

Original Article

Bovine Allergens in a Ruminant Clinic and Dairy Barns: Exposure Levels, Determinants, and Variability

Sadegh Samadi^{1,2*}, Dick J.J. Heederik², Eva Zahradnik³,
Nancy N.J. Rietbroek⁴, Frank van Eerdenburg⁴, Ingrid Sander³,
Monika Raulf³ and Inge M. Wouters²

¹Department of Occupational Health, Health Faculty, Arak University of Medical Sciences, Arak, Iran;

²Division of Environmental Epidemiology, Institute for Risk Assessment Sciences (IRAS), Utrecht University, P.O. Box 80178, 3508 TD Utrecht, The Netherlands; ³Institute for Prevention and Occupational Medicine of the German Social Accident Insurance (IPA), Institute of the Ruhr-Universität Bochum, Bochum, Germany;

⁴Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, P.O. Box 80178, 3584 CN Utrecht, The Netherlands

*Author to whom correspondence should be addressed. Tel: +31-30-253-9480; fax +31-30-253-9499; e-mail: samsad69@gmail.com

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Abstract

Background: Dairy farmers may develop specific sensitization and allergic airway diseases due to bovine allergens. However, dose–response relationships are lacking, and as yet little is known on bovine allergen exposure levels.

Objective: To investigate bovine allergen exposure levels in a ruminant clinic and dairy barns, and to assess exposure determinants and variability of exposure.

Methods: Samples were collected using active and passive airborne dust measurements in a ruminant clinic and several dairy barns. Bovine allergen levels were determined by sandwich enzyme-linked immunosorbent assay. Linear mixed models were applied to explore the association between bovine allergen exposure levels and potential exposure determinants. Day-to-day within-worker and between-worker exposure variability was determined, as well as how exposure determinants affect exposure variability.

Results: Bovine allergens were measurable in all samples. Personal bovine allergen exposure levels in the ruminant clinic ranged from 0.10 to 24.8 $\mu\text{g}/\text{m}^3$, geometric mean (GM) 1.34 $\mu\text{g}/\text{m}^3$. Exposure levels varied dependent on job titles. Personal exposure levels in dairy barns ranged from 0.10 to 46.8 $\mu\text{g}/\text{m}^3$, GM 1.47 $\mu\text{g}/\text{m}^3$. Type of bedding materials in the barns appeared to be a significant determinant of bovine allergen levels. Compost bedding, particularly, increased allergen levels. Milking by robot was the most important determinant explaining between-worker exposure variability, while

bedding was important as well. Bovine allergen levels in stationary measurements were somewhat lower than personal measurements (GM ratio 0.47). Bovine allergens could be readily detected in electrostatic dust-fall collector measurements.

Conclusion: This study provides insight in bovine allergen exposure levels and their determinants, which is a first step to investigate dose–response relationships between sensitization/allergy associated with exposure to bovine allergen levels in future studies.

Keywords: allergy; bovine allergens; dairy barn; determinant; exposure; ruminant clinic; sensitization; variability

Introduction

Workers in agricultural environment settings dealing with animals may be exposed to a variety of proteins originating from animals and plants (Samadi *et al.*, 2013). Proteins derived from animals are classified as high-molecular-weight components, and are considered to be potent allergens (Samadi *et al.*, 2013). Laboratory animal-derived allergens (for rat the main allergen is Rat n 1 and for mouse, Mus n 1) were found in high concentrations in samples of airborne dust at workplaces, and thus sensitization to laboratory animal allergens and subsequent allergic airway diseases are well-known occupational disorders due to animal-derived allergens (Seward, 1999). Farm animals (e.g. cow and horse) are other well-known allergen producers (Chapman and Wood, 2001). Exposure to farm animals, particularly cow as the farm animal most commonly used, takes place in certain jobs within animal settings such as dairy farmers and veterinarians. Several different proteins have been identified as bovine antigens, but only some of them have been characterized as bovine allergens that can be found in cow hair and dander (Prah *et al.*, 1978). The predominant bovine allergen is Bos d 2 (*Bos domesticus* 2) (Rautiainen *et al.*, 1997), which belongs to a protein family termed lipocalins (Mantjarvi *et al.*, 1996). Bos d 2 can be found mainly in hair and dander.

