables and/or clinics in close contact to these animals. Therefore, exposure to bio-aerosols during veterinary practice is perhaps possible, however detailed and comprehensive exposure studies are lacking. The one study performed before starting this thesis, suggests that bio-aerosol exposure in veterinarians, especially during working with livestock or companion animals, can be substantial [9].

Veterinary medicine students may also encounter bio-aerosol exposure during their education. Veterinary medicine students in the first years have to complete a preclinical training in basic veterinary sciences to gain knowledge of the functioning of healthy and ill animals. In later years, students will take part in clinical internships accompanied with clinical-related courses. Practical work with animals is fundamental for veterinary training, providing the required skills for examination and treatment of animals. Work experience may also involve time spent in diverse animal clinics and/or animal housing, and may involve work with several animal species. Biological, chemical, and physical agents may be encountered when veterinary students are working in animal settings. Exposures may impact health of veterinary medicine students. Nonetheless, there is no published information about bio-aerosol exposure during the practical component of veterinary medicine training.

History of bio-aerosol related health effects

The first scientific wordings on health hazards of organic dust exposure were presented by Magnus in 1555 [10]. He described how the exposure to organic dust might affect the airway system of threshers. Bernardino Ramazzini further described these health hazards in his book *"De Morbis Artificum Diatriba"* first published in 1700 and revised in 1713 [11]. Donham *et al*. indicated that exposure to bio-aerosols containing endotoxins might be responsible for the development of respiratory symptoms among pig farmers and veterinarians [12]. Following this, most observational and experimental studies on health effects related to bio-aerosol exposure in agricultural settings have focused on respiratory effects of pig workers, with emphasis on exposure to endotoxin [4,6-8,12-21]. Endotoxins, integral cell wall components of Gram-negative bacteria, can liberate through replication and

cell death lysis [22]. They are known to have strong pro-inflammatory properties causing neutrophilic airway inflammation [23]. Exposure to endotoxin is commonly found in agricultural settings particularly during working with livestock animals (*e.g*. pig barns, poultry houses, dairy barns, and horse stables) [1-3,24]. The most well-known health effects associated with endotoxin exposure are organic dust toxic syndrome (ODTS), chronic bronchitis, asthma-like syndrome (by some references called non-atopic asthma) and chronic obstructive pulmonary diseases (COPD) [5,8,22,25]. There is also evidence indicating that exposure to farm environments in early childhood or as an adult may protect individuals from developing allergy [26-30].

Beta-glucans are other potential biological active components of organic dust [31], which consists of glucose polymers with variable molecular weight and degree of branching [32], of which the predominant form is β(1→3)-glucans [33]. β(1→3)-glucan is a non-allergenic cell wall component that can be found in most fungi, some bacteria, and several plants [34]. β(1→3)-glucans are less potent inflammatory agent than endotoxin, but it still might cause effects when exposure levels are high [35,36]. In the late 1980s, it has been suggested for the first time that exposure to β(1→3)-glucans might be responsible for indoor air-related health effects. Since then, several epidemiological studies have been conducted, resulting in mixed evidence of inflammatory health effects as reviewed by Douwes et al. [31].

High molecular weight (HMW) allergens, usually proteins, of animal, plant or microbial origins are well known as the most important occupational allergens, which can induce allergic reactions with subsequent allergic diseases [37]. Numerous animal proteins (*e.g*. cat, dog, cow, horse, mouse and rat allergens) have potent allergenic properties. These allergens can be found in hair, skin scrapings, and feces; and may originate from epithelia or secretions like plasma, urine and saliva and can become airborne. Most extensive epidemiological studies related to animal allergens focused on rats and mice allergens [38]; consequently these allergens are well established as a risk factor for developing occupational type I (IgE-mediated) allergy in workers exposed to laboratory animals [38-45]. Besides rats and mice allergens, there is relatively little information regarding occupational sensitization/allergy against other animal allergens. Nonetheless, a few studies suggested the importance of exposure to farm animals or pets as a risk factor for the development of sensitization/allergy against farm animal or pet allergens [46,47].

Bio-aerosol exposure assessment methods

Accurate exposure assessment is fundamental to better understand the health consequences of bio-aerosols as well as for the application of measures to reduce the exposure. Various sampling methods for the collection of bio-aerosols have been developed and tested over the last few decades. Glass-impingers (GIMs) were first introduced in the 1920s and then modified in the early 1950s [48]. The six-stage Andersen impaction sampler (AMS) was

introduced after the Second World War to allow simultaneous sizing and counting of viable microbes [49]. Later on, two- and one-stage samplers, as an alternative design of the six-stage, have been introduced for total counting at the expense of sizing ability [50,51]. Collection of airborne bio-aerosols using filter media followed by eluting and plating was first introduced in 1958 [52] which found to be applicable for several microbes. Following the development of specific immune assays, several dust collection methods have been introduced to determine bio-aerosol exposure levels such as collection of settling reservoir dust by vacuuming the surfaces [53], airborne dust collection using pumps and filter holders [1], collection of settling airborne dust using Petri dishes and boxes [54,55], nasal airborne collection by a miniature sampler in the nostril [56], electrostatic dust-fall collectors (EDCs) [57], and collection of settling table surface dust through wipe sampling [58]. Which sampling procedure is used is mainly dependent on the purpose of the exposure measurements. In occupational studies, airborne dust collection on filter media using personal-carried pumps is considered to be the best reflection of a persons' exposure [59].

Two ways of determining endotoxin or LPS exposure have been described including identification in absolute terms (chemical assay) or in a functional way (biological assay measuring biologically available endotoxin). Endotoxin levels in absolute terms (moles or weight per m^3) can be determined with the application of gas chromatography combined with mass spectrometry (GC-MS) which requires the samples to be destroyed for analysis. Biologically available endotoxin levels can be measured using *Limulus amoebocyte lysate* (LAL) assay, as a reaction between LPS present in the sample and lysate (pro-enzyme purified) derived from the horseshoe crab (*limulus polyphemus*). The LAL assay has become available in the early 1980s and later applied as the most commonly method to determine endotoxin exposure. The assay is simple to perform providing specific, rapid and highly sensitive results. It has been described that the exposure levels as determined with the functional assay correlate better with toxic health effects than exposure levels determined in absolute terms [60].

Several methods for the detection of glucans have been described. Initially, β(1→3)-glucans were analyzed using the modified *Limulus amoebocyte lysate* assay [61]. Douwes *et al*. (1996) introduced a simple specific *inhibition enzyme immunoassay* for the detection of β(1→3)-glucans in the mid-1990s [62]. This assay measures β(1→3)-glucans based on detection with antibodies and is extensively applied in large-scale environmental epidemiological studies using dust collected from floors or mattresses [63-66], as well as occasionally in occupational environments such as household waste recycling industry [67]. More recently, glucan *sandwich enzyme immunoassays* (EIAs) [68] have been developed, which are more sensitive and are expected to be applied in future epidemiological studies.

E*nzyme liked immuno-sorbent assays* (ELISAs) using specific antibodies against the allergens are broadly applied to quantify the levels of allergens in settled and airborne dusts.

In general, airborne allergen levels are often much lower, thus, very sensitive analytical assays are needed to detect airborne allergen levels. Recently, multiplex assays have been developed, like the *Multiplex Array for Indoor Allergens* (MARIA), which show to be a highly sensitive analytical method to detect and quantify simultaneously multiple allergens [69].

Bio-aerosol related health effects in veterinary populations

The development of occupational allergic and non-allergic health effects among veterinary populations in relation to bio-aerosol exposure has not been extensively studied. A few cross-sectional studies investigated the prevalence of respiratory symptoms in veterinarians particularly those exposed to pig barns [9,70-74]. These studies demonstrated that veterinarians are likely at risk for the development of occupational allergic and non-allergic respiratory effects, but associated bio-aerosol exposure is largely unknown. Furthermore, dose-response relationships between bio-aerosol exposure and health effects among veterinary populations have not yet been studied, mainly due to the absence of bio-aerosol exposure measurements during working with animals in veterinary practices. Findings of bio-aerosol exposure and related health effects in farmers, exposed to similar animal environments as veterinarians, prompted us to hypothesize that veterinarians might be at risk of developing allergic and non-allergic respiratory problems in relation to bio-aerosol exposure. The main research questions were: (1) what are bio-aerosol exposure levels encountered in veterinary practices within diverse animal clinics and stables, (2) what is the magnitude of risk for development of respiratory effects and sensitization and which factors determine the risk? To answer these questions, the studies described in this thesis have been conducted.

Aims

The main purpose of this thesis was to comprehensively investigate the exposure levels of inhalable dust, endotoxin, β(1→3)-glucans, and animal specific allergens among veterinarians, veterinary students, and animal caretakers (workers) in diverse veterinary clinics, as well as to explore exposure determinants. In addition, we explored the prevalence of sensitization/allergy and respiratory symptoms among veterinary medicine students, which might be related to bio-aerosol exposure. A secondary aim was to investigate the feasibility and efficiency of a newly developed electrostatic dust-fall collector to measure bio-aerosol components especially animal specific allergens.

Outline of the thesis

Chapter 1, the general introduction, gives the background to studies described in this thesis.

Chapter 2, presents the results of a study investigating exposure levels of bio-aerosols in horse stables within a veterinary clinic. The influence of determinants on exposure levels

and exposure variances were investigated. In addition, levels of culturable microbial exposure were explored.

Chapter 3, presents the results of a study exploring exposure levels of allergens and endotoxin in a companion animal hospital with the application of various dust collection methods. The feasibility and efficiency of electrostatic dust-fall collector as a newly developed method for collection of ambient settling dust to determine bio-aerosol components in particular for animal specific allergens were examined.

Chapter 4, describes exposure levels of bio-aerosols during veterinary practice within ruminant and poultry clinics. Moreover, task-based determinants of exposure were investigated.

Chapter 5, presents the results of a study exploring the influence of various bedding materials on bio-aerosol exposure levels in dairy barns.

Chapter 6, describes the findings of a questionnaire survey and blood serological test for (allergic) health symptoms and sensitization among veterinary medicine students in the Netherlands. In this study, the association between the development of sensitization/allergy and respiratory symptoms with study specialization over time as a surrogate of exposure was explored.

Chapter 7, main findings of the studies presented in this thesis are discussed in the context of a review study on bio-aerosol exposure levels in veterinary practices and exploring the implications for health effects associated with bio-aerosol exposure in veterinarians.

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Chapter 2

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

Sadegh Samadi, Inge M. Wouters, Rosa Houben, Ali-Reza Jamshidifard, Frank Van Eerdenburg, and Dick J.J. Heederik

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ABSTRACT

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.

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 [1]. These components may penetrate into the lungs of exposed workers. Two identified major pro-inflammatory components of organic dusts are bacterial endotoxin [2] and mould β(1→3)-glucan [3]. Endotoxin is a component of the cell wall of Gram-negative bacteria and a ubiquitous component of organic dusts [1]. Endotoxin has been proven to be a powerful inflammatory agent 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 [2]. β(1→3)-glucan is an important cell wall constituent of most fungi, some bacteria, and numerous plants [1] 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 [3]. Most evidence comes from epidemiological studies in households in which β(1→3)-glucan levels were associated with respiratory symptoms [1,4].

Recent studies demonstrated that working with horses was associated with an increased risk of respiratory symptoms and/or organic dust toxic syndrome [5,6]. Some small studies found that horses were exposed to high levels of endotoxin in stables [7,8], 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 [9] 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.* [10]. 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.* [11]. 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 [12]. 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 [10], 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 ($R = 0.69$, $P < 0.0001$), 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}) [1]. 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.

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

		Dust (mg m^{-3})						Endotoxin (EU m^{-3})						β(1→3)-glucan (μg m^{-3})					
	Worksite	*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	0.4	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.

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

		Dust (mg m^{-3})					Endotoxin (EU m^{-3})					β(1→3)-glucan (µg m^{-3})				
	Shift	*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.

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.

Table 4. Estimated variance components for personal exposure models.

Exposure models	G[a]	${}_{ww}\sigma^{2b}$	${}_{bw}\sigma^{2b}$	${}_{ww}R^{c}_{0.95}$	${}_{bw}R^{c}_{0.95}$	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]${}_{ww}R_{0.95}$ and ${}_{bw}R_{0.95}$: 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.

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 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.

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.

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 [7,13-15].

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 [16,17]. As it has been reported by others [16,18], 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.* [13], while levels were higher than those found (AM 2.05 mg m^{-3}) by Bartz and Hartung [19]. 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) [20], 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}) [21]. 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 [22], waste management chain [23], and waste composting facility [11]. This might be explained by differences in dust composition, like a relatively large portion 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.* [24]. 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 [14]. Similarly, large variations for dust and endotoxin levels were reported in agricultural industries [21]. 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.* [25]. 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.* [23], 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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Chapter 3

Allergen and Endotoxin Exposure in a Companion Animal Hospital

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ABSTRACT

Background: Exposure to allergens, both in general and occupational environments, is known to result in sensitisation and exacerbation of allergic diseases, while endotoxin exposure might protect against allergic diseases. This may be important for veterinarians and co-workers. However, exposure levels are mostly unknown.

Objective: We investigated the allergen and endotoxin exposure levels of veterinary medicine students and workers in a companion animal hospital.

Methods: Airborne and surface dust was collected using various sampling methods at different locations. Allergen levels in extracts were measured with sandwich ELISAs and/or the multiplex array for indoor allergens (MARIA). Endotoxin was determined by limulus amebocyte lysate (LAL) assay.

Results: Fel d 1 (*Felis domesticus*), Can f 1 (*Canus familiaris*) and endotoxin were detected in all except stationary samples. The geometric mean (GM) level of personal inhalable dust samples for Fel d 1 was 0.3 ng m^{-3} (range: below lower limit of detection (<LOD) to 9.4), for Can f 1 3.6 ng m^{-3} (<LOD to 73.3) and for endotoxin 4.4 EU m^{-3} (<LOD to 75). Exposure levels differed significantly between job titles, with highest allergen exposure for student assistants in the intensive care unit (Fel d 1, GM 1.5 ng m^{-3}; Can f 1, GM 18.5 ng m^{-3}), and highest endotoxin exposure for students (GM 10.1 EU m^{-3}). Exposure levels in dust captured by diverse sampling methods correlated with each other ($p<0.05$).

Conclusion: Allergen exposure likely occurs during veterinary practice, with relatively low endotoxin levels. Future research should investigate dose–response relationship between airborne allergen exposure and health effects.

INTRODUCTION

Exposure to animal allergens has been implicated as a major risk factor for sensitization and allergic diseases like asthma and allergic rhinitis [1,2]. Animal allergen sensitization and asthma are also well-known occupational health risks [3]. This is best described for laboratory animal workers exposed to rat and mouse [4,5], but might also be important for veterinarians [3]. The main animals treated in companion animal hospitals are cats and dogs, which are well known allergen producers. The major allergen for cat is Fel d 1 (*Felis domesticus*) and for dog, Can f 1 (*Canus familiaris*) [4]. Contact with cats and/or dogs has been reported as a cause of self-reported work-related respiratory symptoms in veterinarians [3] and laboratory animal workers [6], but there have been few studies on allergen exposure levels.

During the last decades, a possible protective effect of endotoxin exposure on the development of allergies and asthma has been suggested [7,8]. Endotoxins are integral cell wall component of various Gram-negative bacteria [9]. Exposure to endotoxins is well-known for workers in agricultural industries [10], and has been reported for laboratory animal workers as well [11]. Also little is known concerning exposure among veterinarians

and their assistants [12], we therefore determined allergen and endotoxin exposure levels for veterinarians, animal technicians and students working in a companion animal hospital.
Various sampling methods to collect dust for measuring allergens [13] and endotoxin [14] have been described. In occupational settings, active airborne inhalable dust sampling is the most frequently applied method [15]. Studies that measured airborne allergen levels of Fel d 1 and Can f 1 in homes or public environments reported very low or non-detectable levels [13,14]. We therefore collected dust using several other methods besides personal dust sampling, such as the collection of reservoir dust by vacuuming the floor [16] and the collection of table surface dust through wipe sampling [17]. Furthermore, we explored the feasibility and efficiency of electrostatic dust-fall collectors (EDCs) which collects ambient settling dust for allergen exposure assessment [18].
Thus, the objective of this study was to characterize exposure levels to allergens and endotoxin in a companion animal hospital using various dust collection methods.

METHODS

Study design

This study was performed in a companion animal hospital at the Faculty of Veterinary Medicine of Utrecht University, the Netherlands. Dust was collected using five different sampling methods:

- Active personal inhalable dust collection;
- Stationary inhalable dust collection;
- EDC settling dust collection;
- Floor dust collection;
- Table surface dust collection.

Samples were collected at various worksites within the hospital: operation room, recovery room, intensive care unit, inpatient ward, examination room, practical teaching room, waiting room, ultrasound room, office and canteen.
Personal inhalable dust sampling was conducted among employees, student assistants and students. Student assistants were veterinary medicine students working during their final year rotation in the companion animal hospital. Students were in their first or second year of veterinary training following a practical course in animal handling and physical examination.

Sampling and analytical procedures

Active personal and stationary airborne dust sampling

Personal inhalable dust samples were collected using Gil-Air 5 pumps (Gillian, Clearwater, Florida, USA). Samples were taken in the breathing zone with a PAS-6 sampler equipped

with a 25 mm glass fiber filter (Whatman International, Maidstone, UK) at a flow rate of 2 l min^{-1}. Stationary inhalable dust sampling was performed with the same equipment, positioning the PAS-6 sampler on a tripod at a height of 1.5 m. The glass fiber filters were pre- and post-weighed in a preconditioned room with average temperature 22°C (range 21°C to 23°C), relative humidity 38% (35% to 41%) and air pressure 1026 mbar (1009–1032). One field blank sample was taken on each sampling day. The dust weight of most samples based on filter blanks was below the lower limit of detection (LOD) of 0.26 mg. Filters were stored at −20°C until extraction.

Electrostatic dust-fall collectors

Plastic EDCs equipped with electrostatic cloths (Zeeman, Alphen aan de Rijn, the Netherlands) were used as described previously [18]. Briefly, EDCs were opened and placed on top of cupboards or on manufactured wall holders at approximately 170 cm above floor level, to allow settling dust to be captured by the electrostatic cloths. The sampling area of each cloth was 0.0209 m^2. Cloths were made pyrogen-free by heating at 200°C overnight prior to sampling. Sampling was conducted for three different time periods in parallel by placing three EDCs alongside each other at each location. The following time periods were considered: 10 work days (EDCs were opened at 07:00 h and closed at 16:00 h every day over a 2-week period, Monday to Friday), 10 days (2 weeks, from Monday 07:00 h to Saturday 07:00 h at a stretch, including night time) and 14 days at a stretch (two continuous weeks). Four field blank samples were taken in each sampling period. After sampling, cloths were transferred to 50 ml tubes (Greiner) and stored at −20°C until extraction.

Floor dust sampling

Floor dust samples were collected using a 1200 W vacuum cleaner (Miele, Gütersloh, Germany) equipped with a 25-µm mesh nylon sock (Allied Filter Fabrics, Sydney, Australia) as previously described [19]. An area of 2 m^2 of smooth flooring was vacuumed for 2 min. Samples were collected at the end of the work shift, before cleaning. Socks were pre- and post-weighed in a preconditioned room with average temperature 23°C (range 22°C to 23°C), relative humidity 37% (range 36% to 40%) and air pressure 1022 mbar (range 1013–1031). Dust captured in the sock was transferred to 50 ml tubes prior to extraction. Pre- and post-weighing of the tubes revealed a 12% loss of dust weight while the dust was being transferred from the sock to the tube. Samples were stored at −20°C until extraction.

Table surface dust sampling

Table surface dust sampling [17] was carried out using filter papers (55 mm diameter; Schleicher & Schuell, Dassel, Germany) pre-wetted with 300 µl phosphate-buffered saline containing 0.05% (v/v) Tween-20 (Merck, Schuchardt OHG, Germany). A surface area of ~0.08 m^2 (30×26 cm) was wiped, with the operator wearing clean gloves. Sampling was carried out on the surfaces of animal exam tables in the examination room (n=2), practical teaching room (n=1), ultrasound room (n=1), intensive care unit (n=1) and inpatient ward (n=1); computer tables in the office (n=6), recovery room (n=1), ultrasound room (n=1), waiting room (n=1), practical teaching room (n=1) and examination room (n=2); equipment tables in the examination room (n=2), operation room (n=2), recovery room (n=1), intensive care unit (n=1) and inpatient ward (n=1); and lunch tables in the canteen (n=2). Samples were collected at the end of the work shift. One field blank sample was taken on each sampling day. Filter papers were stored in 15 ml tubes at −20°C until extraction.

Extraction

Extraction for endotoxin and allergens was performed as described elsewhere [20]. Briefly, dust samples were extracted in pyrogen-free water containing 0.05% (v/v) Tween-20. Personal inhalable and table surface dust samples were eluted in 5 ml, EDC dust samples in 20 ml and floor dust samples in 5–20 ml, depending on the weight of dust (<0.5 g: 5 ml; 0.5–1.0 g: 10 ml; 1.0–2.0 g: 20 ml). After shaking in an end-over-end roller for 1 h, the tubes were centrifuged for 15 min at 1000 g, and 10% of the supernatant was harvested and stored at −20°C for endotoxin analysis. The removed volume of supernatant was replenished with the same volume of 10× concentrated phosphate-buffered saline for allergen extraction. Samples were again shaken for 1 h in an end-over-end roller, followed by centrifugation for 15 min at 2000 g. Ten per cent of supernatants were harvested and stored at −20°C prior to allergen analysis.

Allergen and endotoxin detection

Allergen levels of Fel d 1 and Can f 1 in floor dust samples were assessed with sandwich ELISAs (Indoor Biotechnologies, Charlottesville, Virginia, USA) [21]. Samples with a Fel d 1 concentration below the LOD were also analysed using an amplified ELISA, which used streptavidin/HRP (M2051, diluted 1:20000; Sanquin, Amsterdam, the Netherlands) instead of avidin/HRP. Floor dust samples were tested in 1:5 to 1:1600 dilutions.

All personal, stationary, EDC and table surface dust samples were tested undiluted using multiplex array for indoor allergens (MARIA) (Indoor Biotechnologies) as described by Earle

et al. [22] to determine Fel d 1 and Can f 1, and also Mus m 1, Rat n 1, Der p 1, Der f 1, mite group 2 and Bla g 2.

Endotoxin was determined using a limulus amebocyte lysate (LAL) assay [23]. A 12-point standard curve was produced over the concentration range 0.049–100 EU ml^{-1} for floor dust samples and 0.012–25 EU ml^{-1} for other samples. Personal inhalable dust samples were tested in 1:20 dilutions, EDC and table dust samples in 1:50 dilutions, and floor dust samples in 1:500 dilutions.

Allergen exposure levels were expressed as ng m^{-3} for personal inhalable dust samples, ng m^{-2} and ng mg^{-1} of dust for floor dust samples, and ng m^{-2} for the EDC and table surface dust samples. Endotoxin exposure levels were expressed in EU m^{-3} for personal inhalable dust samples, EU m^{-2} and EU mg^{-1} of dust for floor dust samples, and EU m^{-2} for the EDC and table surface dust samples.

Samples with allergen or endotoxin levels below the LOD were replaced with a value of two-thirds of the LOD. The average LOD of the Fel d 1 and Can f 1 of ELISA used for floor dust samples was 3 and 1.5 ng m^{-2}, respectively. For the amplified Fel d 1 ELISA, it was 0.04 ng m^{-2}. The average LOD of MARIA assay for Fel d 1, Can f 1, Mus m 1, Rat n 1, Der p 1, Der f 1, mite group 2 and Bla g 2 corresponds to a LOD of 0.03, 0.10, 0.01, 0.06, 0.1, 0.1, 0.6 and 6.8 ng m^{-3} for personal dust samples; 0.25, 0.75, 0.1, 0.5, 0.8, 0.8, 0.5 and 52 ng m^{-2} for table surface dust samples; and 0.87, 2.61, 0.3, 1.6, 2.6, 2.6, 1.6 and 182 ng m^{-2} for EDC dust samples, respectively. The average LOD of endotoxin for personal dust samples was 4.8 EU m^{-3}, for table surface dust samples 38 EU m^{-2}, for EDC dust samples 830 EU m^{-2}, and for floor samples 45 EU m^{-2}.