Occupational dermatitis related to bovine dander was first described in 1948 (Epstein, 1948). Since then, several other authors published prevalence of bovine allergen sensitization as well as allergic airway diseases as a consequence of bovine allergen exposure (Terho *et al.*, 1985; Rautalahti *et al.*, 1987; Virtanen *et al.*, 1988b; Ylonen *et al.*, 1992; Doekes, 2000; Spiewak, 2001; Heutelbeck *et al.*, 2007; Samadi *et al.*, 2012a). A study among Finish dairy farmers by Rautalahti *et al.* (1987) revealed that 14% of farmers were sensitized to bovine allergen, as observed likewise in later Finnish studies (Terho *et al.*, 1985; Virtanen *et al.*, 1988b; Ylonen *et al.*, 1992). This finding has been followed by a further study showing the importance of bovine-derived proteins as the primarily causative agent for development of occupational asthma, accounting for 609 out of 2609 occupational asthma

cases between 1989 and 1995 (Karjalainen *et al.*, 2000). In contrast, no associations with onset of allergic respiratory symptoms or diseases and bovine allergen sensitization were observed among Dutch, Danish, and Polish dairy farmers (Doekes, 2000; Spiewak, 2001), and a recent study reported cow sensitization to be uncommon in dairy farmers in Denmark (Schlunssen *et al.*, 2015). Although there is no clear answer to the large discrepancies between studies, healthy worker selection bias may play a role and differences in exposure levels of obvious allergens may be part of the explanation as well.

Few studies investigated levels of bovine allergen exposures in agricultural environment settings (Virtanen *et al.*, 1988a; Ylonen *et al.*, 1994; Williams *et al.*, 2011; Zahradnik *et al.*, 2011; Schlunssen *et al.*, 2015; Bohlandt *et al.*, 2016; Williams *et al.*, 2016). A study based on personal and stationary exposure measurements in 18 Finish cowsheds revealed that bovine epithelial antigen in total dust fraction was detectable with a mean level of 0.46 $\mu\text{g}/\text{m}^3$ in personal measurements and a mean between 0.35 and 0.73 $\mu\text{g}/\text{m}^3$ in stationary measurements (Virtanen *et al.*, 1988a). Three recent studies using electrostatic dust-fall collectors (EDCs) for settled airborne dust demonstrated that bovine allergens were measurable in dairy farms, with substantially higher exposure levels in cow stables than within dairy farmers' dwellings (Zahradnik *et al.*, 2011; Schlunssen *et al.*, 2015; Bohlandt *et al.*, 2016). All these studies prove the presence of bovine allergens in dairy barns, but still little is known on personal bovine allergen exposure levels of individuals occupationally involved within different settings in cow facilities. Therefore, the objective of this study was to investigate exposure levels of bovine allergens among veterinary medicine students and workers involved in a ruminant clinic and dairy barns. Exposure determinants and variability of exposure levels were evaluated.

Materials and methods

Exposure measurements

Exposure measurements were carried out at a ruminant clinic and 23 dairy barns as described previously in detail (Samadi *et al.*, 2011, 2012b). Briefly, the ruminant

clinic is sited at the Faculty of Veterinary Medicine of Utrecht University, the Netherlands. This clinic is subversive to education and research. It is a modern building comprising of cow stables, cow examination rooms and a canteen. Regular dairy barns included in the study were located near the city of Utrecht, and consisted of a confined building with freestalls. The floor of each barn consisted of a concrete slates. The freestalls were bedded with four different beddings: compost, sawdust, rubber filled mattress, and rubber mats. On the mattress and mats, an extra top layer of bedding material was also applied such as chopped straw or sawdust with the purpose of keeping cows clean and dry.