Statistical analysis

All data were log-normally distributed ($p<0.05$); therefore, analyses were performed on log-transformed data. Exposure levels were calculated as geometric means (GMs) and geometric standard deviations (GSDs). Differences in exposure concentrations between various locations and jobs were tested using ANOVA, followed by the Tukey–Kramer test as a post hoc test. Pearson correlations were used to assess relationships between parameters. For each location where settling dust samples were collected by EDC, the average allergen concentrations of personal or floor dust samples collected at that location during the EDC sampling period were allocated to the EDC allergen level of the same sampling period in order to compare the different dust sampling methods. Similarly, average concentrations of personal samples collected on the day and at the location of floor dust collection were compared to each other. For this only the 14-day continuous measurements of the EDC samples were used.

Statistical analyses were carried out using SAS v 9.1. Values of $p<0.05$ were considered to be significant.

RESULTS

In total, 293 dust samples for allergens and endotoxin were tested. The average sampling time for personal inhalable dust collection was 4.6 h (range 2–8.5 h). The allergen and endotoxin levels of all stationary inhalable samples were below the LOD. Allergen levels of Mus m 1, Rat n 1, Der p 1, Der f 1, mite group 2 and Bla g 2 were below the LOD for all samples.

Personal inhalable dust sampling

Allergen and endotoxin exposure levels of personal inhalable samples are presented in Table 1. Fel d 1 was not detectable in the majority of personal inhalable dust samples, while Can f 1 was detected. Significant differences in exposure levels between job titles were found for Fel d 1 and Can f 1 ($p<0.05$), with the highest exposure for student assistants in the intensive care unit (Fel d 1, GM 1.5 ng m^{-3}; Can f 1, GM 18.8 ng m^{-3}). Cat allergen exposure levels were significantly higher for students during the practical animal course compared to student assistants in the inpatient ward ($p<0.05$), while other post hoc comparisons based on job titles were found to be non-significant ($p>0.05$). Endotoxins were detectable in the majority of personal inhalable dust samples, but at fairly low levels. The highest GM level was observed for students during the practical animal course (GM 10.1 EU m^{-3}).

EDC dust sampling

Allergen and endotoxin levels in ambient airborne settling dust collected by the EDC are presented in Table 2. Fel d 1 was found in 66% of the samples, and ranged from below the LOD to 579 ng m^{-2}, whereas Can f 1 was detected in 90% of the samples, and ranged from below the LOD to 12105 ng m^{-2}. The highest exposure levels of Fel d 1 (GM 349 ng m^{-2}) and Can f 1 (GM 10818 ng m^{-2}) were observed in the intensive care unit. The allergen levels of Fel d 1 and Can f 1 varied greatly between locations ($p<0.05$). Endotoxin levels in the EDC samples ranged from below the LOD to 24211 EU m^{-2} (GM 2276), with the highest exposure levels in the waiting room (GM 10405 EU m^{-2}).

Table surface dust sampling

Results of allergen and endotoxin exposure levels based on table surface types are presented in Table 3. The GM level of Can f 1 on the surfaces of animal exam tables (779 ng m^{-2}) and computer tables (512 ng m^{-2}) was approximately 7 and 4 times, respectively, higher than on equipment tables (118 ng m^{-2}) ($p<0.05$). In contrast, the GM level of Fel d 1

Table 1. Allergen and endotoxin exposure levels in personal inhalable dust samples.

Job	N	Fel d 1 (ng m^{-3})				Can f 1 (ng m^{-3})				Endotoxin (EU m^{-3})			
		ND	GM	GSD	Range	ND	GM	GSD	Range	ND	GM	GSD	Range
Students													
Practical animal course	17	7	0.7	3.8	<LOD–7.3	–	9.0	2.2	2.9–73.3	–	10.1	2.5	3.7–75
Student assistants													
Inpatient ward	8	6	0.1	3.0	<LOD–1.4	2	1.5	4.1	<LOD–6.8	3	3.6	4.9	<LOD–71
Intensive care unit	3	1	1.5	14.9	<LOD–9.4	–	18.8	2.5	7.1–43.4	–	5.6	1.4	3.7–7.5
Anaesthesiology	5	4	0.1	2.5	<LOD–0.6	2	1.2	4.0	<LOD–6.8	2	2.8	2.2	<LOD–8
Operation room	5	2	0.3	3.6	<LOD–1.7	4	0.6	2.3	<LOD–2.6	2	1.9	1.6	<LOD–3
Animal examination room	17	10	0.3	4.0	<LOD–6.1	–	3.3	2.0	1.1–8.7	7	3.3	2.2	<LOD–20
Total	38	23	0.2	4.6	<LOD–9.4	8	2.3	3.6	<LOD–43.4	14	3.2	2.5	<LOD–71
Animal technicians (employees)													
Inpatient ward	4	1	0.5	3.8	<LOD–1.8	–	2.6	3.7	0.6–14.4	–	5.2	3.3	1.6–24
Intensive care unit	6	4	0.2	5.1	<LOD–2.3	–	7.8	2.5	3.9–45.9	–	4.5	2.3	2.0–16
Anaesthesiology	2	1	0.1	2.4	<LOD–0.3	–	2.2	2.5	1.1–4.2	1	1.5	2.0	<LOD–2
Total	12	6	0.3	4.1	<LOD–2.3	–	4.4	3.1	0.6–45.9	1	3.9	2.7	<LOD–24
Overall	67	36	0.3	4.5	<LOD–9.4	8	3.6	3.8	<LOD–73.3	15	4.4	2.9	<LOD–75

<LOD, below the lower limit of detection; GM, geometric mean; GSD, geometric SD; N, number of measurements;
ND, number of non-detectable samples.

Table 2. Allergen and endotoxin exposure levels in EDC settling dust samples (14 continuous days) at different locations.

Location	N	Fel d 1 (ng m^{-2})				Can f 1 (ng m^{-2})				Endotoxin (EU m^{-2})				
		ND	GM	GSD	Range	ND	GM	GSD	Range	N	ND	GM	GSD	Range
Operation room	4	4	9	–	<LOD	1	91	2.1	<LOD–202	4	2	518	1.6	<LOD–794
Recovery room	2	–	92	1.1	88–97	–	1559	1.9	982–2474	2	–	2618	4.8	870–7922
Intensive care unit	2	–	349	2.0	211–579	–	10818	1.2	9675–12105	2	–	9228	3.0	4182–20366
Inpatient ward	3	2	33	10.1	<LOD–482	–	904	8.2	79–3088	3	–	5378	4.1	1453–24211
Examination room	4	–	158	1.3	123–246	–	1356	1.6	737–2421	4	–	2752	2.0	2437–7288
Practical teaching room	2	–	240	1.1	228–254	–	2018	1.3	1719–2377	2	–	9136	1.9	5773–14536
Waiting room	2	–	94	2.9	44–202	–	2399	3.7	947–6088	2	–	10405	1.1	9698–11204
Ultrasound room	2	–	64	1.3	53–79	–	1711	1.1	1614–1816	2	–	3197	1.0	3140–3296
Office	6	1	72	3.6	<LOD–237	–	668	2.4	228–1781	6	1	1510	2.1	<LOD–4144
Canteen	3	3	–	–	<LOD	2	44	1.5	<LOD–70	3	2	513	1.3	<LOD–707
Overall	30	10	56	4.2	<LOD–579	3	720	5.4	<LOD–12105	30	5	2276	3.5	<LOD–24211

EDC, electrostatic dust-fall collector; <LOD, below the lower limit of detection; GM, geometric mean; GSD, geometric SD; N, number of measurements; ND, number of non-detectable samples.

Table 3. Allergen and endotoxin exposure levels in table surface dust samples by table type.

Table type	N	Fel d 1 (ng m^{-2})				Can f 1 (ng m^{-2})				Endotoxin (EU m^{-2})			
		ND	GM	GSD	Range	ND	GM	GSD	Range	ND	GM	GSD	Range
Equipment table	7	1	11	4.7	<LOD–75	–	118	5.5	5–765	–	12088	4.5	706–59767
Animal exam table	6	1	13	5.7	<LOD–79	–	779	3.5	120–3849	–	4023	5.0	565–30841
Computer table	12	–	22	2.7	4.4–114	–	512	2.0	156–1224	–	3294	13	98–53873
Lunch table	2	2	0.6	0	<LOD–06	–	81	3.4	34–194	–	8266	4.0	3078–22369
Overall	27	4	13	4.7	<LOD–114	–	336	4.0	5–3849	–	5324	8.1	98–59767

<LOD, below the lower limit of detection; GM, geometric mean; GSD, geometric SD; N, number of measurements; ND, number of non-detectable samples.

on the surfaces of computer tables was approximately double that of equipment and animal exam tables. The GM level of endotoxin on the surface of equipment tables was the highest, being threefold higher than on animal exam tables and fourfold higher than on computer tables.

Floor dust sampling

The overall floor dust levels ranged from 13 to 1228 mg m^{-2}, with the highest exposure level in the waiting room (GM 382 mg m^{-2}). The allergen and endotoxin levels of floor dust samples are summarised in Table 4. The highest levels of Fel d 1 (GM 39 ng m^{-2}) were found in the examination room, and of Can f 1 (GM 2101 ng m^{-2}) and endotoxin (GM 7631 EU m^{-2}) in the waiting room. Floor dust allergen and endotoxin content can also be expressed as levels per milligram of dust (ng mg^{-1} or EU mg^{-1}). Fel d 1 levels then ranged from <0.01 to 5.43 ng mg^{-1} (GM 0.2), Can f 1 levels from 0.01 to 82.92 ng mg^{-1} (GM 4.7) and endotoxin levels from below the LOD to 1080 EU mg^{-1} (GM 22). High correlations were found between levels expressed per square metre and per mg of dust (Fel d 1: $r=0.80$, $p<0.0001$; Can f 1: $r=0.90$, $p<0.0001$; endotoxin: $r=0.79$, $p<0.05$).

Comparison between dust sampling methods

Exposure levels observed in personal samples correlated moderately with those in EDC samples for Fel d 1 ($r=0.72$, Fig. 1A), Can f 1 ($r=0.62$, Fig. 1B), and endotoxin ($r=0.70$, Fig. 1C). Exposure levels in EDC samples also correlated moderately with those in floor samples for Fel d 1 ($r=0.61$, Fig. 1G) and Can f 1 ($r=0.64$, Fig. 1H). In contrast, the levels of Fel d 1 ($r=0.34$, Fig. 1D), Can f 1 ($r=0.54$, Fig. 1E), and endotoxin ($r=0.28$, Fig. 1F) measured in personal samples showed poor correlation with floor dust samples.

Levels of allergens and endotoxin obtained with the EDC samplers exposed for different periods of time were similar (GM ratios ranging from 0.76 to 1.0; $p>0.05$). Moreover, Fel d 1, Can f 1 and endotoxin levels correlated well between the different time periods ($r>0.81$, $r>0.94$ and $r>0.70$, respectively; $p<0.05$).

DICUSSION

This is the first study to examine possible exposure to bio-aerosols in a companion animal hospital. In general, the different dust collection methods applied in this study showed similar patterns of allergen and endotoxin exposure levels. As expected, allergens and endotoxin levels in airborne samples including personal and EDC samples, were low and allergen levels could only be detected by the sensitive Luminex assay. In contrast, floor dust samples showed much higher allergen and endotoxin levels, which allergens could be detected with an ELISA.

Table 4. Allergen and endotoxin exposure levels in floor dust samples at different locations.

Location	N	Fel d 1 (ng m^{-2})				Can f 1 (ng m^{-2})				Endotoxin (EU m^{-2})			
		ND	GM	GSD	Range	ND	GM	GSD	Range	ND	GM	GSD	Range
Operation room	16	–	4	3.2	1.0–139	–	46	2.7	7–259	1	403	3.1	<LOD–6996
Recovery room	6	–	15	2.6	4.4–40	–	366	2.2	128–880	–	750	2.9	125–2994
Intensive care unit	8	–	8	6.2	0.2–56	–	441	3.3	29–1432	–	1790	2.2	362–4811
Inpatient ward	7	–	16	3.1	6.0–145	–	324	3.4	43–2462	–	4273	1.9	2005–10448
Examination room	17	–	39	3.4	3.2–183	–	1148	2.0	218–8119	–	2515	3.8	334–21823
Practical teaching room	7	–	24	4.1	1.8–91	–	1206	2.4	489–6111	–	4537	3.6	1978–72506
Waiting room	7	–	36	1.9	16–79	–	2101	3.1	611–13646	–	7631	2.9	2836–66974
Ultrasound room	7	–	34	2.9	6.1–124	–	798	2.5	227–4546	–	1772	1.5	1077–3393
Office	21	–	7	2.1	1.5–28	–	222	3.6	15–1512	–	1863	3.0	254–27655
Canteen	14	–	2	2.6	0.3–9	3	11	5.0	<LOD–110	–	1188	5.4	135–100776
Overall	110	–	11	4.3	0.2–183	3	240	7.1	<LOD–13644	1	1702	4.0	<LOD–100776

<LOD, below the lower limit of detection; GM, geometric mean; GSD, geometric SD; N, number of measurements; ND, number of non-detectable samples.

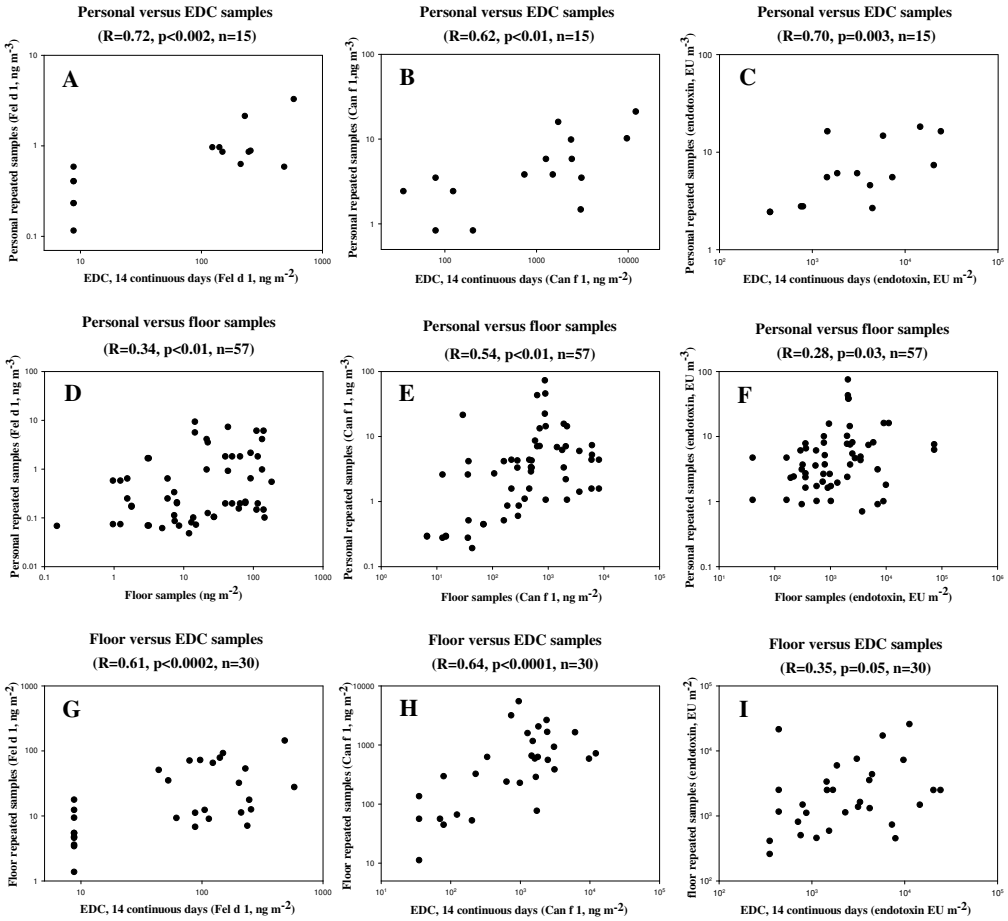

Figure 1. Correlations between various sampling methods for Fel d 1 (A, D, G), Can f 1 (B, E, H) and endotoxin levels (C, F, I). A, B and C, personal versus EDC sampling; D, E and F, personal versus floor dust sampling; G, H and I, floor dust versus EDC sampling.

We were able to measure personal levels of Fel d 1 and Can f 1 despite the low dust levels. Personal allergen exposure levels of Fel d 1 based on job titles were found to be low, often being lower than the LOD with occasional slightly higher levels, suggesting that subjects had only limited exposure to cat allergens. Can f 1 was detected much more often, indicating higher levels of dog allergen than cat allergen. This is likely due to the limited number of cats that are treated in the hospital. During the sampling period, only 15% of the 290 cats and dogs typically treated every week were cats, thus favouring the abundance of dog allergens.

Only a few studies have reported personal exposure levels of Fel d 1 or Can f 1 in homes, offices and schools [24,25], and showed low allergen levels as well. However, a comparison

should be made carefully, since sampling and analysis were different. The higher levels of Fel d 1 and Can f 1 for student assistants in the intensive care unit might reflect the greater proportion of cats and dogs in this area of the hospital, and the nature of the tasks performed. This is in contrast to the low personal allergen exposures in the operation room, likely due to more regular and intensive cleaning and less contact with active animals. Nonetheless, personal allergen exposure levels in this setting occasionally were high and at a level thought to induce asthma symptoms, particularly in those susceptible to cat and dog allergens [26]. The low endotoxin levels in the current study are in agreement with earlier findings for veterinarians working with companion animals [12], in comparison to higher levels for veterinarians working in animal husbandry, poultry or mixed practices [12]. Nonetheless, as also for endotoxin, job titles or areas with closer contact with animals showed the highest exposure levels.

The Fel d 1 levels of floor dust samples in the current study were markedly higher than those previously found in office work places [27], while levels were lower than those reported from floor surfaces in homes with cats [14,28]. A possible explanation for the lower levels of Fel d 1 is the type of floor covering, since most of our sampling was performed on smooth floors, while sampling in homes was frequently conducted on carpeted floors, known to result in higher levels [29]. Unlike for Fel d 1, less data are available for Can f 1. The Can f 1 levels of floor dust samples were comparable to those from public spaces [24] and homes with dogs [30].

This study showed that table surfaces can act as a potential secondary source for exposure. This has rarely been explored in occupational settings. There are a few studies reporting allergens on table surfaces from schools [26,31], but sampling methods are different and levels of allergens are presented in different units. This makes it difficult to compare results; however, those studies also identified table surfaces as a potential secondary source for Fel d 1 and Can f 1 exposure.

We showed that it is feasible to detect allergens and endotoxin in settling dust samples collected with the EDC in an occupational environment, whereas active stationary samples were below the LOD. The better performance of the EDC in determining ambient airborne exposure levels can likely be attributed to the longer average time of sampling. The levels observed at samplers exposed to the air for 14 continuous days, 10 days including night time, and 10 work days were similar, which suggests that the contribution of weekends or nights to the exposure might be negligible. Therefore, measurement of allergens or endotoxin based on 14 continuous days (sampling time) might be preferred for practical reasons.

Moderate to good correlations were found between allergen or endotoxin levels in EDC samples and personal samples or floor dust samples, whereas only weak agreements

between personal samples and floor dust samples were found. This is similar to previous results for endotoxin [18], and for allergens considering the association between floor dust and active airborne dust [32,33]. It seems that the dust collected with the EDC in occupational environmental settings might reflect personal exposure, and is a good alternative for ambient stationary sampling in cases where personal sampling cannot be performed.

We found that the canteen may also be a secondary source for Fel d 1 and Can f 1 exposure. It has previously been shown that Fel d 1 and Can f 1 can transfer to other environments due to transportation via clothes [34] and hair [35]. In this case, transfer to the canteen is likely due to transfer on clothing and the fact that pet owners are allowed to bring their pets into a separated area of the canteen. Avoiding this seems to be the best intervention measure to reduce allergen levels. A four- to sixfold reduction in Fel d 1 levels has been reported after clean clothes were worn [34].

Floor and table surface dust sample results have important implications for investigating the effect of cleaning. Lower floor allergen levels were observed in the inpatient ward, because the floor surface in that area of the hospital was cleaned twice daily with water. In contrast, observed higher allergen levels in the intensive care unit and examination room might be due to once-daily water cleaning and dry cleaning in these locations, respectively. Also differences between levels of dust from computer, equipment and animal exams tables could likely be explained by cleaning patterns.

Up to now, threshold limit values for Fel d 1 and Can f 1 have not been established [36]. Nonetheless, it has been suggested that 1 μg g^{-1} of Fel d 1 was associated with sensitisation, and 8 μg g^{-1} was related to asthma [30,37]. Similarly, levels of Can f 1 to induce sensitisation and asthma symptoms were 2 and 10 μg g^{-1}, respectively [30]. In the present study only floor dust samples can be expressed in such measures. The Fel d 1 levels of all floor samples were below 8 μg g^{-1}, and 10 out of 110 samples were higher than 1 μg g^{-1}. For Can f 1, 82 out of 110 samples were higher than 2 μg g^{-1}, and 41 out of 110 samples were higher than 10 μg g^{-1}, indicating probable health risks for the working population.

CONCLUSION

Our data demonstrated that allergen exposure occurs during veterinary practice, while personal endotoxin levels were low. By using a sensitive allergen assay, quantifying allergen levels in airborne, personal and settling dust samples was possible despite the low dust levels. The EDC sampler can be used as a complementary method. The surfaces of floors and tables were found to be potent secondary sources for bio-aerosol exposure. However, further studies should be conducted to evaluate whether observed exposure levels affect the health of employees and students.

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Chapter 4

An Overview of Bio-aerosol Exposure in Poultry and Ruminant Clinics

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ABSTRACT

Objectives: 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. The aim of this study was 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} (115-49846); 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 endotoxin exposure for veterinarians, caretakers and veterinary students to be considerable. Exposure occurred not only in animal houses, but also in practical teaching rooms. β(1→3)-glucan was occasionally high 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 and endotoxin in pig farms is well-known to be associated with respiratory diseases [9-14]. For other animal farm types, less data is 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 bio-aerosol exposure 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 bio-aerosol exposure of veterinary populations within poultry and ruminant settings [5,24], although respiratory symptoms associated with animal husbandry specialization are common among the veterinary medicine students [25]. We thus questioned 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 among caretakers, veterinarians (teachers), and veterinary students in the Department of Farm Animal Health at the Faculty of Veterinary Medicine of Utrecht University, the Netherlands. Two locations within the department were included in the study: the clinic for (small) ruminants and the poultry clinic. 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. Sampling has been performed repeatedly over time and subjects were included randomly. Caretakers at the clinics of Farm Animal Health were 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 only one caretaker; morning shift, n=17 samples for 4 caretakers; afternoon shift, n=10 samples for 2 caretakers). Also 5 samples were collected for only 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 randomly selected *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 study was described and collaboration of participants 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 design of 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. The floor surfaces area of stables differed between 70 m^2 (10×7 m) and 260 m^2 (20×13 m). The number of cows in each stable varied between 16 and 23. All cows were manually fed with silage, hay and compounds.

Similarly, the poultry clinic was a confined and modern building which was fully reconstructed in 2006. This clinic consists of poultry houses and teaching rooms. The whole building is provided with an automatic heating system. The floor surface area of poultry houses varied between 12 m^2 (3×4 m) and 20 m^2 (4×5 m). Each poultry house divided into 4 pens supplied with automatic watering system, mechanical ventilation, and sawdust on the floor. The number of chicken in each pen varied between 1.5 and 5 per m^2. Concerning poultry farm houses outside the university, the size differed between 1600 and 12000 m^2. The floor surfaces of two poultry farm houses was 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 dust 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 equipments as for personal sampling but then mounted at a tripod at 1.5 m above floor level. Numerous field blank samples were taken without drawing air through the filters.

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 during 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 for endotoxin and β(1→3)-glucan was carried out as described elsewhere [28]. 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 diluted 1:500 to 1:1000. The endotoxin levels of personal and stationary samples were expressed as Endotoxin Units (EU) per cubic meter (m^3) of air, and for EDC samples as Endotoxin Units per squared meter 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.

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 were considered as dependent variables, and time spent on the tasks performed (continuous variables) were 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 <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 (ruminant clinic, 96 samples; poultry clinic, 114 samples) after excluding 10 samples (3.7%) either due to failure of pumps 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 and 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 and poultry farm visits (overall GM: dust, 1.32 mg m^{-3}; endotoxin, 1498 EU m^{-3}; 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}; 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}; 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 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 ($p>0.05$). 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 together with during poultry farm visits 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 of limit of 90 EU m^{-3} proposed by the Health Council of the Netherlands [31]. 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 [32]. 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 measureable 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

Table 1. Personal exposure levels of dust, endotoxin, and β(1→3)-glucan according to work sites and jobs.

		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	115-49846	79	20	3.89	2.29	2.7	<LOD-46.14
Uniform (farm)	5	17	–	6.16	4.89	2.0	1.52-20.90	17	–	5886	4376	2.1	1524-25139	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	115-49846	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	–	16927	–	–	–	1	–	21.7	–	–	
Total	3	11	3	2.05	1.01	3.0	<LOD-12.39	11	–	2813	1221	3.6	237-16927	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	454-10820	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	115-49846	112	21	6.08	3.10	3.2	<LOD-46.14

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; <LOD, below the lower limit of detection

Table 2. Stationary exposure levels of dust, endotoxin, and β(1→3)-glucan according to work sites.

	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	188-10655	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	140-10655	15	2	4.30	1.46	5.7	<LOD-19.86

N, number of samples; ND, number of samples <LOD; AM, arithmetic mean; GM, geometric mean; GSD, geometric standard deviation; <LOD, below the lower limit of detection

Table 3. Settling dust exposure levels of endotoxin, and β(1→3)-glucan according to work sites.

		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 - 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 - 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 - 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 - 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 - 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 - 1.20×10^7	–	2202	1224	4.7	133-4889

N, number of samples; ND, number of samples <LOD; AM, arithmetic mean; GM, geometric mean; GSD, geometric standard deviation;

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

			Dust (mg m^{-3})					Endotoxin (EU m^{-3})				
	Determinants	Median (time-min)	*β*	SE	P value	Adj R-Sq	FEC (95% CI)	*B*	SE	P value	Adj R-Sq	FEC (95% CI)
Ruminant clinic and farm visits												
Preclinical students	Intercept	–	-2.663	–	<0.0001	0.36	–	3.902	–	<0.0001	0.13	–
	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 students	Intercept		-1.418	–	<0.0001	0.48	–	3.925	–	<0.0001	0.57	–
	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)
	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)
	Anastheasiatic injection	150	–	–	–		–	0.012	0.003	0.002		5.88 (2.13-16.3)
Differentiated students	Intercept	–	-2.831	–	0.0006	0.46	–	4.004	–	<0.0001	0.25	–
	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	–
	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)
	Cleaning with water	45	–	–	–		–	–	–	–		–
Poultry clinic and farm visits												
Students	Intercept	–	-3.531	–	<0.0001	0.50	–	3.915	–	<0.0001	0.43	–
	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)
	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)
	Checking of neck & beak	10	0.295	0.052	<0.0001		19.06 (6.94-52)	0.301	0.051	<0.0001		20.25 (7.44-55)
	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)
	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)
	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)
	Evaluating feathers	10	–	–	–		–	–	–	–		–

Table 4. Continued.

			β(1→3)-glucan				
	Determinants	Median (time-min)	*β*	SE	P value	Adj R-Sq	FEC (95% CI)
Ruminant clinic and farm visits							
Preclinical students	Intercept	–	–	–	–	–	–
	Physical examination	125	–	–	–		–
	Listening lectures	35	–	–	–		–
Uniform students	Intercept		-0.704	–	0.2	0.28	–
	Physical examination	97.5	0.031	0.010	0.005		21 (3.04-139)
	Listening lecture	30	0.195	0.061	0.005		0.00 (0.00-0.10)
	Anastheasiatic injection	150	0.011	0.004	0.02		5.21 (1.61-17)
Differentiated students	Intercept	–	–	–	–	–	–
	Stall round in clinic	95	–	–	–		–
	Visiting animals	47.5	–	–	–		–
Caretakers	Intercept	–	2.325	–	<0.0001	0.41	–
	Feeding	45	–	–	–		–
	Bedding	85	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	–	-4.945	–	<0.0001	0.69	–
	Restraining chickens	20	0.176	0.023	<0.0001		34 (14-83)
	Checking of nutrition	10	-0.25	0.026	<0.0001		0.08 (0.14-0.05)
	Checking of neck & beak	10	0.415	0.042	<0.0001		63 (28-144)
	Listening lecture	15	–	–	–		–
	Consulting with owners	15	-0.245	0.033	<0.0001		0.03 (0.01-0.07)
	Taking blood	60	–	–	–		–
	Collecting feces	30	0.092	0.008	<0.0001		15.8 (9.87-25)
	Physical examination	45	0.107	0.01	<0.0001		123 (51-298)
	Evaluating feathers	10	0.101	0.016	<0.0001		2.75 (2.01-3.76)

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

the EDC samples at the clinic of ruminants correlated very well with the endotoxin levels of personal or stationary samples (Figure 1).

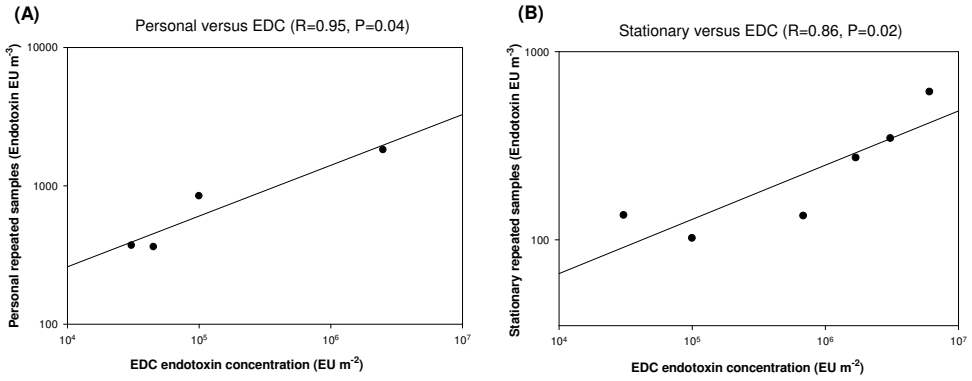

Figure 1. Correlations between various sampling methods: personal versus EDC (A), stationary versus EDC (B).

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. 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 were detectable (83%) with occasionally high levels.

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) [33], 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} [33-35]. Dust levels for caretakers at the poultry clinic (GM 5.72 mg m^{-3}, range 1.62-14.66) were similar with those previously found in the Dutch broiler poultry farm (GM, 4.2 mg m^{-3}, range 4-4.4) [33], but somewhat lower than those reported in the UK broiler poultry farm (GM 10.58 mg m^{-3}, range 8.38-13.34) [35] and the Dutch layer poultry farm (GM 9.5 mg m^{-3}, range 6.6-14) [33]. 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 62-22330) [33], Wisconsin dairy barns (GM 647 EU m^{-3}, 25-348000) [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}) [33]. 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 (such as climate change, meteorology). 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 [33]. Endotoxin levels can be highly influenced by other determinants such as characteristics of ventilation, seasonal variation, and temperature [36-38]. 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}) [33], markedly lower than those reported in the UK broiler poultry farm (GM 8341 ng m^{-3}) [35], and higher than those findings in Dutch broiler poultry farm (GM 880 EU m^{-3}) [33]. Comparison with these studies need 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 [24,39]. 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 reflects 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 were 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 [6,40]. 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 [31] 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 using 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 measurements might underestimate or overestimate of personal exposure.

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 material 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 [3,41]. 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.

CONCLUSIONS

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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Chapter 5

The Influence of Bedding Materials on Bio-aerosol Exposure in Dairy Barns

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ABSTRACT

Background: 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.

Objective: The aim of this study was to explore bio-aerosol exposure levels and its determinants in dairy barns with various bedding materials.

Methods: Dust samples were collected at dairy barns employing various bedding materials. Samples were analyzed for endotoxin and β(1→3)-glucan contents. Culturable bacteria and fungi were sampled by the Anderson N6 impactor. Exposure models were constructed using linear mixed models.

Results: The personal exposure levels to dust, endotoxin, and β(1→3)-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}; β(1→3)-glucan, 7.84 μg m^{-3}) in barns with compost bedding versus the lowest in barns with sawdust bedding (GM: dust, 0.51 mg m^{-3}; endotoxin, 183 EU m^{-3}; β(1→3)-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 β(1→3)-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, while determinants could explain to lesser extent the within-worker variances.

Conclusion: Exposure levels to endotoxin, β(1→3)-glucan, bacteria, and fungi in dairy barns were substantial and differed dependent on bedding materials, suggesting bedding material types as a significant predictor of bio-aerosol 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 β(1→3)-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, *e.g.* 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, β(1→3)-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 5 dairy barns per bedding type was included. Dairy barns applying the following bedding materials were selected: deep litter 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 β(1→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, which were mostly the owners. All workers were included in personal dust sampling and minimally 3 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 the front of the building as main entrance and the other at the end of the building for removing manure during cleaning and taking cows in and out. Both doors were generally open during dust sampling. The animal

buildings were naturally ventilated through an open ridge, openings in the sidewalls and the doors. Tractors were applied to distribute silage for feeding. The number of cows accommodated in each barn ranged from 55 to 185, with a surface area of 3 to 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 2 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 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, Inc., Maidstone, England). A calibrated rotameter was used to adjust the flow rate at 3.5 l min^{-1}. 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 6:00 a.m. when morning activities were started till 2:00 pm. 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 -20ºC until post-weighing and extraction. The levels of dust on filters were calculated gravimetrically using an analytical balance (AX105, Mettler Toledo Inc., Columbus, OH, USA). Filters were acclimatized prior to 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 β(1→3)-glucan detection

Following post-weighing of filters, samples were extracted for endotoxin and β(1→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, Inc, standard *E coli,* lot no. 145394) with a concentration range of 0.01-25 EU ml^{-1}. β(1→3)-glucan was assayed with a specific inhibition enzyme immunoassay (EIA) as described by Douwes et *al.* (1996) [14] but modified by increasing the sample volume and decreasing the antibody amount for improved sensitivity. The β(1→3)-glucan was quantified applying 4-times serial sample dilution (1:2, 1:6, 1:18, 1:54) using a 8-point standard curve with concentration ranging from 9.8 to 1250 µg ml^{-1}. The levels for endotoxin and β(1→3)-glucan were expressed as Endotoxin Units per cubic meter (EU m^{-3}) and µg m^{-3}, respectively. The average lower limit of detection 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 β(1→3)-glucan assay was 0.83 µg per filter or 0.74 µg m^{-3}. 52 out of 191 samples (27.2%) remained undetectable, a concentrations of two third 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^{-1}, and sampling duration was 30 seconds. All samples were collected in duplicate per type of agar in the morning between 8:00 to 10:00 a.m. 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 hours at 37ºC and fungal samples for 3-7 days at 24ºC. Formed colonies on each plate were counted twice and corrected for counts on blanks and then corrected using the positive hole correction factor [15]. The number of bacteria and fungi were expressed as Colony-Forming Unites (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 like main bedding materials, extra bedding materials, and other stable characteristics *e.g*. 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 β(1→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 β(1→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 μg m^{-3} (GM 2.44) for β(1→3)-glucan. The exposure levels of dust, endotoxin, and β(1→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 β(1→3)-glucan (GM 7.84 μg m^{-3}) were found in barns with compost bedding, while 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 β(1→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 case 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 β(1→3)-glucan (48 μg m^{-3}). In barns with only compost utilized as bedding, exposure levels to dust and endotoxin were roughly 2 times higher when compared with the levels collected from barns with adding of either straw or sawdust on compost bedding, while the levels of β(1→3)-glucan were more or less similar for these bedding materials. In barns where chopped straw is employed on the sawdust bedding, the exposure levels of dust and endotoxin were roughly 3 times as high exposure 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 5 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

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

		Dust (mg m^{-3})					Endotoxin (EU m^{-3})					β(1→3)-glucan (µg m^{-3})					
Bedding types	N	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

N, number of measurements; ND, number of measurements <LOD; AM, arithmetic mean; GM, Geometric mean; GSD, geometric standard deviation; range, min-max; <LOD, below the lower limit of detection.

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

		Dust (mg m^{-3})					Endotoxin (EU m^{-3})					β(1→3)-glucan (µg m^{-3})					
Bedding types	n	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

N, number of measurement; ND, number of measurements <LOD; AM, arithmetic mean; GM, Geometric mean; GSD, geometric standard deviation; range, min-max; <LOD, below the lower limit of detection.

Table 3. Culturable bacteria and fungi levels stratified by type of bedding materials.

		Bacteria samples (TSA) (CFU m^{-3})				Fungi samples (DG18) (CFU m^{-3})			
	N	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

n, number of measurement; AM, arithmetic mean; GM, Geometric mean; GSD, geometric standard deviation; range, min-max.

CFU m^{-3}) in barns with compost bedding, roughly 6 times as high exposure 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 exposure as in barns using sawdust and rubber mats, respectively.

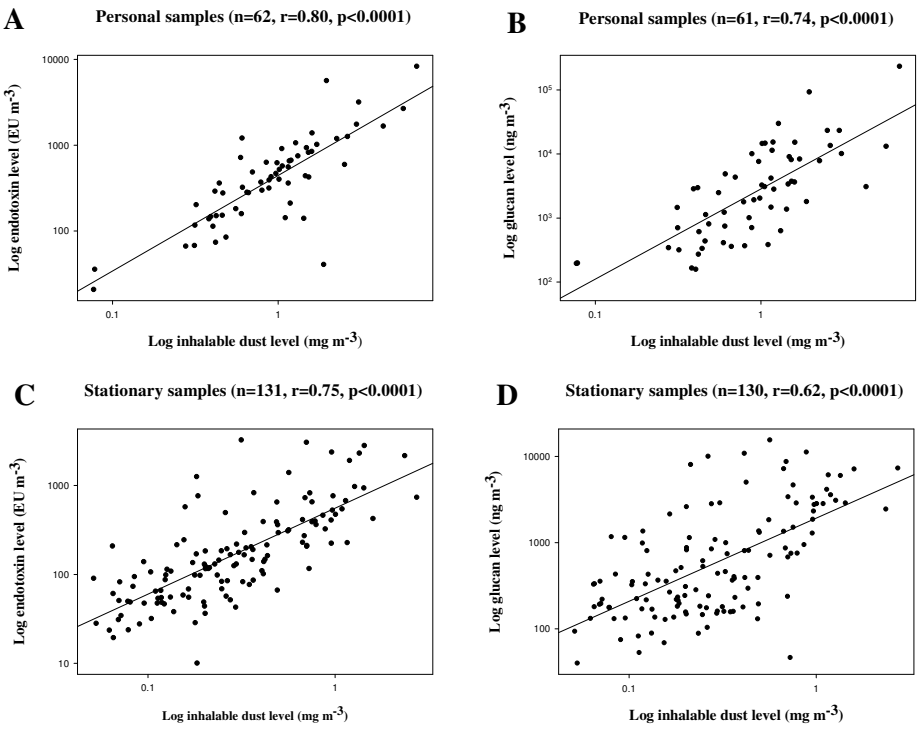

Figure 1. Scatterplot of inhalable dust levels with endotoxin and β(1→3)-glucan levels. Dust vs. endotoxin, Fig. A and C; dust vs. β(1→3)-glucan, Fig. B and D.

Correlations between exposure estimates

Personal inhalable dust levels correlated strongly with endotoxin and β(1→3)-glucan levels (dust vs. endotoxin, $r=0.80$, $p<0.0001$; dust vs. β(1→3)-glucan $r=0.75$, $p<0.0001$; Fig. 1, A and B). The same was found for stationary samples (dust vs. endotoxin, $r=0.75$, $p<0.0001$; dust vs. β(1→3)-glucan $r=0.62$, $p<0.0001$, Fig. 1, C and D). Levels of culturable bacteria were also significantly correlated with endotoxin and dust, but the correlation coefficients were weaker: personal (bacteria vs. endotoxin, $r=0.44$, $p<0.01$; bacteria vs.

dust, r=0.35, p<0.05; Fig. 2, A and B), stationary (bacteria vs. endotoxin, r=0.42, p=0.0002; bacteria vs. dust, r=0.44, p<0.0001; Fig. 2, C and D). Similarly, significant correlations were seen between culturable fungi with β(1→3)-glucan and dust (fungi vs. β(1→3)-glucan r=0.34, p=0.003; fungi vs. dust, r=0.38, p=0.0008; Fig. 2, E and F) for stationary samples, but not for personal samples (p>0.05).

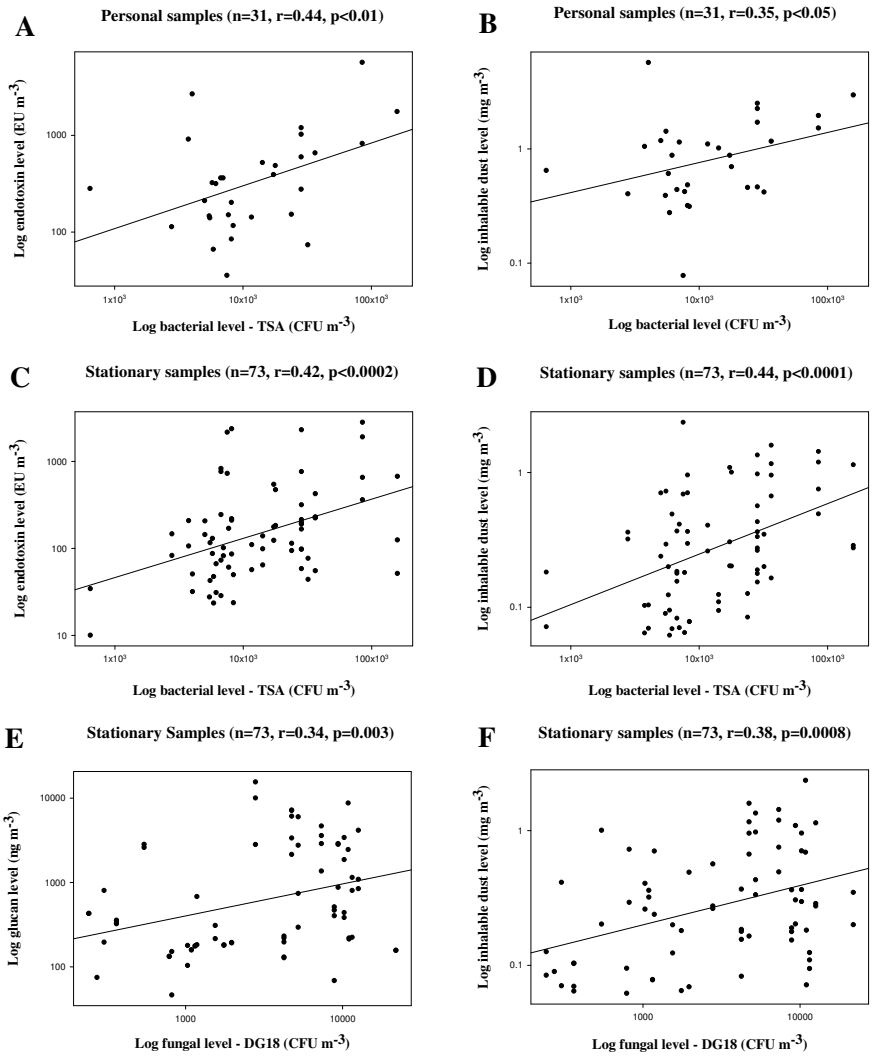

Figure 2. Scatterplot between bacterial or fungal exposure levels with endotoxin, glucan, and dust levels. Bacteria vs. endotoxin, Fig. A and C; bacteria vs. dust, Fig. B and D; fungi vs. β(1→3)-glucan, Fig. E; fungi vs. dust, Fig. F.

Determinants of exposure levels

Several dairy barn characteristics were found to determine dust, endotoxin and β(1→3)-glucan 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 to the other types of bedding. Milking by robot showed higher dust and β(1→3)-glucan exposure than milking in a traditional parlor.

Between- and within-workers variability of exposure were bigger for β(1→3)-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, 58% (from 0.71 to 0.30) for endotoxin, and 70% (from 1.45 to 0.44) for β(1→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 β(1→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 β(1→3)-glucan.

Table 4. Parameter estimates of the final mixed effects multivariate model of the log-transformed 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.

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, β(1→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.

Table 5. Variance components and confidence intervals (95% CI) of the log-transformed 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] $=[(S^2_{bw}$ empty model$-S^2_{bw}$ full model$)/S^2_{bw}$ empty model$]*100$

[D] $=[(S^2_{ww}$ empty model$-S^2_{ww}$ full model$)/S^2_{ww}$ empty model$]*100$

The overall GM of personal endotoxin concentrations (392 EU m^{-3}) measured in this study was slightly lower than those levels reported in Dutch dairy farms (560 EU m^{-3}) [7] and Wisconsin dairy barns (647 EU m^{-3}) [2]. The personal GM Levels of β(1→3)-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 (Chapter 4). Levels were also substantially lower than those levels reported in grain farming (120 µg m^{-3}) [17], but higher than the levels obtained from household green waste-composting plants (1.22 µg m^{-3}) [18], 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 due to the absence of similar data. 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}, endotoxin 1006 EU m^{-3}) than dust particles collected from barns with only using sawdust (dust 0.40 mg m^{-3}, endotoxin 137 EU m^{-3}; dust 0.99 mg m^{-3}, endotoxin 366 EU m^{-3}) or straw (dust 0.53 mg m^{-3}, 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. Further this finding is supported by Wouters *et al.* [18] indicating compost as a potential source for endotoxin. 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 [19]. Samples collected from barns with a mixture of chopped straw and sawdust yielded 3 times higher levels of endotoxin (574 EU m^{-3}) compared to those barns utilized only sawdust bedding (137 EU m^{-3}). This finding is consistent with a previous study reporting higher bacteria counts in straw than in sawdust [20]. Chopped straw together with chalk employed 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 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 [21], it has been reported that the load of bacteria in bedding materials treated with chalk were elevated compared to untreated bedding materials [21,22]. This is likely related to pH changes of bedding

materials that steeply reduced from alkaline towards acidity after the first 2 days of adding chalk to bedding [21,22].

Similar to endotoxin, we found significantly higher levels of β(1→3)-glucan in barns with compost bedding compared to other bedding materials. No data are available for comparison, but results from recent studies by Cyprowski *et al*. and Wouters *et al*. exploring β(1→3)-glucan exposure in compost plants suggested compost as an potential source for β(1→3)-glucan exposure [18,23]. Chopped straw together with chalk applied on compost bedding was associated with substantial higher levels of β(1→3)-glucan (2 times higher) when compared with only compost bedding. The same trend but with a much larger increase (18 times) of β(1→3)-glucan was observed in barns utilizing straw together with sawdust compared to barns with only sawdust bedding. This observation is plausible since plant materials utilized for bedding are likely contribute as a source for β(1→3)-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,[2,9,24] 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 [25], 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 due 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 due 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 [25,26].

The culturabe fungal levels (GM 2.37 × 10^3 CFU m^{-3}) in this study were comparable to 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}) [25] and lower than levels in Danish pig farms (GM 3.8 × 10^5 CFU m^{-3}) [1]. The absence of a significant correlation between β(1→3)-glucan and culturable fungal levels for personal samples was in agreement with earlier findings reported by Halstensen *et al*. [17]. In contrast to personal samples, we found a significant correlation for stationary samples which was in accordance with those reported by Adhikari *et al*. in greenhouses [26]. β(1→3)-glucan is a component of fungal cell walls and it has often been considered as an indicator for fungal exposure [27], but plant materials utilized for bedding are possibly additional

sources for β(1→3)-glucan exposure besides fungi [28]. This result was supported by higher β(1→3)-glucan levels in barns utilized 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 to be influenced by other potential determinants that we did not include such as climate conditions on the measuring days, type of ventilation and ventilation rate, methods and intervals on cleaning, and sampling season as suggested elsewhere [3,6,29].