Personal inhalable dust was collected repeatedly over time for individuals dependent on job title, work-shift, and location. Repeated stationary sampling took place at different locations as well. A portable pump operating at a flow rate of 3.5 l/min was applied for personal and stationary inhalable dust measurements, using a plastic conical inhalable sampler equipped with glass fiber filters (Whatman International Ltd, Maidstone, UK). In dairy barns, the measurements were performed throughout a full work-shift, in most cases from 6:00 a.m. until 2:00 pm. Measurements for workers in the ruminant clinic were carried out during three different full work-shifts: daytime, 8:00–16:00; morning, 6:00–14:00; and afternoon, 14:00–22:00. Students of veterinary medicine are categorized based on their study phase in preclinical students (1–4th years), clinical students performing uniform internships (5th year), and clinical students performing differential internships (6th year). For students, measurements were conducted throughout practical teaching work. The lower limit of detections (LODs) for personal and stationary dust measurements ranged from 0.12 to 0.13 mg per filter. Also, 10.1 and 13.9% of personal and stationary inhalable dust measurements were, respectively, below these LODs.

EDCs collecting settled airborne dust were only applied within the ruminant clinic due to logistic constraints (Noss *et al.*, 2008). Each EDC sampler consisted of two electrostatic cloths, with an exposed area of 0.0209 m² for each cloth, of which one was used for allergen analysis. The EDCs were placed in different areas within the clinic at roughly 1.7 m above the ground level and left exposed for 14 days.

Extraction and detection of bovine allergens

Sequential extraction has been applied as previously described elsewhere (Schram *et al.*, 2005), of which the supernatant of the second extraction step was used for bovine allergen analysis. Briefly, each personal or stationary dust sample was first eluted in 5 ml pyrogen-free

water containing 0.05% Tween-20 (PBST). EDC-settled dust samples were extracted in 20 ml. Samples were shaken for 1 hr in an end-over-end roller at room temperature, following centrifugation for 15 min at 1000 g. 10% of supernatant was harvested for endotoxin analyses. This amount was replenished with 10 times concentrated phosphate buffered saline as a second extraction step. Samples were again shaken for 1 hr in an end-over-end roller at room temperature, following centrifugation for 15 min at 2000 g. Ten percent of supernatant was stored in aliquots at –20°C until allergen analysis.

The levels of bovine allergens were determined through a sensitive sandwich enzyme-linked immunosorbent assay as described by Zahradnik *et al.* (Zahradnik *et al.*, 2011). In brief, microtiter plates (Thermo Fisher Scientific, Roskilde, Denmark) were coated over night with 100 µl/well anti-cow hair polyclonal antibodies (pAbs) and blocked for 2 hr with 200 µl/well of PBST with 1% gelatin. Afterwards, the plates were incubated for 1 hr at 22°C with standards, assay controls and samples diluted in PBST. A standard curve was produced using a commercial cow dander extract (Allergon, Angelholm, Sweden) over the concentrations range 0.08–10 ng/ml. Cow shed settled dust and wheat flour extract were used, respectively, as positive and negative controls. The plates were incubated for 1 hr at 22°C with biotinylated anti-cow hair polyclonal antibody, followed by streptavidin–peroxidase conjugate (Poly-HRP80-SA, Fitzgerald, Concord, MA, USA) and finally the plates were developed with ABTS substrate (Sigma-Aldrich, Steinheim, Germany). The optical density (OD) was read at 414 nm. Sample concentrations were interpolated from a four-parameter fitted standard curve using Softmax Pro 4.7.1 (Molecular Devices, Sunnyvale, CA, USA). The lower limit of detection (LOD) was the concentration corresponding to the minimal value of the four-parameter curve fit function plus the 6-fold standard deviation of the zero standard. Each sample was analyzed using three serial dilutions in PBST (1:50, 1:100 and 1:200), and the mean of diluted concentrations was utilized as the final concentration. Bovine allergen levels were expressed as µg/m³ or µg/mg of dust for personal and stationary samples, and µg/m² for EDC-settled dust samples. The average LOD of the allergen assay was 0.118 ng/ml. All field blank samples had bovine allergen levels below this LOD. A total of 336 samples were analyzed. The bovine allergen levels of all dust measurements exceeded the LOD of the allergen assay.