The variability of exposure levels between workers and within workers over time were high in our study, consistent with findings from previous studies [1,4,7,18]. Final exposure models in this study explained a significant proportion of between-worker variability for inhalable dust, endotoxin, and β(1→3)-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 spent 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 due 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 which favor bacterial growth such as humidity and temperature [25]. The application of extra bedding material explained between-worker variability for β(1→3)-glucan very well (61%) much more than for dust (19%) or endotoxin (35%). This may be explained in part by the fact bedding materials with plant origin might serve as a main source for β(1→3)-glucan. This was supported by the low levels of β(1→3)-glucan in sawdust bedding compared to 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 versus 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.

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Chapter 6

Allergy among Veterinary Medicine Students in the Netherlands

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ABSTRACT

Background: Veterinary medicine students who practice with animals are potentially exposed to many occupational agents, yet sensitisation and allergic symptoms among this group have not been studied extensively.

Objective: The objective of this study was to estimate the prevalence of sensitisation and allergic symptoms in veterinary medicine students in association with study specialisation over time.

Methods: A questionnaire-based cross-sectional study was conducted. Blood was collected and tested for total and specific serum IgE for 16 different common and study-specific allergens using enzyme immunoassay.

Results: New development of self-reported allergic symptoms to various allergens occurred in 8.7%, of which 44% was deducted against animals. Handling farm animals was strongly associated with self-reported allergies to various allergens (OR=6.9, 95% CI 1.9 to 25) and animal allergens (OR=12, 95% CI 1.4 to 103). Sensitisation to at least one allergen occurred in 33.1%. Sensitisation prevalence tended to be elevated in later years of the equine study program. In contrast to self-reported allergies, the prevalence of sensitisation to any allergen decreased with prolonged study duration for those specialising in farm animal health (years 3–5: OR=0.5, 95% CI 0.3 to 1.1; year 6: OR=0.2, 95% CI 0.1 to 0.5). This was independent of whether people were raised on a farm, which is in itself a protective factor for allergy and sensitisation.

Conclusion: This study provides evidence of an elevated prevalence of allergic symptoms with increasing years of veterinary study, suggesting that contact with animals, more specifically contact to farm animals, is a risk factor for the development of symptoms.

INTRODUCTION

Veterinarians and coworkers are potentially exposed to various occupational hazardous agents such as allergens shed by animals and plants [1], microbial agents (*e.g*. endotoxins) [1-3], and chemical agents (*e.g*. disinfectants) [4]. Exposure to animal-derived allergens are well known to induce immediate (IgE-mediated) sensitisation [5] and development of respiratory, eye and skin symptoms, as well as allergic asthma [6-9]. There are only a few published studies describing allergic symptoms among veterinary populations [6,7,9-14]. These studies suggest that veterinary populations are at risk of developing allergic sensitisation, allergic rhinitis, conjunctivitis, asthma and dermatitis, with prevalences ranging between 40% and 69% for respiratory symptom [7,10,15] and 11% and 46% for dermal symptoms [12-14].

Studies among laboratory animal workers exposed to rodents show that allergies can develop within months after first exposure [16,17]. This prompted us to hypothesise that veterinary medicine students, who come into contact with animals during their education,

might be at risk of developing allergy. We thus conducted a cross-sectional investigation on the prevalence and self-reported incidence of allergic symptoms in all veterinary medicine students studying in the year 2006. In addition, sensitisation to various allergens was investigated. Since students follow specialisation-specific study paths (individually kept animals, equine, companion animals and farm animals), this information could be used as a surrogate of exposure to specific agents. The main objective of this study was to explore the occurrence of self-reported allergic symptoms and sensitisation among veterinary medicine students in association with study specialisation and study duration.

METHODS

Study design and population

For this cross-sectional investigation, all 1416 students who were registered as a veterinary medicine student in 2006 at the Utrecht University in the Netherlands were invited to participate and asked to complete an internet-based self-administrated questionnaire. Additionally, they were invited to provide a 20 ml blood sample for serological IgE testing. Up to two reminders were sent to non-responders.

Questionnaire

The questionnaire included questions on demographic characteristics, history of and current contact with animals outside the study program, growing up on a farm (farm childhood) and smoking status. Information on previous and current contact with animals besides animal contacts during the study was ascertained for horse, farm animals (cow, sheep, pig, goat and poultry) and some of the more important pet species (cat, dog and rodent). A farm childhood was derived from the question 'Did you live on a farm during childhood?' Smoking status was divided into current smokers, non-smokers and former smokers.

The health assessment part of the questionnaire addressed questions on airway and allergic symptoms. Questions on airway symptoms were adopted from the Dutch version of the European Community Respiratory Health Survey questionnaire [18], questions on allergic symptoms were ascertained as previously [19], with the main question on allergy: 'Are you sensitive or allergic to one or more agents?' If the answer was 'yes', then the participants were asked to report whether they have nose, eye, respiratory or skin symptoms to a possible list of allergens, including animals, house dust or grass pollen. Information on the time course of the allergy being before or after starting the veterinary medicine study was ascertained. The self-reported allergy was considered as new onset when the initiation of the allergy was reported to occur after the veterinary medicine study was started. Self-reported bronchial hyperresponsiveness was defined as experiencing difficulties in breathing

in one of the following situations: fog, baking and frying, change in temperature, freezing cold or smoke [19].

As a potential risk factor, we studied the diversity and duration of specialisation within the veterinary medicine curriculum. The veterinary curriculum was divided into two main categories: individually kept animals and farm animal health. After the first and second year in these study directions, the curriculum was subdivided into specialisation as companion animals or equine for the individually kept animal direction and as animal husbandry together with veterinary public health or farm animals for the farm animal health direction. During the third to fifth year within the specialisation, the students have most theoretical and some practical courses. In the sixth year of the specialisation, the students follow internships for their specific study direction. The type of animals being encountered mostly during the curriculum differed for the different specialisations, being mainly cats and dogs for companion animal specialisation, horses for equine specialisation and farm animals such as cows, sheep, goats, pigs and poultry for farm animal health.

Total and specific serum IgE

Sera were stored at −20°C until IgE analysis was performed. Total serum IgE antibody was quantified using a sandwich enzyme immunoassay as described previously [20]. The results were expressed in kilo units per litre. Enhanced total IgE was defined as ≥100 kU/l. Specific serum IgE antibodies for 16 different allergens—including common allergens (house dust mites, grass mixture (1:1 mixture of *Lolium perenne* and *Phleum pratense*), birch pollen (*Betula verrucosa*), cat fur and dog fur), laboratory or domestic animal allergens (rat urine, mouse urine, budgerigar feather, guinea pig hair and skin scrape), horse allergen (horse hair and dander), farm occupational allergens (cow hair and dander, goat hair and skin scrape, pig feces, pig skin scrape and chicken feces)—were measured by means of enzyme immunoassay as described elsewhere [20]. This method previously showed to be very well correlated to skin prick test positivity, as well as to Phadiazym RAST [20]. Serum was tested in a 1:5 dilution. Sera with an optical density (OD) of 0.05 and above, following correction for the OD of serum blank and OD of reagent blank, were considered to contain specific IgE for the tested allergen. Participants were considered to be sensitised to an allergen group, for example, common allergens, domestic allergens and farm animal allergens, if they tested positive for at least one of the specific allergens within the group.

Statistical analysis

Total IgE levels were best described by a log normal distribution; subsequently, geometric means and geometric SDs were calculated. Multiple logistic regression analysis was performed to associate the prevalence of self-reported respiratory and skin symptoms, self-

reported allergy and sensitisation with different exposure groups based on study specialisations over time. The first 2 years of the specific study specialisations are the reference. ORs and 95% CIs were calculated and adjusted for potentially confounding variables including gender, smoking status and farm childhood. All statistical analyses were performed with SAS V.9.1 (Statistical Analysis Software; SAS Institute Inc).

RESULTS

Of the 1416 veterinary medicine students approached, 968 (68.4%) responded to the questionnaire, of which 673 (70.3%) provided a blood sample as well. Seven participants were excluded for further analyses: three because they did not complete the whole questionnaire and four because of a too small number in their specific specialisation. Numbers and percentage of participants per study specialisation and phase are depicted in Figure 1.

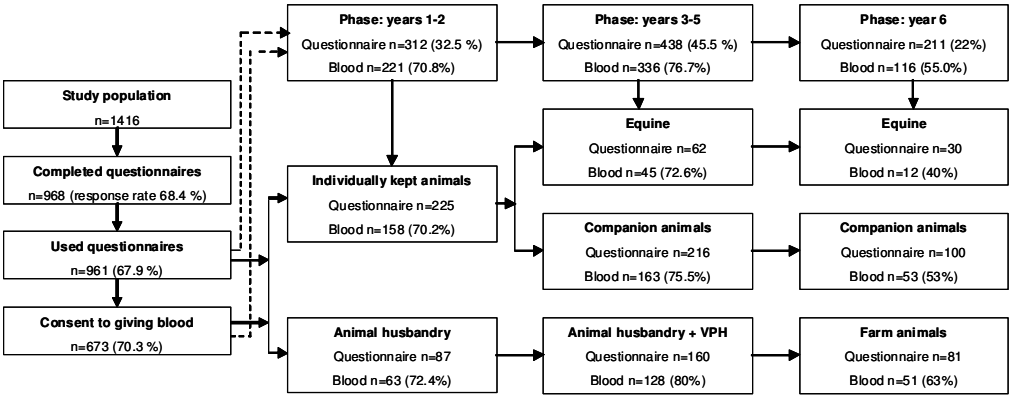

Figure 1. Flow diagram shows recruitment of the study population, number and relative proportion of participants in the different specialisations. VPH, veterinary public health.

Table 1 presents the demographic characteristics of the study population. The population was predominately women (80.3%). Only 5.5% was older than 30 years (ranged from 18 to 47 years). Current smokers were more likely to be men (16.4%) than women (9.2%). Most subjects had previous or current contact with animals outside the study program. About 25.8% of the study population reported a history of allergy prior to commencement of veterinary medicine study. This was independent of study specialisation or gender (26.2% women and 24.7% men). Participants with a history of allergy were more likely to provide a blood sample (27.8% vs 21.2%, p=0.03). The geometric means total IgE was 20.1 kU/l (geometric SDs 9.5), with 24.2% of sera being above 100 kU/l.

The association between self-reported health symptoms and categories of study specialisation is presented in Table 2. The most commonly reported symptom was rhinitis, with an overall prevalence of 58.8%. The prevalence of various symptoms were more likely to report in the higher years of study, particularly during farm animal specialisation, compared with the reference groups, except rhinitis where companion animal students reported less symptom of rhinitis with increasing years of study (years 3–5, OR=0.7 (95% CI 0.5 to 1.0); year 6, OR=0.5 (95% CI 0.3 to 0.8)) compared with the reference group. Dermatitis was reported more often during practice with animal husbandry in years 3–5 (OR=4.2 (95% CI 1.5 to 14)) and farm animals in year 6 (OR=2.4 (95% CI 1.7 to 8.5)) than the reference group.

Table 1. Descriptive characteristics of study participants

	Total	Giving blood	Non-giving blood
Population characteristics	961	673 (70%)	288 (30%)
Female, n (%)	772 (80.3)	539 (80.1)	233 (80.9)
Weigh (kg), AM (SD)	68.5 (11.2)	68.3 (10.7)	69.1 (12.2)
Height (cm), AM (SD)	175 (8.3)	174 (8.2)	175 (8.5)
Age, AM (SD)	23.7 (3.7)	23.7 (3.6)	23.8 (3.8)
Smoking status			
Current smoker, n (%)	102 (10.6)	69 (10.3)	33 (11.5)
Former-smoker, n (%)	85 (8.8)	60 (8.9)	25 (8.7)
Non-smoker, n (%)	774 (80.5)	544 (80.8)	230 (79.9)
History of allergy			
Yes, n (%)	248 (25.8)	187 (27.8)	61 (21.2)
Farm childhood			
Yes, n (%)	110 (11.5)	25 (8.7)	85 (12.6)
Previous or current contact with animals outside the study			
Horse, n (%)	648 (67.2)	451 (67.0)	195 (67.7)
Farm animals, n (%)	491 (51.1)	348 (51.7)	143 (50.0)
Pets, n (%)	938 (97.6)	659 (98.0)	279 (97.0)

n, number; AM, arithmetic mean; SD, standard deviation.

Self-reported new-onset allergies (developed during the veterinary education) occurred in 8.7% of the population (men 9.0% vs women 8.7%,Table 3), with a tendency of higher prevalence with increasing years of study. Of those reporting allergic symptoms to various allergens, 44% reported animal allergy, with sneezing or runny nose as the most common symptoms (59.4%). About 10.8% of those with animal allergy were also allergic to grass and house dust, while 51.4% exclusively reported animal allergy. Working with farm animals appeared to be the strongest risk factor associated with self-reported symptoms of allergy to various allergens (OR=6.9, 95% CI 1.9 to 25, $p<0.05$) and to animal allergens (OR=12,

95% CI 1.4 to 103, p<0.05). Students in the final year of the companion animal or equine specialisation also tended to report more often the development of allergies.

Based on the serological evaluation, 33.1% of participants appeared to be sensitised to at least one specific allergen (Table 4). Highest sensitisation prevalence was found for grass pollen (16.2%) and house dust mite (12.9%). Sensitisation to animal allergens was less common with the highest prevalence for cow allergen (3.7%). Only 1.3% of all tested students were sensitised to dog, and none were sensitised to chicken.

Presence of positive specific IgE to any allergen was diminished for participants in years 3–5 (overall: OR=0.5, 95% CI 0.3 to 1.1, p<0.05) and in year 6 (overall: OR=0.2, 95% CI 0.1 to 0.5, p<0.05) of the farm animal specialisation compared with the reference group (Table 4). This is in contrast to the self-perceived (allergic) symptoms (Table 3). In the equine specialisation group, participants in the sixth year were more likely to be sensitised to horse allergen, albeit not being statistically significant (p>0.05).

Growing up on a farm is in itself protective against sensitisation to allergens (OR=0.6, 95% CI 0.3 to 1.0). Nonetheless, adjusting for farm childhood did not change the association between allergic symptoms or sensitisation with study specialisations and over time.

A substantial number of participants with new onset of self-reported allergic symptoms did not have specific IgE. Of those reporting allergic symptoms to various allergens or to specific animal allergens, 47% and 77%, respectively, had no specific IgE, suggesting an over-representation of self-perceived symptoms. Conversely, 86.5% of sensitised individuals had no new self-reported symptoms of allergy. This number is an overestimation though, as those who reported symptoms prior to commencement of veterinary medicine study were excluded in the analyses. When taking into account self-reported symptoms before the study, the number of participants with sensitisation who did not report symptoms decreased to 38.3%.

Being sensitised to farm animals, horse, cat or dog allergens was in itself strongly and positively associated with the prevalence of symptoms, including wheezing (in the last year or without a cold), wheezing with shortness of breath, asthma attack (ever, last year or diagnosed by doctor) and itchy and red skin (range in ORs 2.3–15, p<0.05).

Table 2. Prevalence and adjusted ORs (95% CIs) for symptoms per study specialisation.

		Individually kept animals								
		Individually kept animals*	Equine				Companion animals			
	Total (n=961)	Years 1-2 (n=225)	Years 3-5 (n=62)		Year 6 (n=30)		Years 3-5 (n=216)		Year 6 (n=100)	
	n (%)	n (%)	n (%)	OR (95% CI)	n (%)	OR (95% CI)	n (%)	OR (95% CI)	n (%)	OR (95% CI)
Chronic cough (3 months in last year)	78 (8.1)	16 (7.1)	4 (6.5)	0.9 (0.3-2.6)	3 (10.0)	1.2 (0.3-4.5)	23 (10.7)	1.5 (0.8-2.9)	6 (6.0)	0.8 (0.3-2.0)
Chronic phlegm (3 months in last year)	55 (5.7)	7 (3.1)	3 (4.8)	1.6 (0.4-6.4)	2 (6.7)	1.5 (0.3-8.1)	22 (10.2)	3.3 (1.4-7.9)	3 (3.0)	0.9 (0.2-3.5)
SOB	54 (5.6)	10 (4.4)	6 (9.7)	2.3 (0.8-6.6)	1 (3.3)	0.7 (0.1-6.0)	14 (6.5)	1.5 (0.6-3.4)	6 (6.0)	1.4 (0.5-3.9)
Wheezing (last year)	108 (11.2)	24 (10.7)	7 (11.3)	1.0 (0.4-2.4)	4 (13.3)	1.1 (0.3-3.3)	32 (14.8)	1.4 (0.8-2.4)	16 (16.0)	1.5 (0.8-3.3)
Wheezing (without a cold)	61 (6.4)	11 (4.9)	4 (6.4)	1.2 (0.4-3.9)	1 (3.3)	0.7 (0.1-5.4)	18 (8.3)	1.6 (0.7-3.6)	11 (11.0)	2.3 (0.9-5.7)
Wheezing with SOB	73 (7.6)	12 (5.3)	4 (6.4)	1.2 (0.3-4.3)	3 (10.0)	2.0 (0.6-7.4)	22 (10.2)	2.0 (1.0-4.2)	10 (10.0)	2.0 (0.9-4.7)
Breathlessness with wheezing	26 (2.7)	4 (1.8)	3 (4.8)	1.8 (0.7-5.2)	NA	–	6 (2.8)	2.1 (1.0-4.3)	3 (3.0)	1.6 (0.6-4.0)
Chest tightness	81 (8.4)	23 (10.2)	5 (8.1)	0.7 (0.3-2.1)	4 (13.3)	1.4 (0.4-4.2)	20 (9.3)	0.9 (0.5-1.8)	6 (6.0)	0.6 (0.2-1.5)
Asthma										
Asthma attack (ever)	77 (8.0)	21 (9.3)	7 (11.3)	1.2 (0.5-3.0)	2 (6.7)	0.7 (0.2-3.1)	14 (6.5)	0.7 (0.3-1.4)	9 (9.0)	1.0 (0.4-2.3)
Asthma attack (last year)	27 (2.8)	8 (3.6)	3 (4.8)	1.4 (0.3-5.2)	2 (6.7)	1.9 (0.4-9.6)	4 (1.9)	0.5 (0.2-1.8)	4 (4.0)	1.2 (0.3-3.8)
Asthma diagnosed by doctor	70 (7.3)	19 (8.4)	7 (11.3)	1.4 (0.6-3.5)	1 (3.3)	0.4 (0.0-2.9)	14 (6.5)	0.8 (0.4-1.5)	8 (8.0)	0.9 (0.4-2.2)
Asthma-like symptoms	153 (15.9)	37 (16.4)	9 (14.5)	0.8 (0.3-1.8)	7 (23.3)	1.4 (0.5-3.8)	43 (19.9)	1.2 (0.7-2.0)	18 (18.0)	1.1 (0.6-2.0)
BHR	305 (31.8)	75 (33.3)	14 (22.6)	0.6 (0.3-1.1)	11 (36.7)	1.2 (0.6-2.8)	78 (36.1)	1.2 (0.8-1.7)	29 (29.0)	0.9 (0.5-1.4)
Rhinitis	565 (58.8)	150 (66.7)	36 (58.1)	0.6 (0.4-1.2)	19 (63.3)	0.9 (0.4-2.1)	125 (57.8)	0.7 (0.5-1.0)	50 (50.0)	0.5 (0.3-0.8)
Conjunctivitis	199 (20.7)	47 (20.9)	18 (29.0)	1.6 (0.8-3.0)	8 (26.7)	1.4 (0.6-3.2)	39 (18.1)	0.9 (0.5-1.3)	31 (31.0)	1.7 (1.0-2.9)
Dermatitis	110 (11.5)	26 (11.6)	6 (9.7)	0.8 (0.3-2.1)	2 (6.7)	0.6 (0.1-2.6)	24 (11.1)	1.0 (0.5-1.8)	15 (15.0)	1.4 (0.7-2.9)

Table 2. Continued.

		Farm animal health				
		Animal husbandry†	Animal husbandry+VPH		Farm animal	
	Total (n=961)	Years 1-2 (n=87)	Years 3-5 (n=160)		Year 6 (n=81)	
	n (%)	n (%)	n (%)	OR (95% CI)	n (%)	OR (95% CI)
Chronic cough (3 months in last year)	78 (8.1)	6 (6.9)	9 (5.6)	0.7 (0.2-2.2)	11 (13.6)	2.1 (0.7-6.0)
Chronic phlegm (3 months in last year)	55 (5.7)	4 (4.6)	5 (3.1)	0.6 (0.2-2.2)	9 (11.1)	2.3 (0.7-7.8)
SOB	54 (5.6)	3 (3.5)	8 (5.0)	1.5 (0.4-6.0)	6 (7.4)	2.2 (0.5-8.9)
Wheezing (last year)	108 (11.2)	5 (5.8)	13 (8.1)	1.4 (0.5-4.2)	7 (8.6)	1.6 (0.6-5.2)
Wheezing (without a cold)	61 (6.4)	3 (3.5)	9 (5.6)	1.7 (0.5-6.3)	4 (4.9)	1.5 (0.3-6.7)
Wheezing with SOB	73 (7.6)	4 (4.6)	11 (6.9)	1.9 (0.5-6.7)	7 (8.6)	2.0 (0.4-7.0)
Breathlessness with wheezing	26 (2.7)	2 (2.3)	6 (3.8)	1.4 (0.4-4.5)	2 (2.5)	2.1 (0.6-7.0)
Chest tightness	81 (8.4)	8 (9.2)	6 (3.8)	0.4 (0.1-1.1)	6 (7.4)	1.2 (0.5-3.2)
Asthma						
Asthma attack (ever)	77 (8.0)	5 (5.8)	11 (6.9)	1.5 (0.5-4.6)	9 (11.1)	1.8 (0.5-5.6)
Asthma attack (last year)	27 (2.8)	1 (1.2)	3 (1.9)	1.6 (0.2-16.0)	8 (9.9)	2.2 (0.2-24)
Asthma diagnosed by doctor	70 (7.3)	5 (5.7)	10 (6.3)	1.1 (0.4-3.3)	2 (2.5)	1.2 (0.4-4.2)
Asthma-like symptoms	153 (15.9)	10 (11.5)	16 (10)	0.9 (0.4-2.0)	6 (7.4)	1.5 (0.5-3.5)
BHR	305 (31.8)	28 (32.2)	39 (24.4)	0.7 (0.4-1.2)	13 (16.1)	1.3 (0.7-2.5)
Rhinitis	565 (58.8)	46 (52.9)	91 (56.9)	1.1 (0.7-2.0)	48 (59.3)	1.1 (0.6-2.0)
Conjunctivitis	199 (20.7)	12 (13.8)	26 (16.3)	1.2 (0.6-2.5)	18 (22.2)	1.8 (0.8-4.3)
Dermatitis	110 (11.5)	4 (4.6)	24 (15.0)	4.2 (1.5-14.0)	4 (4.9)	2.4 (1.7-8.5)

OR adjusted for gender, smoking status and farm childhood.

*Individually kept animals used as an internal reference group for subcategories of equine or companion animals.

†Animal husbandry used as an internal reference group for subcategories of animal husbandry+VPH or farm animals.

NA, not available; SOB, shortness of breath (asthma-like, wheezing or chest tightness); VPH, veterinary public health.

Table 3. Prevalence and adjusted ORs (95% CI) for newly self-reported allergy and related symptoms per study specialisation upon starting the veterinary medicine study program.

		Individually kept animals								
		Individually kept animals*	Equine				Companion animals			
	Total (n=961)	Years 1-2 (n=225)	Years 3-5 (n=62)		Year 6 (n=30)		Years 3-5 (n=216)		Year 6 (n=100)	
	n (%)	n (%)	n (%)	OR (95% CI)	n (%)	OR (95% CI)	n (%)	OR (95% CI)	n (%)	OR (95% CI)
Allergy to various allergens	84 (8.7)	16 (7.1)	3 (4.8)	0.6 (0.2-2.3)	6 (20)	3.5 (1.2-10)	15 (6.9)	1.0 (0.5-2.0)	12 (12)	1.8 (0.8-3.9)
Allergy to animals	37 (3.9)	5 (2.2)	2 (3.2)	1.4 (0.3-7.5)	1 (3.3)	1.7 (0.2-15)	6 (2.8)	1.3 (0.4-4.4)	6 (6.0)	3.0 (0.9-10)
Breathing problem	15 (1.6)	3 (1.3)	1 (1.6)	1.1 (0.1-11)	1 (3.3)	2.2 (0.2-23)	2 (0.9)	0.7 (0.1-4.5)	3 (3.0)	2.5 (0.5-13)
Sneezing or runny nose	22 (2.3)	4 (1.8)	2 (3.2)	1.8 (0.3-10)	1 (3.3)	1.9 (0.2-18)	1 (0.5)	0.3 (0.1-2.5)	4 (4.0)	2.5 (0.6-11)
Itchy and red skin	20 (2.1)	1 (0.4)	NA	–	NA	–	5 (2.3)	5.3 (0.6-46)	4 (4.0)	9.3 (1.0-85)
Itching eyes	20 (2.1)	3 (1.3)	NA	–	NA	–	1 (0.5)	0.2 (0.0-2.4)	5 (5.0)	3.1 (0.6-15)
Confirmed by blood or skin prick test	11 (1.1)	1 (0.4)	NA	–	NA	–	1 (0.5)	1.1 (0.1-18)	3 (3.0)	5.6 (0.6-56)
Allergy to grass pollen	52 (5.4)	12 (5.3)	NA	–	4 (13.3)	2.7 (0.8-9.0)	9 (4.2)	0.8 (0.3-1.9)	7 (7.0)	1.4 (0.5-3.5)
Breathing problem	19 (2.0)	3 (1.3)	NA	–	3 (10.0)	8.2 (1.6 -43)	6 (2.8)	2.1 (0.5-8.6)	2 (2.0)	1.5 (0.3-9.2)
Sneezing or runny nose	45 (4.7)	11 (4.9)	NA	–	4 (13.3)	2.5 (0.7-8.7)	7 (3.2)	0.7 (0.2-1.7)	7 (7.0)	1.5 (0.6-3.9)
Itchy and red skin	2 (0.2)	1 (0.4)	NA	–	NA	–	1 (0.5)	1.0 (0.1-17)	NA	–
Itching eyes	35 (3.6)	10 (4.4)	NA	–	3 (11.0)	2.4 (0.6-9.2)	7 (3.2)	0.7 (0.3-1.9)	2 (2.0)	0.4 (0.1-2.0)
Confirmed by blood or skin prick test	10 (1.0)	NA	NA	–	1 (3.3)	–	2 (0.9)	–	1 (1.0)	–
Allergy to house dust	24 (2.5)	3 (1.3)	1 (1.6)	1.1 (0.1-11)	2 (6.7)	6.3 (1.0-41)	4 (1.9)	1.4 (0.3-6.6)	4 (4.0)	3.2 (0.7-15)
Breathing problem	16 (1.7)	3 (1.3)	NA	–	2 (6.7)	6.1 (0.9-40)	3 (1.4)	1.1 (0.2-5.5)	3 (3.0)	2.4 (0.5-12)
Sneezing or runny nose	21 (2.2)	2 (0.9)	NA	–	2 (6.7)	8.0 (1.1-59)	4 (1.9)	2.2 (0.4-12)	4 (4.0)	4.8 (0.9-27)
Itchy and red skin	3 (0.3)	NA	1 (1.6)	–	NA	–	NA	–	1 (1.0)	–
Itching eyes	12 (1.3)	3 (1.3)	NA	–	2 (6.7)	6.1 (0.9-40)	1 (0.5)	0.4 (0.0-3.6)	2 (2.0)	1.6 (0.3-10)
Confirmed by blood or skin prick test	17 (1.8)	1 (0.4)	1 (1.6)	3.5 (0.2-57)	1 (3.3)	8.9 (0.5-152)	2 (0.9)	2.2 (0.2-24)	4 (4.0)	9.8 (1.1-90)

Table 3. Continued.

		Farm animal health				
		Animal husbandry†	Animal husbandry+VPH		Farm animals	
	Total (n=961)	Years1-2 (n=87)	Years 3-5 (n=160)		Year 6 (n=81)	
	n (%)	n (%)	n (%)	OR (95% CI)	n (%)	OR (95% CI)
Allergy to various allergens	84 (8.7)	3 (3.4)	13 (8.1)	2.5 (0.7-8.9)	16 (20)	6.9 (1.9-25)
Allergy to animals	37 (3.9)	1 (1.2)	7 (4.4)	5.5 (0.6-47)	9 (11.1)	12 (1.4-103)
Breathing problem	15 (1.6)	NA	2 (1.3)	1.1‡	3 (3.7)	3.3‡
Sneezing or runny nose	22 (2.3)	NA	5 (3.1)	2.8‡	5 (6.2)	5.7‡
Itchy and red skin	20 (2.1)	1 (1.2)	4 (2.5)	2.5 (0.3-25)	5 (6.2)	5.4 (0.6-50)
Itching eyes	20 (2.1)	NA	5 (3.1)	2.8‡	6 (7.2)	6.9‡
Confirmed by blood or skin prick test	11 (1.1)	NA	3 (1.9)	1.7‡	3 (3.7)	3.3‡
Allergy to grass pollen	52 (5.4)	2 (2.3)	7 (4.4)	1.9 (0.4-9.6)	11 (13.6)	6.7 (1.4-31)
Breathing problem	19 (2.0)	NA	1 (0.6)	0.5‡	4 (4.9)	4.5‡
Sneezing or runny nose	45 (4.7)	2 (2.3)	6 (3.8)	1.7 (0.3-8.4)	8 (10.0)	4.7 (1.0-23)
Itchy and red skin	2 (0.2)	NA	NA	–	NA	–
Itching eyes	35 (3.6)	2 (2.3)	5 (3.1)	1.4 (0.3-7.2)	6 (7.4)	3.4 (0.7-17)
Confirmed by blood or skin prick test	10 (1.0)	NA	3 (1.9)	1.6‡	3 (3.7)	3.3‡
Allergy to house dust	24 (2.5)	NA	5 (3.1)	2.8‡	5 (6.2)	5.7‡
Breathing problem	16 (1.7)	NA	2 (1.3)	1.1‡	3 (3.7)	3.3‡
Sneezing or runny nose	21 (2.2)	NA	4 (2.5)	2.2‡	5 (6.2)	5.7‡
Itchy and red skin	3 (0.3)	NA	NA	–	1 (1.2)	1.1‡
Itching eyes	12 (1.3)	NA	1 (0.6)	0.5‡	3 (3.7)	3.3‡
Confirmed by blood or skin prick test	17 (1.8)	NA	3 (1.9)	1.6‡	4 (4.9)	4.5‡

OR adjusted for sex, allergy, smoking status and farm childhood.
*Individually kept animals used as an internal reference group for subcategories of equine or companion animals.
†Animal husbandry used as an internal reference group for subcategories of animal husbandry+VPH or farm animals.
‡Estimate of the OR calculated by adding one person at the reference group.
–unable to calculate as no participants in the groups; NA, not available; VPH, veterinary public health.

Table 4. Prevalence and ORs (95% CI) for sensitisation against various allergens per study specialisation throughout the veterinary medicine study program.

		Individually kept animals								
		Individually kept animals*	Equine				Companion animal			
	Total (n=673)	Years 1-2 (n=158)	Years 3-5 (n=45)		Year 6 (n=12)		Years 3-5 (n=163)		Year 6 (n=53)	
	n (%)	n (%)	n (%)	OR (95% CI)	n (%)	OR (95% CI)	n (%)	OR (95% CI)	n (%)	OR (95% CI)
Common allergens										
House dust mite	87 (12.9)	18 (11.4)	11 (24.4)	2.7 (1.0-7.1)	3 (25.0)	1.8 (0.4-8.7)	17 (10.4)	0.9 (0.4-1.9)	6 (11.3)	1.0 (0.3-2.9)
Grass Pollen	109 (16.2)	26 (16.5)	12 (26.7)	1.8 (0.8-4.5)	1 (8.3)	0.3 (0.0-2.3)	25 (15.3)	0.9 (0.5-1.8)	7 (12)	1.1 (0.4-2.6)
Birch pollen	23 (3.4)	8 (5.1)	2 (4.4)	0.8 (0.2-3.9)	1 (8.3)	1.2 (0.1-11)	5 (3.1)	0.6 (0.2-1.9)	1 (1.9)	0.4 (0.1-2.9)
Cat	28 (4.2)	7 (4.4)	2 (4.4)	1.0 (0.2-5.0)	NA	–	7 (4.2)	1.0 (0.3-3.0)	4 (7.6)	2.9 (0.5-7.5)
Dog	9 (1.3)	2 (1.3)	1 (2.2)	1.7 (0.2-20)	NA	–	7 (4.2)	2.0 (0.4-11)	1 (1.9)	1.5 (0.1-17)
Total	168 (25.0)	39 (24.7)	15 (33.3)	1.5 (0.6-3.6)	5 (41.6)	1.5 (0.4-6.6)	37 (22.7)	0.9 (0.5-1.7)	12 (22.6)	0.8 (0.3-2.0)
Laboratory and domestic animal allergens										
Rat (urine)	13 (1.9)	3 (1.9)	NA	–	NA	–	4 (2.5)	1.4 (0.3-6.5)	NA	–
Mouse (Urine)	19 (2.8)	3 (1.9)	1 (2.3)	1.1 (0.1-11)	1 (8.3)	4.2 (0.4-49)	7 (4.3)	2.5 (0.6-10)	1 (1.9)	0.9 (0.1-9.1)
Budgerigar	21 (3.1)	7 (4.4)	1 (2.3)	0.4 (0.1-3.6)	NA	–	6 (3.9)	0.8 (0.3-2.5)	3 (5.7)	1.3 (0.3-5.0)
Guinea pig	14 (2.1)	5 (3.2)	2 (4.6)	1.4 (0.3-7.7)	NA	–	3 (1.8)	0.6 (0.1-2.4)	NA	–
Total	56 (8.4)	14 (8.9)	4 (8.9)	1.0 (0.3-3.2)	1 (8.3)	0.8 (0.1-6.9)	15 (9.2)	1.0 (0.4-2.2)	4 (7.5)	0.8 (0.3-2.8)
Cat + dog	32 (4.8)	9 (5.7)	3 (6.6)	1.0 (0.2-4.3)	NA	–	8 (4.9)	0.9 (0.3-2.4)	4 (7.5)	1.4 (0.4-5.1)
Horse	11 (1.6)	3 (1.9)	2 (4.4)	2.4 (0.4-15)	1 (8.3)	4.7 (0.4-49)	4 (2.5)	1.0 (0.3-5.9)	1 (1.2)	1.0 (0.1-9.8)
Farm occupational allergens										
Cow	25 (3.7)	3 (1.9)	1 (2.2)	1.2 (0.1-11)	NA	–	8 (4.9)	2.6 (0.6-10)	3 (5.7)	3.0 (0.6-16)
Goat	12 (1.8)	3 (1.9)	NA	–	NA	–	4 (2.5)	1.3 (0.3-5.9)	2 (3.8)	2.0 (0.3-12)
Pig (skin)	1 (0.2)	1 (0.6)	NA	–	NA	–	NA	–	NA	–
Chicken	NA	NA	NA	–	NA	–	NA	–	NA	–
Total	29 (4.3)	5 (3.2)	1 (2.2)	0.7 (0.1-6.3)	NA	–	9 (5.5)	1.8 (0.6-5.6)	3 (5.6)	1.9 (0.4-8.2)
Total (sensitization)	222 (33.1)	51 (32.5)	18 (40.9)	1.4 (0.6-3.1)	6 (50.0)	1.7 (0.4-6.7)	55 (33.7)	1.1 (0.7-1.9)	18 (34.0)	1.1 (0.5-2.2)

Table 4. Continued.

		Farm animal health				
		Animal husbandry†	Animal husbandry+VPH		Farm animals	
	Total	Years 1-2				
	n=673)	n=63)	Years 3-5 (n=128)		Year 6 (n=51)	
	n (%)	n (%)	n (%)	OR (95% CI)	n (%)	OR (95% CI)
Common allergens						
House dust mite	87 (12.9)	13 (20.6)	16 (12.5)	0.7 (0.3-1.7)	3 (5.9)	0.2 (0.1-0.9)
Grass Pollen	109 (16.2)	13 (20.6)	19 (14.8)	0.8 (0.3-1.8)	4 (7.8)	0.3 (0.1-1.0)
Birch pollen	23 (3.4)	3 (4.7)	2 (1.6)	0.3 (0.1-2.0)	1 (2.0)	0.4 (0.0-4.0)
Cat	28 (4.2)	2 (3.2)	5 (3.9)	1.2 (0.2-6.6)	1 (2.0)	0.6 (0.1-7.0)
Dog	9 (1.3)	NA	1 (0.8)	–	NA	–
Total	168 (25.0)	22 (34.9)	31 (24.2)	0.7 (0.3-1.5)	7 (13.7)	0.2 (0.1-0.6)
Laboratory and domestic animal allergens						
Rat (urine)	13 (1.9)	1 (1.6)	5 (3.9)	2.5 (0.3-22)	NA	–
Mouse (Urine)	19 (2.8)	2 (3.2)	4 (3.1)	1.0 (0.2-5.4)	NA	–
Budgerigar	21 (3.1)	3 (4.8)	1 (0.8)	0.2 (0.0-1.5)	NA	–
Guinea pig	14 (2.1)	1 (1.6)	1 (0.8)	0.5 (0.0-7.8)	2 (3.9)	2.5 (0.2-28)
Total	56 (8.4)	7 (11.1)	9 (7.0)	0.6 (0.2-1.7)	2 (3.9)	0.3 (0.1-1.6)
Cat + dog	32 (4.8)	2 (3.2)	5 (3.9)	1.2 (0.2-6.6)	1 (2.0)	0.6 (0.1-7.0)
Horse	11 (1.6)	NA	NA	–	NA	–
Farm occupational allergens						
Cow	25 (3.7)	5 (7.9)	4 (3.1)	0.4 (0.1-1.7)	1 (2.0)	0.2 (0.0-2.1)
Goat	12 (1.8)	1 (1.6)	1 (0.8)	0.5 (0.0-7.9)	1 (2.0)	1.2 (0.1-20)
Pig (skin)	1 (0.2)	NA	NA	–	NA	–
Chicken	NA	NA	NA	–	NA	–
Total	29 (4.3)	5 (7.9)	4 (3.1)	0.4 (0.1-1.7)	2 (3.9)	0.5 (0.1-2.7)
Total (sensitization)	222 (33.1)	28 (45.2)	37 (28.9)	0.5 (0.3-1.1)	9 (17.7)	0.2 (0.1-0.5)

*Individually kept animals used as an internal reference group for subcategories of equine or companion animals.
†Animal husbandry used as an internal reference group for subcategories of animal husbandry+VPH or farm animals.
–, unable to calculate as no participants in the groups; NA, not available; VPH, veterinary public health.

DISCUSSION

To the best of our knowledge, this is the first study to document the prevalence of adverse health outcomes among veterinary medicine students, in which the associations between self-reported allergic symptoms and sensitisation determined by specific IgE with study specialisations as a surrogate of specific animal exposure have been investigated.

The overall prevalence of new-onset self-reported symptoms of allergy to animals was 3.9% (n=37). This figure should be interpreted as an incidence as only new-onset allergic symptoms were included. An incidence of 3.9% is lower than the 20% incidence, which has been reported for laboratory animal sensitisation [21]. This seems a plausible estimate though, because participants in the early years of their education are involved more in theoretical courses and consequently less in practical work; thus, the exposure intensity and exposure frequency is probably too low to induce the development of allergic symptoms in early years.

We used study specialisation groups as a surrogate of exposure and observed that the prevalence of self-reported symptoms of allergy was elevated in later years, but the onset of self-reported symptoms based on different study specialisation groups was variable. Handling farm animals emerged as the strongest risk factor for self-reported allergic symptoms to animals (11.1%). This risk is lower than the 20% risk of laboratory animal allergy among laboratory workers reported by a review study [5], suggesting that those participants without allergy at the end of their education might still remain at a high risk of developing allergy in their future career.

The widespread symptoms of sneezing or runny nose (59.4%) among those with self-reported allergic symptoms to animals are in line with previously reported symptoms in Californian veterinarians (62%) [7], and also similar to the figure reported for swine veterinarians (69%) [10]. Symptoms of itchy and red skin attributed to animals were less likely to occur (2.3%) similarly as reported for Californian veterinarians (11%) [7]. The comparison should be interpreted with caution as we only included new-onset symptoms compared with the overall prevalence in the Californian study. The animals that were most commonly implicated in causing itchy and red skin were farm animals, followed by cat and dog. These findings are consistent with those reported earlier by Sustitaival *et al*. [7]. Dermatitis might partially occur due to contact with animal dander resulting in urticaria [22]. Urticaria derived from cow dander has been reported as a major cause of occupational dermatitis among veterinarians before. Furthermore, dermatitis could be partially associated with the use of gloves [13] and antibiotics [23].

The allergy prevalence noticeably differs whether derived by questionnaire or serological assessment [5]. A high number of participants with newly self-reported allergic symptoms without evidence of positive IgE are in line with what has been reported previously [16,19].

Veterinarians are very likely exposed to proinflammatory agents such as bacterial endotoxin and fungal β-(1–3)glucan, as well as irritant agents such as ammonia and disinfectants in animal houses [1,2,24]. Therefore, it is reasonable to assume that reported allergic symptoms without IgE could also be caused by these agents and thus might be mediated through other than type II allergic mechanisms. We also found a substantial number of sensitised individuals (38.3%) without self-reported symptoms of allergy, either before or after starting veterinary medicine study. This observation is in agreement with a previous study [19]. However, sensitised participants without any symptoms are known to be more prone to develop symptoms in the near future [25].

In the present study, cat sensitisation was the most prevalent animal sensitisation (4.2%), with a lower sensitisation risk for dog (1.3%). The pattern of sensitisation to cat is known to be inconsistent among cat owners in comparison with those people without having cat [26]. In contrast to cat owners, sensitisation risk for dog is consistently reduced among dog owners [26]. Although most animals treated in the companion animal hospital were dogs (≥95%) and, to a lesser extent, cats (<5%), less sensitisation to dogs and more sensitisation to cats in the present study support earlier studies and might probably reflect previous contacts with cats and dogs since 97% of the study population had previous contact with cats and/or dogs.

We found an inverse risk of sensitisation in the study specialisations of animal husbandry (years 3–5) and farm animals (year 6) compared with the reference group, while the prevalence of self-reported symptoms was elevated. Previous studies described tolerance associated with IgG and IgG4 antibody responses to animal allergens [27,28], consequently specific IgE antibodies against animal allergens could not be detected. However, a longitudinal study is needed to investigate the relationship between exposure to specific animal allergens and risk of sensitisation.

Several important limitations of this study need to be considered. Two of the participants in this study changed study specialisation during the course of their education because they had been seriously affected by allergy. This might be an underestimation because participants with allergy that completely end their education might not be included [29]. Most of the participants in this study have had previous and/or current contact with animals, especially domestic animals, besides the study program. As a result, it is difficult to distinguish between the exposure to animals through the study program and the exposure outside the study program in association with the development of allergy.

Participation bias needs to be considered. Although participants with a history of allergy more likely provided a blood sample than those without a history of allergy, this suggests a possible source of bias in the sensitisation part of this cross-sectional study. However, as the relative number of participants with history of allergy who provided a blood sample was the

same for the different study durations, the association between sensitisation and study over time is not affected. For some specialisation groups, the number of participants sensitised or reporting allergic symptoms was low, limiting our ability to estimate associations over time. The self-reported time course of symptoms might be a source of bias as well due to misclassification of the time of initiation of symptoms. How this would have affected the outcomes cannot be said.

However, known factors previously associated with enhanced or decreased sensitisation such as enhanced total IgE and growing up on a farm behaved similarly in the current study. Enhanced total IgE ≥100 kU/l was strongly associated with sensitisation as in other studies demonstrating enhanced total IgE as a strong determinant for sensitisation [19,21,30]. Farm childhood exposure was associated with a reduced risk of self-reported allergy or sensitisation in adulthood. There is consistent evidence for this finding as illustrated for French farmers [31], Danish farmers [32], and Dutch farmers [33]. This suggests that veterinary medicine students with an earlier exposure to farm environments might be protected from developing allergy, although current farm animal exposure still is associated with an increased risk to report respiratory symptoms. A similar paradoxical finding had been reported for Dutch agricultural population previously [33].

CONCLUSIONS

The findings of this study provide evidence of an increased prevalence of (allergic) symptoms with elevated years of veterinary study, proposing that contact with animals, more specifically contact to farm animals, is a risk factor for developing symptoms. There is still a need for further investigation to determine the incidence of sensitisation and allergic symptoms in a follow-up study and also to find out a dose–response relationship between bioaerosol exposure and health outcomes.

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Chapter 7

General Discussion

A Review of Bio-aerosol Exposures and Associated Health Effects in Veterinary Practice

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ABSTRACT

Background: Occupational exposure to bio-aerosols has been linked to various health effects. Whether this is of importance in veterinary settings is not much studied. This review will present an overview of bio-aerosol exposure levels in veterinary practices and investigate the possibility of health effects associated with bio-aerosol exposure.

Methods: A systematic literature search was carried out in PubMed. Publications were included if they provide information on bio-aerosol exposure and related health effects through veterinary practice and other professions with similar exposures, occupationally exposed to animals.

Results: Bio-aerosol exposure in veterinary settings is investigated in only a few studies which showed that substantial endotoxin and β(1→3)-glucan exposure levels likely occur when handling farm animals and horses. Exposure levels are comparable to those levels observed in farming which have been associated with respiratory health effects. Animal specific allergen exposures have hardly been studied, but showed to be measurable in companion animal clinics and dairy barns. Findings of few studies available among veterinary populations, particularly those working with farm animals and horses, are indicative of an elevated risk for developing respiratory symptoms. Dose-response relationships between exposure to bio-aerosols and health effects during veterinary practice have not yet been conducted. However, studies among pig farmers, exposed to similar environments as veterinarians, strongly confirm that veterinary populations are at an increased risk of developing respiratory diseases in relation to bio-aerosol exposure in particular endotoxin. Exposure to animal allergens during veterinary practice may cause allergic inflammation, characterized by IgE-mediated reactions to animal allergens. Nonetheless, the occurrence of sensitization or allergy against animal allergens is poorly described, apart from laboratory animal allergy especially known from exposure to rats and mice.

Conclusion: Veterinary populations are likely to be exposed to elevated levels of bio-aerosols such as endotoxins, β(1→3)-glucans, and some specific animal allergens. Exposures to these agents in animal farmers are associated with allergic and non-allergic respiratory effects, proposing similar health effects in veterinary populations.

INTRODUCTION

Practitioners of veterinary medicine typically perform clinical work and deliver healthcare to animals including farm animals (*e.g*. cows, sheep, pigs, and goats), companion animals (*e.g*. cats, dogs, and birds), and horses. Most veterinarians work in private medical practices. They treat animals suffering from infectious and non-infectious diseases and vaccinate against infectious diseases. Some veterinarians are animal-food-product inspectors; their job involves inspection of alive animals and their food-products for transmittable diseases. A small proportion of veterinarians work in universities both as physicians and researchers.

Veterinary professions usually involve shift work, and veterinarians working with farm animals and horses regularly commute between their office/clinic and farms to provide veterinary services at the farms/stables. Veterinary practices often use medical equipment such as diagnostic and surgical instruments (*e.g*. radiographic and ultrasound equipment). Collectively, veterinary professions are extremely diverse because of multiple work environments and the performance of various activities. Therefore, veterinarians experience several known occupational hazards that can be categorized into exposures to biological agents (*e.g*. organic dust, microorganisms), chemical agents (*e.g.* anesthetic gases, pesticides, insecticides, pharmaceuticals), physical agents (*e.g*. radiation, noise), and trauma hazards (*e.g*. needle-stick injuries, bites) [1-10]. Exposure to all of these hazardous agents can potentially result in a broad range of adverse health effects such as respiratory problems [11-19], dermatitis [20,21], zoonotic infectious diseases [22], pesticides-associated toxicity [23], certain cancers [24-28] and physical trauma [8,29,30]. It is known for a long time that occupational exposure to farm animals is linked to a wide variety of respiratory health effects [13,14,19,31-34], with biological agents as primary causal agents. Working conditions of veterinary professionals are to a large extent comparable to farmers exposed to animals with subsequent similar exposure, although gradual differences may exist. However, the occupational health risks of veterinary professionals associated with bio-aerosol exposure have so far been poorly described.

The main purpose of this review was to systematically summarize the literature on bio-aerosol exposure in veterinary practice and relate to possible health effects. We do acknowledge that, at present, only few studies have been performed investigating health effects associated with bio-aerosol exposure during veterinary practice, while the body of evidence on health effects of similar exposure among farmers exposed to animals is considerable. Therefore, the literature on farmers' exposures will be considered where relevant, to fill knowledge gaps.

METHODS

Definition of bio-aerosols

"*Organic dust*", dust of biological origin, also referred to as "*bio-aerosol*", is dust originating from microbial, animal or plant materials. Organic dust generally has a heterogeneous composition containing many toxic and immunogenic particles, for instance, pathogenic and/or non-pathogenic microorganisms (*e.g.* bacteria, viruses, and fungi) and their biological active components (*e.g*. bacterial endotoxin, mould glucan, and mycotoxin), plant fragments (*e.g*. pollen), and animal-derived materials (*e.g*. hair, dander, and allergens) [35].

Literature search

Publications investigating bio-aerosol exposure as well as health effects associated with bio-aerosol exposure through veterinary practice were searched in the PubMed database. Because the publications on the topic are relatively rare, similar studies related to other animal environment settings were included as well. The following search terms were utilized: "respiratory symptoms", "allergy", "sensitization", "infectious diseases", "biological agents", "bio-aerosol", "organic dust", "endotoxin", "glucan", or "allergen", linked with the use of the word "veterinary", "veterinarian", or "animal". Publications were judged to be covered in the review when following inclusion criteria applied:

- Articles should be published in English language;
- Studies reported bio-aerosol measurements during contact with animals;
- Studies concerned respiratory health effects associated with exposure to animals;
- Studies concerned allergy and/or sensitization associated with exposure to animals;
- Studies concerned zoonotic infectious diseases associated with exposure to animals.

We explicitly explored the type of evidence available, ranging from case series, surveys focusing on health endpoints only to surveys with (simple) exposure categorizations up to quantitative exposure-response studies. The latter types of evidence are stronger than the first types of evidence.

Levels of bio-aerosol exposure

Initially bio-aerosol exposure in animal settings was measured as culturable levels of airborne microorganisms [36-38]. Duchaine *et al*. [38] in pig barns found 4.25 x 10^5 CFU m^{-3} (1.67 x 10^5 to 9.30 x 10^5) of total bacteria and 883 CFU m^{-3} (547 to 2862) of molds. Donham *et al*. [36] and Chang *et al*. [37] also showed similar results with a mean airborne level around 10^5 CFU m^{-3}. A factor lower total bacterial and fungal exposure levels were found in horse stables [39] and dairy barns (Chapter 5) with a geometric mean of 3.1 x 10^3 to 1.1 x 10^4 CFU m^{-3} for total bacteria and 1.9 x 10^3 to 2.3 x 10^3 CFU m^{-3} for fungi. Later on, culture-independent approaches using *e.g*. direct coloring of bacteria or biological assays like the *limulus amebocyte lysate* assay to determine endotoxin as general marker of bacterial exposure as well as molecular biological techniques specifically quantitative real-time PCR for certain microbial products like mycotoxin were deployed [35]. With the fast development of molecular based techniques during last decades and the availability of probes, they are now applied to investigate airborne bacterial diversity [40]. Interestingly, a recent study by *Nehme et al*. [41] shift focus from aerobic to anaerobic microbial burden in farm environments by showing that airborne archaea could be detected by PCR. The authors

found high levels of archaea up to 10^8 16S rRNA gene copies per m^3 of air, which was in the same order of magnitude as total bacteria reported previously [40].

Table 1 summarizes studies reporting occupational endotoxin and β(1→3)-glucan exposure levels for studies which have been conducted in veterinary settings as well as in agricultural settings in which animals were involved. Not many studies have investigated bio-aerosol exposure in veterinary practice, except for the series of studies performed by us, on bio-aerosol measurements in a broad spectrum of veterinary practices within animal clinics and farms. Overall, exposure levels to dust, endotoxin and β(1→3)-glucan were found to be distinctly high although dependent on animal species involved, sampling sites, and job titles. Highest personal levels of endotoxin exposure were found during veterinary practice with poultry (GM 1498 EU m^{-3}) (Chapter 4), second highest in horse stables (GM 608 EU m^{-3}) [39], followed by ruminant clinics (GM 520 EU m^{-3}). In contrast to farm animals and horses, levels of dust and endotoxin during veterinary practice with companion animals were found to be low and close to background [42,43]. The dust and endotoxin exposure of veterinarians dealing with farm animals are in the same range as has been reported previously for farmers involved in similar farm animal settings, of which a recent selection is presented in table 1. Concerning β(1→3)-glucan, the highest personal levels were observed in horse stables (GM 9.5 μg m^{-3}) [39], followed by clinics for poultry (GM 3.39 μg m^{-3}) and ruminants (GM 3.10 μg m^{-3}). To our knowledge, no published studies are available on personal exposure of β(1→3)-glucan related to animal settings. Levels of β-(1→3)-glucan within different farm animal clinics, however, were much higher than those previously reported from greenhouses [44] and green waste-composting plants [45,46]. There are two studies available determining cat and dog specific allergen levels during veterinary practice [42,43]. Findings of these studies showed the presence of cat (Fel d 1) and dog (Can f 1) allergens in the air of companion animal clinics, although exposure levels differ significantly between job titles. Similarly, two studies reported personal exposure levels of Fel d 1 and Can f 1 in homes, offices and schools [47,48], however, comparisons with these studies need to be taken with caution because different sampling and analysis methods were utilized. Allergen exposure levels in farm animal settings were not often investigated. However, a few recent studies showed that specific allergens of cow [49] and horse [50] within or around animal buildings were measureable and occasionally high.

Potential health effects

The most well-known occupational health effects related to bio-aerosol exposure are respiratory symptoms [13,16,17,19,51-58], ranging from acute mild and self-limiting to sever chronic, even life-threatening. Respiratory symptoms can be classified on the basis of inflammatory mechanisms into allergic and non-allergic respiratory diseases. Allergic

respiratory symptoms are caused by an immune-specific airway inflammation in which antibodies of IgE (type I) or IgG (type III) may play a role in the inflammatory reactions. Allergic asthma and rhinitis are two well-known allergic respiratory diseases that may occur due to exposure of specific allergens present in organic dust (*e.g.* animal specific allergens) [59,60]. In addition to allergic asthma and rhinitis, organic dust exposed workers might develop extrinsic allergic alveolitis (EAA), referred to as hypersensitivity pneumonitis (HP) or farmer's lung [61]. Asthmatic symptoms may occur in the absence of an immune-specific reaction. A considerable proportion of work-related asthma symptoms are known to be non-atopic asthma. This form of asthma, sometimes referred to as asthma-like syndrome or non-allergic asthma [34], is supposed to be caused by inflammatory components of bio-aerosols such as endotoxin. The underlying mechanism is considered a neutrophil-mediated inflammatory reaction [62]. Workers exposed to organic dust contaminated with a very high endotoxin level may also develop non-allergic systematic inflammatory reactions which are accompanied by flu-like symptoms. This is referred to as "*organic dust toxic syndrome*" (ODTS) [57]. Additionally, exposure to organic dust has also been associated to chronic obstructive pulmonary diseases (COPD) [34].

Besides respiratory health effects, other possible adverse health effects have been suggested or proven to be associated with bio-aerosol exposure such as infectious diseases (*e.g.* Q-fever, anthrax, tuberculosis, swine influenza) [63-70], certain cancers [24,26,71] and dermatitis [72-74]. Nonetheless, these health effects have not been studied extensively and information about their occurrence is extremely limited. Paradoxically, studies also suggested a possible protective effect of exposure to microbial agents on development of allergic diseases [18,75-77]. In following paragraphs we will explain more about the mechanisms and the occurrence of the different health endpoints in relation to veterinarians.

Respiratory health effects

Table 2 summarizes a selection of studies on adverse health effects associated with bio-aerosol exposure in veterinarians and related other settings. Respiratory health effects associated with bio-aerosol exposure through veterinary practice have not been investigated extensively. Andersen *et al*. [78] investigated the prevalence of self-reported respiratory symptoms and lung function changes in a cross-sectional study among veterinarians during the annual meeting of American association of pig veterinarians. Pig veterinarians in this study reported a high prevalence of work-related respiratory symptoms including rhinitis (69%), cough and chest tightness (53%), wheeze (31%), and wheeze accompanied with airway obstruction (24%). This study also showed that veterinarians with airway obstruction spent more hours per week in pig barns than those veterinarians with normal lung function [78]. Tielen *et al*. [79] evaluated the prevalence of self-reported respiratory symptoms in a

cross-sectional questionnaire-based study among Dutch veterinarians. The authors found that the veterinary practitioners exposed to farm animals had a distinctly higher prevalence of chronic cough (OR 1.8, 95% CI 1.1-2.8), chronic phlegm production (OR 2.1, 95% CI 1.1-3.7), and wheeze (OR 2.8, 95% CI 1.3-6.3) compared to veterinarians with other specialties. Jolie *et al*. [80] investigated the health respiratory problems in veterinary students after visiting of a pig farm for 3 hours. Overall, 72.5% of veterinary students reported respiratory symptoms in relation to pig farm exposure. Symptoms (87.1%) mostly developed the same day of visiting pig farm and disappeared 3 days after exposure. A more recent study [81], carried out among veterinary medicine students, similarly showed a higher prevalence of respiratory symptoms in those veterinary students exposed to farm animals compared to other animal species. This very well matched with the observed trends in endotoxin levels which were high in farm animal related clinics and low in companion animal hospital [39,43] and also comparable to those endotoxin levels previously reported in farms [39,43,82-84]. Both studies by Tielen *et al*. [79] and Samadi *et al*. [81] showed an association between the onset of respiratory symptoms and duration of animal exposure. This finding is also consistent with the reported association between time exposed to organic dust in pig barns and observed adverse respiratory symptoms [14,32,78,79,85].

In contrast to veterinary populations, respiratory health effects associated with bio-aerosol exposure among pig farmers is probably one of the best-studied setting considering bio-aerosol related health effects. Studies showed that exposure to organic dust from pig barns are associated with elevated respiratory symptoms, chronic bronchitis, increased bronchial hyper-responsiveness, and accelerated lung function decline. The evidence of these health effects is based on a series of experimental and observational studies among pig farmers. Donham *et al*. [33] first proposed in 1977 that exposure to organic dust, especially in large pig confinement operations, might play a role for the development of respiratory symptoms in pig farmers and veterinarians. This finding has further been confirmed since, by several other epidemiological studies among pig farmers [17,33,78]. Results of experimental studies on naïve and non-naïve subjects, healthy volunteers, showed that a short-term exposure to organic dust from pig barns induced airway inflammation, characterized by elevated bronchial hyper-responsiveness and increased number of inflammatory cells (mainly neutrophilic granulocytes) in nasal lavage [62,86-90]. Dosman *et al*. [91,92] reported 7 cases of occupational asthma in newly employed pig workers. All these cases developed symptoms within months after starting employment. A clinical evaluation indicated occupational asthma in all cases in the absence of a clear immunological response to common or work specific allergens. All cases were bronchial hyper-responsive and thus showed lower metacholine or histamine thresholds and the findings were, according to the authors, indicative of non-atopic asthma. In a Norwegian study among farmers, non-atopic

asthma accounted for more than 75% of all current asthma and was more frequently observed in livestock farmers particularly in pig farmers [18]. Several studies give indications for an increased risk of ODTS among pig farmers; characterized by fever, chills, chest tightness, shortness of breath, dry cough, myalgias, and/or fatigue [16,31,93,94], with a prevalence ranging from 26.3% to 34% [31,95]. ODTS can not be differentiated from HP by clinical symptoms. However, ODTS is a systemic toxic response caused by pro-inflammatory agents such as endotoxins [57], while HP is an immune-mediated response [61]. Studies also give indications for an elevated risk of chronic bronchitis and COPD among pig farmers [34,96].

Besides exposure to pig barns, which is relatively well-established as an occupational health risk, exposure to dairy barns [14,19,32,52,97-102], poultry houses [14,55,103], and horse stables [11,104-106] are also considered to be a risk factor for the development of respiratory problems such as respiratory symptoms, airway responsiveness, chronic bronchitis and ODTS.

Two well-established pro-inflammatory components of organic dust are endotoxins [35,107] and glucans [108], of which endotoxin has been the most widely studied, also because of its role in sepsis. Endotoxin, often referred to as lipopolysaccharide (LPS), is a non-allergic constituent of the outer cell wall of Gram-negative bacteria and an ubiquitous component of organic dust [83]. The lipid A portion of LPS is known to cause inflammatory reactions [107] with lung function decline as the most serious effect of both short- and long-term exposure. In cross-sectional dose-response studies, an inverse association between endotoxin exposure and lung function changes in pig farmers was first established by Donham *et al*. [109], which was supported further by similar studies [13,93]. This finding was also corroborated with an experimental study, in naïve healthy volunteers, showing an association between endotoxin exposure and FEV_1 decline [51]. Similarly, a few longitudinal studies among pig farmers found a clear dose-response relationship between endotoxin exposure and lung function decline [12,96,110]. Dose-response studies in pig farmers also showed a stronger inverse association between exposure to endotoxin and lung function changes when compared with dust exposure [93,96]. Similar observations have also been found in workers exposed to grain [110] and cotton dust [51,111]. Smit *et al*. [57] in a study among workers involved in agricultural seed processing industry proposed exposure to organic dust highly contaminated with endotoxin (GM 1800 EU m^{-3}) as the primarily causative agent for developing ODTS. Smit *et al*. [112] also observed in a dose-response study in agricultural workers including animal and crop workers that high endotoxin exposure (GM 319 EU m^{-3}) was a risk factor for bronchial hyper-responsiveness and wheeze, which were characterized by a predominantly non-atopic nature. One study among pig farmers found a clear exposure-response relationship between endotoxin exposure and lung

function decline in a longitudinal study over a period of 3 years, indicating that long-term exposure to high endotoxin levels (GM 105 ng m^{-3} ~1050 EU m^{-3}) is likely a risk factor for developing COPD [96].

β(1→3)-glucans are polysaccharides of D-glucose molecules with different molecular weights and degrees of branching [113] which can be found in most fungi, some bacteria, and a number of plants [108]. Occupational exposure data for this component are very limited. When considering animal settings, we only found one study in poultry workers investigating health effects related to β(1→3)-glucan exposure [103]. This study showed that poultry workers had an elevated prevalence of toxic pneumonitis, chronic bronchitis, and increased airway responsiveness indicative of airway inflammation compared to controls; however, dose-response relationships were not determined and high endotoxin levels were reported as well. Epidemiological studies in other occupational settings suggest that exposure to β(1→3)-glucan is associated with respiratory symptoms, airway responsiveness, toxic pneumonitis, chronic bronchitis, and lung function decline [45,103,114-118], although the evidence is still inconclusive. Few studies also found similar respiratory effects associated with β(1→3)-glucan exposure after adjusting for the levels of endotoxin exposure [115,116]. Two experimental studies showed that the combination of β(1→3)-glucan and endotoxin synergistically enhances their toxicity causing inflammation [119,120].

To summarize, both experimental and observational studies strongly support the proposition that exposure to endotoxin is casually related to the development of work-related respiratory effects. Besides endotoxin, exposure to β(1→3)-glucan may also be responsible to certain extent for work-related respiratory effects. With the knowledge of high endotoxin exposure in veterinary practices handling farm animals and horses, it seems logical to assume that veterinary populations suffer from respiratory diseases related to endotoxin exposure as previously reported for farmers. Similar to endotoxin, occasionally high exposure to β(1→3)-glucan during veterinary practice might also play a role for the development of respiratory effects as reported for other studies.

Sensitization/allergy to animal allergens

Proteins derived from animals and plants are the most important group of high molecular weight occupational allergens. Exposure to these allergens, especially animal allergens, is more specifically associated with working in animal settings, although not much studied. Exposure to chemical agents (low molecular weight) which form a hapten (*e.g*. disinfectants), also regularly occurs during working in animal settings [2]. Thus, certain jobs dealing with animals likely put people at risk of exposure to allergens, *e.g*. veterinarians, animal caretakers and farmers [50,121-125]. The most potent animal allergens are associated with mammalians such as cow, horse, cat, dog, rat, and mice [126], which may

originate from multiple sources such as hair, dander, saliva, urine, and serum. Inhalation of animal allergens is considered the most common route of occupational exposure, although skin and eyes might be routes of exposure as well [127]. Following exposure, individuals might become sensitized (IgE-mediated) [128], subsequently allergic symptoms develop, with allergic rhinitis as the most common symptom, followed by allergic conjunctivitis [129], and ultimately resulting in work-related asthma [130,131].

Rodents

Rats and mice are the animals most commonly used in scientific experimental studies. Occupational exposure to these animals often occurs when working at animal laboratories. The most important allergen for rat is Rat n 1, and for mouse, Mus n 1; which both belong to a family of proteins termed Lipocalins [132]. A study by Krakowiak *et al*. [133] among veterinarians exposed to laboratory animals showed that the majority of veterinarians were sensitized to rats and mice allergens. The authors gave explanations for this finding including more frequent contact with these animals, as well as increased susceptibility to become allergic when being sensitized to other allergens as well. Rats and mice seem to be the most important animals inducing sensitization (IgE-mediated) in laboratory animal workers, many of whom are veterinarians as well, and probably is one of the best described adverse health effect associated with laboratory animal exposures [129,131,132,134-142]. The prevalence of allergy against rats in laboratory animal workers ranged from 12 to 31% in some recent studies [129,143-146], and for mice ranged from 10 to 32% [129,144]. Several epidemiological studies have shown a strong association between increased intensity of exposure to laboratory animal allergens and elevated prevalence of laboratory animal allergy (LAA) [141,147,148]. In a recent study, cumulative exposure to Mus n 1 (median 0.29 ng m^{-3} per years) in a dose-response dependent manner has been shown to be a significant risk factor for IgE-mediated mouse sensitization [142]. Hollander *et al*. [141] in a dose-response study found a clear relationship between rat allergen exposure (median 0.68 ng equivalent per m^3) and sensitization only in a group of workers who had worked with rats for less than 4 years. It is important to note that the observed higher prevalence of occupational allergy against rats and mice compared to other animal allergens, is likely due to the more frequent use of these animals in experimental studies, and not to lesser ability of other animal allergens to trigger allergy.

Farm animals (ruminants)

The most important cow allergen is Bos d 2 (*Bos domesticus 2)* that is found mainly in cow hair and dander, and it belongs to the lipocalin family of proteins [149]. The occurrence of sensitization against cow allergen in veterinary populations has only been studied specifically

in veterinary medicine students, showing sensitization to cow allergens to be present in 3.7% (25 cases) of all participants [81]. Cow allergy is much more studied among Finish dairy farmers. Investigations have confirmed the role of cow-derived proteins as important occupational allergens on developing allergy among dairy workers [150,151], subsequently causing asthma [152,153]. High prevalence of positive IgE anti-Bos d 2 reactions have been reported among Dutch dairy farmers as well [154], which is in agreement with previously reported results from Finland [155-157], but the occurrence of respiratory symptoms or the development of airway diseases in Dutch dairy farmers was rare [154] contrary to observations among Finish farmers. Similarly, either positive specific IgE-antibodies (8.8%) or skin prick test (7.4%) against cow allergens have been reported in Polish farmers [158], but allergic symptoms relevant to cow allergen were rare [158].

There is no data available concerning sensitization/allergy to other farm animals like sheep, goat, and poultry. Nonetheless, we can not ignore that allergy against these animals might occur.

Horse

The most important horse allergen is Equ c 1 which is a lipocalin protein [159] and can be found in horse dander and hair [160,161]. Exposure to horse allergen often occurs through direct contact with horses [50] or indirect contact due to transfer of horse allergen on clothes or hair [162]. Occupational exposure to horse allergen mainly occurs among farmers, veterinarians, as well as those individuals who handle horses either for professional or recreational purposes. Only one study [81] investigated sensitization to horse specific allergen among veterinary medicine students and showed that 1.6% (11 cases) of all participants was sensitized to horse allergen. In this study, the prevalence of sensitization in those students specializing in equine veterinary medicine increased over time (years 3-5: OR 2.4, 95% CI 0.4-15; year 6^{th}: OR 4.7, 95% CI 0.4-49 compared to the year 1-2 students), indicating prolonged years of exposure to horses as a possible determinant of sensitization. Similarly, Tutlouglu *et al*. [106] found in a cross-sectional study that horse grooms had a significantly higher prevalence of sensitization to horse hair (OR 3.75, 95% CI 1.1–12.82) compared to controls. Sensitization to horse hair was associated with an increased risk of allergic conjunctivitis (OR 1.5, 95% CI 0.4–5.1), asthma (OR 4.5, 95% CI 1.5–13.3), and lung function decline [106]. A recent study by Liccardi *et al*. [122] in an urban population in Italy demonstrated that 35 out of 1822 adults (3.43%) were sensitized to horse dander. Of these sensitized people, 6 reported direct contact with horse, 10 had indirect contact with horse owners, and 19 reported no direct or indirect contact with horse or horse owners. Twenty sensitized people reported having both nasal and bronchial symptoms and one reported asthma without rhinitis. Ronmark *et al*. [163] in a cross-

sectional study found that sensitization to horse specific allergen was a significant risk factor for the development of rhinitis and asthma.

Domestic animals

Fel d 1 has been described as the major cat allergen and Can f 1 as the most important dog allergen [132]. Cat allergen is often attached to particles less than 10 µm (range 1-20 µm) [164-167] and the particle size distribution for dog allergen appears to be very similar to that of cat allergen [168]. The small size makes it possible that these two allergens are easily transmitted through the air. Spread into the environment by contact with clothing, hair or other surfaces have been described for these allergens [169,170]. Occupational exposure to cat and dog may cause respiratory symptoms in veterinarians [130] and laboratory animal workers [129]. In a recent study, 4.8% (32 cases) of all veterinary medicine students were sensitized to cat and dog allergens, but the prevalence of sensitization in the specialty of domestic animals did not clearly change over time (years 3-5: OR 0.9, 95% CI 0.3-2.4; year 6th year: OR 1.4, 95% CI 0.4-5.1 compared to the year 1-2 students) [81]. These results, however, are unadjusted for previous exposure to these animals because most subjects had earlier domestic exposures. Further investigation, including measurement of specific cat and dog allergens, has corroborated the presence of cat and dog allergens as important occupational airborne allergens in a companion animal hospital [43]. However, most epidemiological evidence on sensitization/allergy in relation to cat and dog allergen exposure comes from studies conducted in general population and residential and public spaces [171-174], indicating the importance of exposure to cat and dog inducing sensitization/allergy against these allergens.

In addition to studies investigating sensitization and related allergic respiratory symptoms, a few studies have also reported a high prevalence of atopic symptoms among veterinarians such as allergic rhinitis and asthma, but no information is given regarding specific underlying immune reactions against animal allergens [130,175-178].

To summarize, exposure to rats and mice are well-established to cause sensitization/allergy among laboratory animal workers. Less information is available about sensitization/allergy against other animal allergens; however few limited studies among animal workers and veterinary populations still suggest the importance of exposure to animals (*e.g.* cow, horse, cat, and dog) as a risk factor for development of animal specific sensitization/allergy.

Protective effects of bio-aerosol against allergy

A reduced risk of sensitization and self-reported allergy was observed among veterinary medicine students who grew up on a farm [81]. In parallel to veterinary populations,

numerous publications related to farmers indicating that growing up on a farm may have a protective effect against development of allergy which are summarized in table 3. A large number of epidemiological studies consistently showed that childhood exposure to farm environments is associated with a reduced risk of developing atopy and atopic asthma [75,76,179-181]. Several epidemiological studies have also found that this protective effect of early childhood exposure may still be present during adulthood [58,112,182-185]. Recent studies among farmers and workers in agricultural industries also strongly showed inverse associations between endotoxin exposure with atopic asthma [18], sensitization [77,186] and hay fever [187]. The underlying mechanisms behind these protective effects are still poorly understood. However, it has been hypothesized that bio-aerosol components particularly endotoxin may protect from the development of allergic diseases by modifying the immune responses against allergens. The initial explanation was that bio-aerosol components particularly LPS shift towards a TH_1 (innate)-type response that further suppresses the development of TH_2 response against allergens [188,189]. More recently, an alternative concept has been suggested to explain TH_1/TH_2 paradigm; T-regulatory (Treg) cells balance both TH_1 and TH_2 responses [188].

Infectious diseases

Biological agents may contain a large variety of pathogenic microorganisms like bacteria, viruses, fungi, and parasites that can pose a threat to human and animals. More than 1400 microorganism species are known to be pathogens for human [190]. Of these, 175 can be categorized into "*emerging or reemerging pathogens*" [190]. Emerging and reemerging pathogens are those that either have been seen in human for the first time or have been occurred previously, but the incidence is increasing or they expand in locations where they have not previously been observed. About 75% of the emerging and reemerging pathogens are capable of causing infectious diseases in animals (termed as zoonotic pathogens), proposing that they can be transmitted from animals to human. Zoonotic infections (*e.g*. Q-fever, avian and swine influenza, and anthrax) in humans are predominantly attributed to exposure in specific occupational settings such as livestock farms, animal stores, and veterinary practices, but accurate information for most is absent. Veterinarians are probably at high risk of developing infectious diseases because of their high likelihood of contact with infected animals [191]. A study among all 565 US members of the American Association of Zoo Veterinarians has shown that 30.2% of veterinarians reported to have had a zoonotic infection [192]. A recent review [193] summarized published literature about infectious diseases among veterinarians, authors concluded that veterinary populations are at an increased risk of several zoonotic pathogens like *Coxiella burnetii,* swine and avian influenza A virus, *Brucella* spp, methicillin-resistant *Staphylococcus aureus (*MRSA*),* avian and feline *C*

psittaci and swine hepatitis E virus. However, exact numbers on the prevalence of most zoonotic infections is lacking. It has also been suggested that veterinary populations may act as biological sentinels for emerging pathogens and could potentially spread zoonotic pathogens to their family and community members [193]. Exposure assessment studies which involve infectious agents have hardly been published. Some examples of recent encountered infectious diseases will be discussed in more detail.

Q fever

Q fever is generally an occupational disease caused by the bacterium called *Coxiella burnetii*. Occupational exposure to *Coxiella burnetii* often occurs through contact with infected farm animals (*e.g*. cattle, sheep, and goats), as well as their birth-products [64]. In sero-epidemiological studies among veterinarians, elevated specific IgG antibodies against *Coxiella burnetii* were found in 13.5% in Japan [64], 12.9% in Sweden [66], 7.5% in Turkey [67], 9.5% in Australia [65], 22% in the USA [194] and 36% in Slovakia [195], which were higher than those reported for the general population. Amongst others working with ruminants was identified as a risk factor.

Influenza A viruses

Infections with influenza A viruses have been reported in several animal species (*e.g.* birds, swine, and horse). Avian (bird) and swine influenza are two of the well-known infectious diseases caused by influenza A viruses. All birds are thought to be susceptible to avian influenza disease (*e.g*. chickens, ducks, and turkeys). The transmission risk of influenza viruses to human is low, but some cases of human infection have been reported since 1976 [196]. During an outbreak of highly pathogenic H7N7 avian influenza virus in Dutch poultry farms in 2003, the highest self-reported influenza-like symptoms were found among veterinarians of all those exposed to poultries [70]. In sero-epidemiological studies among American veterinarians exposed to poultry, positive specific IgG antibodies against avian influenza viruses were observed in 12.2% (type H5), 23.8% (type H6), and 14.6% (type H7) [197]. In another study among American veterinarians exposed to swine, 10.9% and 19.1% had positive serological evidence to swine influenza viruses of N1H1 and N1H2 [198]. A 57-year-old Dutch veterinarian has died because of infection by H7N7 avian influenza virus following visiting an infected poultry farm [69].

Methicillin-resistant Staphylococcus aureus (MRSA)

Staphylococcus aureus is a Gram-positive bacterium that can be found in humans and numerous animal species [199]. After the introduction of antibiotics, *Staphylococcus aureus* has become resistance to certain antibiotics such as methicillin, oxacillin, penicillin, and

amoxicillin; which is called as methicillin/(oxacillin)-resistant *Staphylococcus aureus* (MRSA). Animals can act as a reservoir for MRSA, thus humans can be infected through close contact with MRSA colonized animals. In recent years, two outbreaks of MRSA infections were reported in veterinary clinics in Canada and the United States [200,201]. In the American study the outbreak had most likely a human source and animals became carrier through the owner or in the clinic, but the source was not identified [201]. Of particular interest is the Canadian study. After recognition of a cluster of MRSA infection in horses and humans at the Ontario Veterinary College Veterinary Teaching Hospital, environmental contamination with MRSA was evaluated [200]. Relatively widespread contamination of the hospital environment was observed and suggests that the environment may be an important source of MRSA infection. In an Irish study the occurrence of MRSA during veterinary practice was studied [202]. The pulsed field gel electrophoresis patterns of the isolates showed that transmission of two strains of MRSA is occurring in veterinary practices in Ireland and that one strain may have arisen from human hospitals. The source of the second strain remains to be determined [202]. Since 2004, MRSA has been found to be emerging in livestock animals, especially in pigs and veal calves [203]. From 2007 a specific MRSA strain (ST398) emerged in animal husbandry not seen before in hospitals, termed as livestock associated-MRSA [204]. MRSA strain ST398 can cause invasive infections and outbreaks, although so far only incidentally reported [204]. Exposure to livestock animals in particular pigs among Dutch veterinarians [205] and pig farmers [206] considered a risk factor for MRSA (4.6 and 26%, respectively) compared to the general population (0.03%) [207]. However, occupational epidemiological studies which involve MRSA associated to exposure have not yet been investigated.

Occupational threshold limit values

Occupational exposure limits (OELs) or threshold limit values (TLVs) of hazardous agents provide reference levels for which it is assumed that workers can be exposed continually for a working lifetime without adverse health effects. Although several health risks associated with bio-aerosol exposure have been described, exposure-response relationships have been shown for some components of bio-aerosol only, particularly for endotoxin in relation to non-infectious health effects and attempts have been undertaken to derive occupational exposure limits. The Dutch expert committee on occupational standards (DECOS) [208] has recently established a health-based recommended occupational exposure limit (HBROEL) of endotoxin of 90 EU m^{-3} (eight hours time-weighted-average) based on acute respiratory effects resulting from airway inflammation. This exposure limit was based on a study in which healthy volunteers without respiratory symptoms were exposed to endotoxin in cotton dust [51], a cross-sectional study of the chronic lung function changes of animal feed mill

workers [209], and a five years follow-up study of such workers [210]. Exposure levels in veterinary practices, as presented in table 1, indicate that exceedance of the standard regularly occurs in clinics related to farm animals [43] and horses [39], proposing that veterinary populations during working in these animal settings probably experience health effects related to endotoxin exposure. It is obvious that endotoxin levels during veterinary practice in the companion animal hospital [43] is lower than the recommended health-based exposure limit of 90 EU m^{-3}, presumably leading to no adverse health effects on the basis of low endotoxin exposure. Up to now, no OELs have yet been established for β(1→3)-glucan exposure due to inconclusive evidence of heath effects. In addition, there are no OELs for allergen exposure levels, although few exposure-response studies showed an association between exposure to some animal specific allergens and health effects [141,142]. Nonetheless, a framework for deriving OELs for allergens has been proposed [211], however, methods for exposure assessment of animal specific allergens have not yet been standardized, which compromises development of standards and are not commercially available.

CONCLUSIONS

There are only a few studies available that investigated bio-aerosol exposure in veterinary settings. These studies showed veterinary populations especially those working with farm animals such as cows and poultry as well as horses are exposed to substantial levels of inhalable dust, endotoxin, and β(1→3)-glucan. Exposure levels of animal specific allergens have hardly been investigated, but animal specific allergens showed to be measurable in companion animal clinics (cat and dog allergens), dairy barns (cow allergen), and horse stables (horse allergen). The limited available information on health effects related to veterinary practice give some indications for an increased risk of respiratory effects, especially for those veterinarians handling farm animals and horses. Nonetheless, accurate estimates of the occurrence and prevalence figures of respiratory diseases are lacking. Dose-response studies between exposure to bio-aerosols and health effects during veterinary practices have not yet been performed. Since exposure levels through veterinary practices especially for endotoxin are similar to those previously found in farming, one can speculate that similar to results of experimental and observational studies among farming populations, veterinary populations are at an elevated risk of developing respiratory diseases in relation to bio-aerosol exposure in particular endotoxin. Workers in animal settings are not frequently exposed to just one biologically active agent of organic dust, but to a mixture with different exposure levels. Animal workers in some situations may also come into contact with chemical agents such as ammonia [18]. In such cases, it seems

logical to assume that at least a part of respiratory effects among veterinary populations are likely attributed with exposure to other agents rather than endotoxin.

The occurrence of work-related sensitization and allergic symptoms among veterinary populations and animal workers has not yet been extensively studied, except for laboratory animal workers exposed to rats and mice. Nonetheless, few studies available give indications for sensitization and allergic respiratory symptoms in veterinary populations being exposed to animals such as rats, mice, cats, dogs, cows, and horses, but the role of exposure pattern and level to these animal allergens is still poorly described. So far, dose-response relationships between allergen exposure and health effects through veterinary practices have not yet been conducted. In general, it seems logical to assume that reactions to animal allergens in veterinary populations would be an important issue because they are likely often exposed to a number of animal allergens for prolonged periods of their working time.

Besides adverse health effects, some protective effects of bio-aerosol exposure on developing sensitization/allergy have been proposed among veterinary populations. However, respiratory health effects seem to occur at the same levels as the protective effect of allergy, thus the protective effect is counterbalanced and symptoms in higher exposed individuals are more likely to be due to non-allergic mechanisms.

Suggestion for further studies

A large variety of respiratory symptoms associated with animal environmental settings containing bio-aerosols during veterinary practice has been reported. Nonetheless, it is not obvious which bio-aerosols are primarily responsible mainly due to the absence of exposure data. As a result, cross-sectional and longitudinal exposure-response studies need to be conducted in order to investigate allergic and non-allergic respiratory diseases associated with exposure to bio-aerosol components. Measurement of inflammatory markers could assist to prove the occurrence of airway inflammations and subsequent respiratory diseases. Moreover, there has been no evidence on the incidence of sensitization against animal allergens among veterinary populations. For this reason, new studies are required to investigate the incidence and the prevalence of sensitization/allergy during veterinary practices.

Bio-aerosol exposure is inherent through veterinary practice with animals. Thus it is necessary to apply measures to reduce bio-aerosol exposure in particular endotoxin, with a priority of removal of exposure sources as well as exposure reduction through substitution of bedding material or other exposure reducing approaches like ventilation.

Table 1. Exposure levels to inhalable dust, endotoxin, and β(1→3)-glucan as determined in various animal facilities.

	Sample type	N	Dust mg m^{-3} GM (range)	Endotoxin EU m^{-3} GM (range)	β(1→3)-glucan μg m^{-3} GM (range)	Reference
Veterinary practice						
Companion animal clinic						
Veterinary students	P	55	<LOD	3.2 (<LOD-75)	NM	Samadi et al. [43]
Veterinarians	P	12	<LOD	3.9 (<LOD-24)	NM	Samadi et al. [43]
Poultry clinic						
Veterinary students	P	98	1.27 (<LOD-20.9)	1485 (115-49846)	3.0 (<LOD-46)	Samadi et al. (chapter 4)
Veterinarians	P	11	1.01 (<LOD-12.4)	1221 (237-16927)	2.2 (<LOD-22)	
Caretakers	P	5	5.72 (1.62-14.7)	2749 (454-10820)	9.7 (2.5-27)	
	S	16	1.25 (0.18-5.37)	938 (140-10655)	1.5 (<LOD-20)	
Ruminant clinic						
Veterinary students	P	64	0.37 (<LOD-1.5)	368 (67-3047)	2.1 (<LOD-106)	Samadi et al. (chapter 4)
Caretakers	P	32	1.56 (0.14-20.8)	1042 (60-7492)	8.6 (<LOD-11)	
	S	36	0.15 (<LOD-0.49)	173 (27-1475)	1.4 (0.2-12)	
Horse clinic						
Caretakers	P	42	1.40 (0.20–9.5)	608 (<LOD–9846)	9.5 (<LOD–631)	Samadi et al.[39]
	S	32	0.40 (<LOD-1.1)	167 (<LOD-1385)	2.6 (<LOD-39)	
Animal farming						
Cow						
Dairy farming	P	8	1.30 (0.40–2.3)	560 (62–2230)	NM	Spaan et al.[83]
Dairy farming and cattle breeding	P	4	1.50 (0.70–2.7)	1570 (444–3860)	NM	Spaan et al.[83]
Dairy barns	?	159	1.78 (0.01-53.6)	647 (25.4-34800)	NM	Kullman et al.[82]
Dairy cattle	S	22	NA	16.9 (2.8- 66)	NM	Schierl et al.[212]
Beef cattle (breeding)	S	6	NA	558 (124-1025)	NM	Schierl et al.[212]
Cow sheds	P	23	1.78 (0.25-58.2)	NA	NM	Berger et al. [213]
	S	31	0.22 (0.01-2.43)	36 (4-561)	NM	

Table 1. Continued.

	Sample type	N	Dust mg m^{-3} GM (range)	Endotoxin EU m^{-3} GM (range)	β(1→3)-glucan μg m^{-3} GM (range)	Reference
Pig						
Pig barns	S	18	NA	669 (43- 7469)	NM	Schierl et al.[212]
Pig barns	P	6	2.60 (1.6–5.4)	1510 (992–6970)	NM	Spaan et al. [83]
Pig feeding	P	?	3.65 (0.16-37.2)	? (95-147885)	? (0.006-5.2)	Szadkowska-Stańczyk et al.[214]
Pig barns	S	236	NA	111.3 (<1-4153)	NM	Ko et al. [215]
Pig barns	P	360	2.40 (0.30-26.6)	92 (5.6-1503)	NM	Preller et al. [216]
Poultry						
Laying hens	S	18	NA	463 (21.8- 21933)	NM	Schierl et al.[212]
Poultry farm (eggs)	P	2	9.50 (6.60–14)	2090 (1716–2550)	NM	Spaan et al.[83]
Poultry farm (meat)	P	2	4.20 (4.00–4.4)	880 (520–1500)	NM	Spaan et al.[83]
Poultry farm (free-range hens)	P	5	3.60 (1.60–11)	2140 (360–8120)	NM	Spaan et al.[83]
Turkeys	S	6	NA	1902 (467- 5292)	NM	Schierl et al.[212]

N, number of samples; <LOD, below the lower limit of detection, P, personal; S, stationary; NA, not available, NM; not measured.

Table 2. A selection of epidemiological and experimental studies of adverse health effects associated with bio-aerosol exposure in veterinarians and related other settings.

Study design	Study population	Outcome measures	Reference
Veterinary studies			
Cross-sectional questionnaire-based	Swine veterinarians	An increase of work-related respiratory symptoms and airway obstruction was observed.	Andersen et al.[78]
Cross-sectional questionnaire-based	Pig workers and veterinarians	Exposure to organic dust suggested to play a role for observed respiratory problems.	Donham et al.[33]
Cross-sectional questionnaire-based	Veterinarians	Large animal practitioners reported higher symptoms of chronic cough, chronic phlegm production, chest wheezing, compared to veterinarians with other specialties.	Tielen et al.[79]
Cross-sectional questionnaire-based	Veterinary medicine students	An elevated prevalence of sensitization and self-reported symptoms with increasing years of veterinary study was found, suggesting contact with animals, is a risk factor for developing sensitization and symptoms.	Samadi et al.[81]
Cross-sectional questionnaire-based	Veterinarians	About 40% of veterinarians reported animal-related respiratory and/or skin symptoms. The most commonly reported animals inducing symptoms were cats and dogs.	Susitaival et al.[130]
Cross-sectional questionnaire-based	Veterinarians	The majority of subjects were sensitized to rat and mouse. The prevalence of asthmatic and ocular symptoms was more prevalent in sensitized veterinarians versus non-sensitized veterinarians.	Krakowiak A.[133]
Cross-sectional questionnaire-based	Veterinary students	Acute health problems in terms of ODTS appeared in previously unexposed veterinary students following contact to dairy barns	Jolie et al.[80]
Case report	Veterinary surgeon	Occupational urticaria dermatitis	Roger et al.[217]
Laboratory animal workers			
Cross-sectional questionnaire-based	Laboratory animal workers	23.1% of workers had at least one allergic symptom against laboratory animals and two-thirds of them developed allergic symptoms during first three years of exposure. Atopy, animal species handled, and time spent in handling associated with developing LAA.	Aoyama et al.[129]
Cross-sectional questionnaire-based	Laboratory animal workers	Prevalence of allergic symptoms caused by rats and mice were 19% and 10%, respectively. Allergic symptoms strongly correlated with sensitization measured by specific serum IgE to RUAs or MUAs.	Hollander et al.[146]
Cross-sectional exposure-response	Laboratory animal workers	Sensitization to rat allergens in sub-group of workers with less than 4 years of exposure was clearly associated with exposure levels: 15, 9.5, and 7.3 times higher in the high, medium, and low exposure groups compared with internal reference group.	Hollander et al.[141]

Table 2. Continued.

Study design	Study population	Outcome measures	Reference
Cross-sectional exposure-response	Laboratory animal workers	Prevalence of sensitization to rat allergens was 9.7%. About 57% of the sensitized workers had work-related symptoms (asthma or rhinitis). The risk of sensitization elevated with increasing allergen exposure.	Heederik et al.[138]
Retrospective cohort exposure-response	Laboratory animal workers	19.2% of workers reported LAA. The intensity of exposure and atopy were significant predictors for developing LAA.	Kruize et al.[148]
Livestock farmers			
Cross-sectional questionnaire-based	Pig workers	Exposure to pig barns associated with a range of respiratory symptoms such as chronic cough, chronic phlegm production, wheeze, shortness of breath, as well as lung function decline.	Donham et al.[17]
Cross-sectional questionnaire-based	Pig workers	An increased risk of non-allergic flu-like symptoms (ODTS)	Donham et al.[16] Holness et al.[94]
Cross-sectional exposure-response	Pig workers	A positive association between respiratory symptoms indicative ODTS and endotoxin level was observed. Workers with a high endotoxin exposure had a lower lung function.	Heederik et al. [93]
Cross-sectional exposure-response	Pig workers	A positive association between asthma-like symptoms with endotoxin exposure was seen.	Smit et al.[218]
Cross-sectional exposure-response	Pig workers	An inverse association between endotoxin exposure with lung function was found.	Donham et al.[109] Zejda et al.[13]
Cohort (longitudinal) Exposure-response	Pig workers	Long-term average exposure to endotoxin (105 ng m-3) was clearly associated with lung function decline.	Vogelzang et al.[96]
Cohort (longitudinal) Exposure-response	Pig workers	Lung function decline clearly associated with endotoxin exposure.	Schwartz et al.[110] Kirychuk et al.[12]
Cross-sectional	Pig workers	The prevalence of ODTS was elevated in pig farmers compared to controls.	Vogelzang et al.[31]
Cohort study exposure-response	Pig workers	Exposure to dust and ammonia in pig barns associated with increase in bronchial responsiveness expressed as steps for provocative concentration causing FEV_1 decline.	Vogelzang et al.[219]
Case-report	Pig workers	Authors describes the onset of non-atopic asthma in 7 pig farmers after a short-term exposure to pig barns.	Dosman et al.[91,92]
Experimental exposure-response	Healthy naïve volunteers	Short-term exposure (3-hr) to pig barns associated with elevated bronchial responsiveness to methacholine and also increased number of neutrophilic inflammatory cells.	Larsson et al.[62]
Experimental exposure-response	Healthy naive non-farmers and pig farmers	Exposure to dust from pig barn altered lung function and bronchial responsiveness, as well as cell number and cytokines in blood and nasal lavage fluid in non-farmers, while only minor alterations were found in pig farmers.	Palmberg et al.[86]

Table 2. Continued.

Study design	Study population	Outcome measures	Reference
Cross-sectional questionnaire-based	Dairy workers	Dairy farmers had significantly reactions than teachers to cow epithelium, suggesting the importance of cow epithelium as occupational source of allergen among dairy farmers.	Rautalahti et al.[220]
Cross-sectional	Dairy workers	An increase of respiratory symptoms such as rhinitis, asthma, bronchitis, hypersensitivities inhumanities, and ODTS.	Radon et al.[14] Choudat et al.[19] Chaudemanche et al.[52] Choma et al.[99] Cormier et al.[32]
Longitudinal	Dairy workers	An increase of respiratory symptoms such as rhinitis, asthma, bronchitis, hypersensitivities inhumanities, and ODTS.	Gianet et al.[97] Kronqvist et al.[98] Dalphin et al.[101] Manuy et al.[102]
Cross-sectional	Poultry workers	An increase of respiratory problems such as airway responsiveness, toxic pneumonitis, and chronic bronchitis.	Radon et al.[14] Morris et al.[55] Rylander et al.[103]
Cross-sectional	Poultry workers	Lung functions inversely associated with exposure to bio-aerosol particularly endotoxin.	Olenchock et al.106 Clark et al.[221]
Dose-response	Poultry workers	Lung functions inversely associated with endotoxin exposure.	Donham et al.[53]
Cross-sectional	Poultry workers	Significantly higher prevalence of work-related respiratory symptoms, eye and skin symptoms was found in poultry workers compared to controls.	Rimac et al.[222]
Cross-sectional questionnaire-based	Horse workers	Exposure to horse environments associated with an elevated prevalence of respiratory symptoms such as shortness of breath, chronic bronchitis, ODTS, and asthma.	Kimbell-Dunn et al.[11] Mackiewicz et al.[105] Mazan et al.[104] Tautuoglu et al.[106]
Cross-sectional	Children living in northern Swede	Sensitization to horse allergen considered as a risk factor inducing rhinitis and asthma.	Ronmark et al.[163]
Cross-sectional exposure-response	Livestock farmers	Livestock farmers had significantly higher prevalence of chronic bronchitis and COPD than crop farmers. These symptoms was associated with organic dust and endotoxin.	Eduard et al.[34]

Table 3. Epidemiological studies regarding on association between allergic diseases and farm childhood and/or adulthood exposure.

Study design	Study population	Childhood/ adulthood	Major findings	Reference
Cross-sectional	Farmers' children	Childhood	Farmers' children had lower prevalence of hay fever (OR 0.52, 95% CI 0.28-0.99), asthma (0.65, 0.39-1.09), and wheeze (0.55, 0. 36-0.86) than their peers not living in a farm.	Von Ehrenstein et al.[179]
Cross-sectional	Farmers' children	Childhood	Long-term exposure to stables until age 5 years had a protect effect of asthma, hay fever, atopic sensitization.	Riedler et al.[180]
Cross-sectional	Farmer's children	Childhood	Living on a farm during childhood associated with a lower risk of atopy in Wagga (OR 0.47, 0.32-0.72) but not in Moree (OR 0.97, 0.62-1.53). Authors concluded that children in Wagga were more likely lived on a livestock farm than children from Moree .	Downs et al.[75]
Cross-sectional	Adults	Childhood	Living on a farm during childhood associated with a reduced risk of atopic sensitization (OR 0.76, CI 95% 0.60-0.97).	Leynaert et al.[181]
Cross-sectional	Adults	Childhood	Individuals who lived on a farm during their first 5 years of life had lower prevalence of allergic rhinitis than all other age groups.	Eriksson et al.[76]
Cross-sectional exposure-response	Pig farmers	Adulthood	Strong inverse relationship was found between endotoxin exposure and sensitization to common allergens.	Portengen et al.[77]
Cross-sectional exposure-response	Farmers	Adulthood	Exposure to endotoxin appears to have a protective effect on atopic asthma.	Eduard et al.[18]
Nested case-control	Adults	Adulthood	Current exposure to high levels of house dust endotoxin inversely associated with allergic sensitization to at least one common allergens (OR 0.80, 0.64-1.00).	Gehring et al.[186]
Cross-sectional exposure-response	Workers from diverse agricultural sectors	Childhood/ adulthood	A significant inverse exposure-response relationship between endotoxin exposure and atopic sensitization was observed during both childhood and adulthood farm exposures.	Smit et al.[112]
Cross-sectional	Adults	Childhood/ adulthood	The risk of sensitization to pollens was inversely associated with farming exposures during adulthood (OR=0.93, 95% CI 0.44-0.2.0), childhood (OR=0.55, 95% CI 0.26-0.1.2), and both childhood and adulthood (OR=0.18, 95% CI 0.08-0.42).	Koskela et al.[182]
Cross-sectional	Farmers	Childhood /adulthood	Exposure to farms during either childhood or adulthood associated with a lower risk for atopy (identified by positive SPT or IgE to common allergens) and allergic respiratory symptoms.	Portengen et al.[183]

Table 3. Continued.

Study design	Study population	Childhood/ adulthood	Major findings	Reference
Cross-sectional	Adults	Childhood /adulthood	The risk of sensitization to common allergens was inversely associated with farming exposure during childhood (OR 0.7, 0.5-0.9) and in both childhood and adulthood (OR 0.4, 0.3-0.6).	Radon et al.[184]
Cross-sectional exposure-response	Farmers	Childhood /adulthood	Combination of adulthood and childhood exposure to farm environment was more inversely associated with asthma symptoms than adulthood or childhood exposure alone.	Douwes et al.[185]
Cross-sectional	Conventional and organic farmers	Childhood /adulthood	Living on a farm during childhood , combined with current livestock farming, is associated with a lower prevalence of hay fever in both conventional and organic farmers.	Smit et al.[58]
Cross-sectional exposure-response	Farmers and agricultural industry workers	Childhood /adulthood	Endotoxin exposure inversely associated with hay fever and self-reported allergy: hay fever [childhood OR 0.64 (0.43-0.95), adulthood 0.59 (0.44-0.80)], self-reported allergy [childhood OR 0.89 (0.70-1.12), adulthood OR 0.75 (0.60-0.93)]	Smit et al.[187]

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SUMMARY

Studies showed that workers handling animals especially in livestock farms (*e.g*. pig, cow, and poultry) are likely exposed to high levels of bio-aerosol exposure. Working with animals during veterinary practice has great similarities with livestock farming because veterinary populations spent a large amount of their time in stables or clinics in close contact with animals. Thus, exposure to bio-aerosols during veterinary practice is perhaps possible, but detailed and comprehensive studies on bio-aerosol exposure are lacking. A few studies also demonstrated that veterinary populations are probably at risk for the development of allergic and non-allergic respiratory effects, but associated bio-aerosol exposure is largely unknown.

The main purpose of this thesis was to comprehensively investigate the exposure levels of inhalable dust, endotoxin, β(1→3)-glucans, and animal specific allergens among veterinarians, veterinary students, and animal caretakers (workers) in diverse veterinary clinics, as well as to explore exposure determinants. In addition, the presence of sensitization/allergy and respiratory symptoms among veterinary medicine students, which might be related to bio-aerosol exposure was studied. A secondary aim was to explore the feasibility and efficiency of a newly developed EDC (electrostatic dust-fall collector) to measure bio-aerosol components especially animal specific allergens.

In **Chapter 2**, dust, endotoxin, β(1→3)-glucan, and culturable microorganisms exposure levels in horse stables are reported. 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, to resemble exposure for horses. Samples were analyzed for endotoxin and β(1→3)-glucan by Limulus amebocyte lysate (LAL) assay and an inhibition Enzyme Immunoassay (EIA), respectively. Culturable bacteria and fungi were collected with an Anderson impaction sampler. 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), 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. This study showed that exposure to dust, endotoxin, and β(1→3)-glucan were 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.

Chapter 3 gives an overview of allergens and endotoxin exposure levels during veterinary practice in a companion animal hospital. Airborne and surface dust was collected using various sampling methods at different locations within the companion animal hospital. Allergen levels in extracts were measured with sandwich ELISAs and/or the multiplex array for indoor allergens (MARIA). Endotoxin was determined by LAL assay. Fel d 1 (*Felis domesticus*), Can f 1 (*Canus familiaris*) and endotoxin were detected in all samples except stationary samples. The geometric mean (GM) level of personal inhalable dust samples for Fel d 1 was 0.3 ng m^{-3} (range: below lower limit of detection (<LOD) to 9.4), for Can f 1 3.6 ng m^{-3} (<LOD to 73.3) and for endotoxin 4.4 EU m^{-3} (<LOD to 75). Exposure levels differed significantly between job titles, with highest allergen exposure for student assistants in the intensive care unit (GM: Fel d 1, 1.5 ng m^{-3}; Can f 1, 18.5 ng m^{-3}), and highest endotoxin exposure for students (GM 10.1 EU m^{-3}). Exposure levels in dust captured by diverse sampling methods correlated with each other ($p<0.05$). This study showed that allergen exposure likely occurs during veterinary practice in a companion animal hospital, alongside with relatively low endotoxin levels.

In **Chapter 4** we explored exposure levels of inhalable dust, endotoxin, and β(1→3)-glucan among animal caretakers, veterinarians, and veterinary students in ruminant and poultry clinics. Furthermore, task-based determinants of exposure were studied. 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} (115-49846); 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, although tasks were dependent on the job-title. This study showed endotoxin exposure for veterinarians, caretakers and veterinary students to be considerable. Exposure occurred not only in animal houses, but also in practical teaching rooms. β(1→3)-glucan was occasionally high as well. Observed exposure levels might present an occupational respiratory health risk for veterinary populations.

Exposure to bio-aerosols has been reported previously in dairy barns, but little is known about sources of bio-aerosol. It can be hypothesized that type of bedding materials applied in dairy barns might affect bio-aerosol exposure levels. In **Chapter 5**, a study which investigates this is presented. Dairy barns that apply deep litter bedding of either compost or sawdust, as well as mattress bedding of either rubber mattress or rubber filled mats were

included. Personal exposure levels of dust, endotoxin, and β(1→3)-glucan differed significantly between barns utilizing the 4 different main bedding types ($p<0.05$), with the highest levels (GM: dust, 1.38 mg m^{-3}; endotoxin, 895 EU m^{-3}; β(1→3)-glucan, 7.84 µg m^{-3}) in barns with compost bedding versus the lowest in barns with sawdust bedding (GM: dust, 0.51 mg m^{-3}; endotoxin, 183 EU m^{-3}; β(1→3)-glucan, 1.11 µg m^{-3}). The exposure levels were also highly variable depended on various extra bedding materials applied on top of the main bedding. Plant materials, particularly straw appeared to be a significant source for β(1→3)-glucan. Compost bedding was significantly associated with elevated personal and stationary exposure levels. Between-worker variances of exposure were well explained by determinants of exposure, while determinants could explain the within-worker variances to lesser extent. This study showed that exposure levels to endotoxin, β(1→3)-glucan, bacteria, and fungi in dairy barns were substantial and differed dependent on bedding materials applied, suggesting bedding material types as a significant predictor of bio-aerosol exposure.

In **Chapter 6** occurrence of bio-aerosol related health effects in veterinary medicine students is presented. This study estimated prevalence of sensitization and respiratory (allergic) symptoms in association with study specialization and study duration. Contact with type of animal species over time was used as a proxy of bio-aerosol exposure. New development of self-reported allergic symptoms to various allergens occurred in 8.7%, of which 44% was deducted against animals. Handling farm animals was strongly associated with self-reported allergies to various allergens (OR=6.9, 95% CI 1.9 to 25) and animal allergens (OR=12, 95% CI 1.4 to 103). Sensitization to at least one allergen occurred in 33.1%. Sensitization prevalence tended to be elevated in later years of the equine study program. In contrast to self-reported allergies, the prevalence of sensitization to any allergen decreased with prolonged study duration for those specializing in farm animal health (years 3-5: OR=0.5, 95% CI 0.3 to 1.1; year 6: OR=0.2, 95% CI 0.1 to 0.5). This was independent of whether people were raised on a farm, which is in itself a protective factor for allergy and sensitization. This study provides evidence of an elevated prevalence of (allergic) symptoms with longer duration of veterinary study, suggesting that contact with animals, more specifically contact to farm animals, is a risk factor for the development of symptoms.

Finally, in the general discussion, **Chapter 7**, main findings of the studies presented in this thesis are discussed in the context of a systematic literature review on bio-aerosol exposure and related health effects. The few studies on bio-aerosol exposure in veterinary settings showed that substantial endotoxin and β(1→3)-glucan exposure levels likely occur when handling farm animals and horses. Exposure levels are comparable to those levels observed in farming which have been associated with respiratory health effects. Animal specific

allergen exposure have hardly been studied, but showed to be measurable in companion animal clinics and dairy barns. Hardly any bio-aerosol related health effects studies are available for veterinarians. Those available suggest that veterinary populations, particularly those working with farm animals and horses, are at increased risk of developing respiratory symptoms. Dose-response relationships between bio-aerosol exposure and health effects during veterinary practice have not yet been conducted. However, studies among pig farmers, exposed to similar environments as veterinarians, strongly confirm the proposition that bio-aerosol exposed veterinary populations are at an increased risk of developing respiratory diseases. Exposure to animal allergens during veterinary practice is probably causing allergic inflammation, characterized by IgE-mediated reactions to animal allergens. Nonetheless, the occurrence of sensitization or allergy against animal allergens is poorly described, apart from laboratory animal allergy which involves exposure to rats and mice. Overall, veterinary populations are likely to be exposed to elevated levels of bio-aerosols such as endotoxins, β(1→3)-glucans, and some specific animal allergens. Exposure to these agents in similar settings like livestock farmers and laboratory animal workers were previously associated with allergic and non-allergic respiratory effects, suggesting that similar health effects can occur in veterinary populations.

SAMENVATTING

Het is bekend dat werknemers van veehouderijbedrijven die veel contact hebben met dieren (bijv. varkens, koeien en pluimvee) blootgesteld worden aan hoge concentraties van bio-aerosolen. Het begrip 'bio-aerosolen' is een verzamelnaam voor stoffen die afkomstig zijn van dieren, planten of micro-organismen. Veterinairen werken regelmatigmet dieren. Het werken met dieren tijdens de veterinaire praktijk vertoont grote overeenkomsten met de veehouderij, aangezien veterinairen een groot deel van hun tijd in stallen of klinieken doorbrengen in nauw contact met dieren. Het is daarom plausibel dat veterinairen tijdens hun werk blootgesteld worden aan bio-aerosolen, maar gedetailleerde en uitgebreide studies naar bio-aërosol blootstelling ontbreken. Daarnaast is beschreven dat veterinaire populaties een verhoogd risico hebben met betrekking tot het ontwikkelen van allergische en niet-allergische respiratoire aandoeningen, maar de daarmee geassocieerde bio-aerosol blootstelling is grotendeels onbekend.

Hoofddoel van dit proefschrift was om uitvoerig blootstelling aan inhaleerbaar stof, endotoxinen (een celwand component van gram-negatieve bacterien), β(1→3)-glucanen (celwand bestanddeel van schimmels), en specifieke allergenen afkomstig van dieren te onderzoeken bij dierenartsen, studenten diergeneeskunde en dierverzorgers in diverse veterinaire klinieken, alsook om de determinanten van de blootstelling te verkennen. Daarnaast werd de aanwezigheid van sensibilisatie en/of allergie en gezondheidsklachten die verband kunnen houden met bio-aerosol blootstelling bestudeerd bij studenten diergeneeskunde. Een tweede doel was om de bruikbaarheid met betrekking tot bepaling van blootstelling aan dierlijke allergenen te onderzoeken van een nieuw ontwikkelde methode om luchtgedragen stof te verzamelen met behulp van een passieve meetmethode, de zogenaamde 'EDC' (electrostatic dust-fall collector).

In **hoofdstuk 2** worden blootstellingsniveaus van stof, endotoxinen, β(1→3)-glucanen en levensvatbare micro-organismen in paardenstallen beschreven. Stationaire (n = 38) en persoonlijke (n = 42) inhaleerbaar stof monsters werden verzameld met behulp van de PAS-6 inhaleerbaarstof monsternemer. Als een speciale meting werd bemonstering in de buurt van de paardenhoofden uitgevoerd, om de potentiële blootstelling van paarden te onderzoeken. Monsters werden geanalyseerd op endotoxine en β(1→3)-glucanenconcentratie. Kweekbare bacteriën en schimmels werden verzameld met een impactie monsternemer voor levende kiemen, gevolgd door incubatie in een stoof. Geometrische gemiddelde concentraties voor de persoonlijke blootstelling aan stof, endotoxine, en β(1→3)-glucanen waren respectievelijk 1.4 mg m^{-3} (range 0.2-9.5), 608 EU m^{-3} (20-9846) en 9.5 µg m^{-3} (0.4-631). Blootstelling in de ochtendploeg was hoger in vergelijking tot andere ploegen gedurende de dag. De gemiddelde concentraties van

bacteriën en schimmels waren respectievelijk 3.1 × 10^3 kolonievormende eenheid (KVE).m^{-3} (range 6.7 x 10 tot 1.9 × 10^4) en 1.9 × 10^3 KVE.m^{-3} (7.4 x 10 tot 2.4 × 10^4). De variatie in blootstelling voor endotoxine en β(1→3)-glucanen was aanzienlijk hoger dan voor stof. De belangrijkste taak die de blootstelling aan stof, endotoxine en β(1→3)-glucanen bepaalde was het vegen van de vloer. Voor β(1→3)-glucanen was het voeren van de paarden ook een belangrijke determinant.

Hoofdstuk 3 geeft een overzicht van blootstelling aan kat- en hondallergenen en endotoxine voor veterinairen en studenten diergeneeskunde in een gezelschapsdierenkliniek. Luchtgedragen en oppervlakte stof werd verzameld met behulp van verschillende bemonsteringsmethoden op verschillende plaatsen binnen de kliniek. Kat- en hondallergeen en endotoxine werden gedetecteerd in alle typen monsters, behalve in stationaire monsters. Geometrisch gemiddelde concentraties in de persoonlijke inhaleerbaar stof monsters was 0.3 ng m^{-3} voor katallergeen (range: lager dan de detectielimiet (<DL)-9.4), voor hondallergeen 3.6 ng m^{-3} (<DL-73.3) en voor endotoxine 4.4 EU m^{-3} (DL-75). Blootstellingsniveaus waren significant verschillend voor de diverse functies. De hoogste allergenenblootstelling trad op bij student-assistenten op de intensive care unit (GM: katallergeen, 1.5 ng m^{-3}; hondallergeen, 18.5 ng m^{-3}), en de hoogste endotoxinenblootstelling bij studenten diergeneeskunde tijdens het praktikum (GM 10.1 EU m^{-3}). Blootstellingsniveaus van bio-aerosolen in stof verzameld met de diverse methoden correleerden met elkaar ($p<0.05$). Deze studie toont aan dat blootstelling aan allergenen optreedt tijdens veterinaire werkzaamheden in een gezelschapsdieren kliniek, waarbij blootstelling aan endotoxine relatief laag is.

In **hoofdstuk 4** werd blootstelling aan inhaleerbaar stof, endotoxinen, en β(1→3)-glucanen onderzocht bij de dierverzorgers, dierenartsen en veterinaire studenten in de kliniek landbouwhuisdieren, meer specifiek bij de afdelingen herkauwers en pluimvee. Taakgerelateerde determinanten van blootstelling werden bestudeerd. Persoonlijke blootstelling aan stof, endotoxine, en β(1→3)-glucanen waren hoger voor pluimvee [geometrisch gemiddelde (GM): stof, 1.32 mg m^{-3} (range: <DL tot 20.9); endotoxinen, 1498 EU m^{-3} (115-49846) en β(1→3)-glucanen, 3.10 µg m^{-3} (<DL-46.1)] dan voor herkauwers [GM: stof, 0.60 mg m^{-3} (<DL-20.8), endotoxinen, 520 EU m^{-3} (60-7492) en β(1→3)-glucanen, 3,39 µg m^{-3} (<DL-111)]. Stof- en endotoxineconcentraties waren significant gecorreleerd, behalve voor verzorgers in de herkauwerskliniek. Afhankelijk van de functie (verzorger, veterinair of student) konden enkele activiteiten in verband worden gebracht met de hoogte van blootstelling. Deze studie toont aan dat endotoxinen blootstelling voor dierenartsen, dierenverzorgers en veterinaire studenten aanzienlijk is in de herkauwers- en pluimveekliniek. Blootstelling doet zich niet alleen in de stallen voor, maar

ook in de praktijk- en onderwijsruimtes. β(1→3)-glucanen concentraties waren zo nu en dan hoog.

Blootstelling aan bio-aerosolen is eerder beschreven in melkveehouderijen, maar er is weinig bekend over mogelijke bronnen van bio-aerosolen. Het kan worden verondersteld dat type bodembedekking toegepast in de stallen de bio-aerosol blootstelling zou kunnen beïnvloeden. In **hoofdstuk 5** wordt een studie die dit onderzoekt gepresenteerd. Melkveehouderijen met vier typen stalbodembedekking werden geincludeerd in het onderzoek: diep strooisel van zaagsel of compost, rubberen koematrassen of rubber gevulde matten. Persoonlijke blootstelling aan stof, endotoxinen, en β(1→3)-glucanen verschilden significant tussen de stallen waar de verschillende bodembedekkingen werden toegepast ($p<0.05$). De de hoogste concentraties (GM: stof, 1.38 mg m^{-3}; endotoxine , 895 EU m^{-3}; β(1→3)-glucanen, 7.84 mg m^{-3}) werden gevonden in stallen met compost bodembedekking, de laagste in stallen met zaagsel (GM: stof, 0.51 mg m^{-3}, endotoxine, 183 EU m^{-3}; β(1→3)-glucanen, 1.11 µg m^{-3}). De hoogte van blootstelling werd ook beinvloed door diverse extra strooisel bodembedekking gebruikt als aanvulling op de hoofd bodembedekking. Gebruik van plantaardige materialen, met name stro, bleek een belangrijke bron voor β(1→3)-glucanen blootstelling. Een bodembedekking van compost was significant geassocieerd met verhoogde persoonlijke en stationaire bio-aerosol concentraties. Tussen-persoons variatie van de blootstelling werd goed verklaard door de determinanten van blootstelling, terwijl de binnenpersoons, dag-tot-dag, verschillen in concentraties in veel mindere mate konden worden verklaard. Deze studie toont aan dat blootstelling aan endotoxinen, β(1→3)-glucanen, bacteriën en schimmels in melkveehouderijen aanzienlijk waren en verschilden afhankelijk van de toegepaste bodembedekking, wat suggereert dat type bodembedekking een belangrijke voorspeller van bio-aerosol blootstelling is.

Hoofdstuk 6 beschrijft het voorkomen van sensibilisatie voor algemene en dierspecifieke allergenen en respiratoire (allergische) aandoeningen bij studenten diergeneeskunde. Studiespecialisatie, synoniem voor type dieren waarmee contact optreedt gedurende de studie, en duur van de studie werden gebruikt als een proxy van bio-aerosol blootstelling. Nieuw optredende zelf-gerapporteerde allergische symptomen trad op in 8.7% van de populatie, 44% hiervan waren allergische symptomen geassocieerd met dieren. Contact met landbouwhuisdieren was sterk geassocieerd met het optreden van zelf-gerapporteerde symptomen voor diverse allergenen (odds ratio (OR) = 6.9, 95% betrouwbaarheidsinterval (BI) 1.9-25) en dieren (OR = 12, 95%BI 1.4-103). Sensitisatie, de aanwezigheid van IgE antilichamen, voor ten minste één allergeen trad op in 33.1%. De prevalentie van sensitisatie voor 1 of meer allergenen lijkt toe te nemen in de latere jaren van de studiespecialisatie paard. In tegenstelling tot de zelf-gerapporteerde allergieën, neemt de prevalentie van sensitisatie $\geq$ 1 allergeen af bij langere studieduur voor diegenen die

gespecialiseerd zijn in richting landbouwhuisdieren (jaar 3-5: OR = 0.5, 95% CI 0.3-1.1; jaar 6: OR = 0.2 , 95% BI 0.1-0.5). Correctie voor opgroeien op een boerderij, an sich een beschermende factor voor allergie en sensibilisatie, verandert de schattingen niet. Deze studie toont aan dat een verhoogde prevalentie van respiratoire symptomen geassocieerd is met een langere duur van de veterinaire studie. Dit suggereert dat contact met dieren, meer specifiek contact met landbouwhuisdieren, een risicofactor is voor de ontwikkeling van symptomen.

Ten slotte worden in de algemene discussie, **hoofdstuk 7**, de belangrijkste bevindingen van de studies in dit proefschrift besproken in de context van een systematische literatuurstudie van bio-aerosol blootstelling en de daarmee samenhangende effecten op de gezondheid. Uit de weinige studies uitgevoerd om bio-aërosol blootstelling in veterinaire praktijken in kaart te brengen, waaronder die beschreven in dit proefschrift, bleek dat aanzienlijke endotoxinen en β(1→3)-glucanen blootstelling kan optreden bij het hanteren van landbouwhuisdieren en paarden. Blootstellingsniveaus zijn vergelijkbaar met niveaus zoals eerder waargenomen bij boeren welke geassocieerd zijn met gezondheidseffecten van de luchtwegen. Blootstelling aan dierspecifieke allergenen is nauwelijks onderzocht, maar blijkt wel degelijk aanwezig (dit proefschrift). Bio-aerosol gerelateerde gezondheidseffectenstudies zijn niet beschikbaar voor dierenartsen, met name dose-respons studies ontbreken. Echter, vergelijkbaar onderzoek onder veehouders, met name varkenshouders, die blootgesteld zijn aan een vergelijkbare omgeving als dierenartsen, bevestigen dat bio-aërosol blootstelling een verhoogd risico op het ontwikkelen aandoeningen aan de luchtwegen geeft. Blootstelling aan dierlijke allergenen tijdens de veterinaire praktijk kunnen mogelijk allergische reacties veroorzaken. Toch is het optreden van sensitisatie of allergie klachten door dierlijke allergenen slechts weinig beschreven voor veterinairen, behalve het optreden van proefdierallergie als gevolg van blootstelling aan ratten en muizen. Andere bio-aerosol gerelateerde gezondheidsrisico's als gevolg van infectieuze agentia afkomstig van dieren zijn ook beschreven. Concluderend, veterinaire populaties worden blootgesteld aan verhoogde concentraties van bio-aerosolen, zoals endotoxinen, β(1→3)-glucanen, en een aantal specifieke dierlijke allergenen en infectieuze agentia die zoonosen kunnen veroorzaken. Blootstelling aan deze stoffen werd eerder in verband gebracht met allergische en niet-allergische luchtwegklachten bij veehouders, wat suggereert dat vergelijkbare effecten kunnen optreden in veterinaire populaties. Ook voor wat betreft infectieuze risico's worden vergelijkbare zoonosen gevonden bij veterinaire in vergelijking met name veehouders.

Curriculum vita

Sadegh samadi was born on 22nd May 1969 in Shazand, Iran. He grew up in Arak (Iran), where he graduated from high school. He studied at Tehran University of Medical Sciences, where he obtained a BSc degree in Occupational Health. After graduation he joined the occupational health unit at the health center of Arak University of Medical Sciences. He received his MSc degree in Occupational Health from Tarbiat Modares University, Tehran, Iran in 1997. Then he joined the division of health at Paramedical Faculty in Arak University of Medical Sciences where he worked as a teaching staff member. He was twice awarded during his career: the yearly lecturer of Paramedical Faculty as well as yearly researcher in Markazy province in 2003. He participated in an exam which held by Health of Ministry and Medical Education of Iran and finally he was awarded a grant to pursue his education as a PhD abroad. From October 2006 he has started to working on his PhD at the Division of Environmental Epidemiology (EEPI) of the Institute for Risk Assessment Sciences (IRAS), Utrecht University, the Netherlands.

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