Statistical analysis

Statistical analyses were carried out applying statistical software SAS, version 9.4 (SAS Institute, Cary, NC, USA).

The values of dust measurements below the LODs were replaced using imputation and maximum likelihood estimation (Lubin *et al.*, 2004). This method supposes that the probability distribution of values below the LOD pursue the same as observed data and substitute each non-detectable value with a random value between zero and the estimated LOD. Relationships between dust and allergen levels were explored using Pearson correlation coefficients. Linear mixed models were fitted using the log-transformed bovine allergen levels. A forward step-wise modeling procedure was used to explore the influential exposure determinants. Variables were held in the final model when they were significantly associated with exposure ($P < 0.05$). ‘Worker’ was considered a random effect, while ‘type of bedding materials’ and other determinants were assumed as fixed effects. Exposure variability was modeled as personal (between-worker variability [σ_{bw}^2] in average levels) and temporal (within-worker variability [σ_{ww}^2] from day-to-day) variability. The estimation method applied was the restricted maximum likelihood method.

Results

Exposure levels

In this study we were able to detect levels of bovine allergens in all personal and stationary measurements even with non-detectable levels of dust on some filters. The distribution of personal exposure measurements for both inhalable dust and bovine allergens stratified by job title and type of bedding are shown in Table 1. Overall, personal exposure levels to bovine allergens ranged from 0.10 to 46.8 $\mu\text{g}/\text{m}^3$, with a GM of 1.39 $\mu\text{g}/\text{m}^3$. Significant differences in bovine allergen levels were observed between job titles for students ($P < 0.0001$), while this was not significant for inhalable dust. The highest bovine allergens levels appeared to be among preclinical students with a GM of 3.16 $\mu\text{g}/\text{m}^3$ (range 0.90–24.8 $\mu\text{g}/\text{m}^3$), which was roughly five times higher than the GM observed for uniform students (0.65 $\mu\text{g}/\text{m}^3$). The levels of bovine allergens for workers within the ruminant clinic were the highest during the afternoon shift (GM 1.85 $\mu\text{g}/\text{m}^3$), roughly double the exposure during the morning (GM 0.93 $\mu\text{g}/\text{m}^3$) and daytime (GM 1.14 $\mu\text{g}/\text{m}^3$) shifts, but these differences were not statistically significant ($P > 0.05$). Significant differences in personal bovine allergen levels ($P < 0.05$, Table 1) were observed between barns using different bedding types. The highest levels to bovine allergens (GM 2.95 $\mu\text{g}/\text{m}^3$) were observed in barns using compost bedding and these levels were significantly higher than the levels in barns with sawdust bedding (GM ratio 4.8, $P < 0.05$), while other

post hoc comparisons based on bedding types were not significant ($P > 0.05$). The estimated bovine allergen content levels expressed as $\mu\text{g}/\text{mg}$ of dust ranged from 0.05 to 20.3, with a GM of 1.96 $\mu\text{g}/\text{m}$. The pattern of allergen content ($\mu\text{g}/\text{mg}$) levels over job title, location and work-shift were different than the pattern for allergen concentration ($\mu\text{g}/\text{m}^3$), suggesting that dust formation may partly drive the observed higher levels with certain determinants.

Stationary levels of bovine allergens ranged from 0.28 to 35.6 $\mu\text{g}/\text{m}^3$, with a GM of 0.66 $\mu\text{g}/\text{m}^3$ (Table 2). Overall, bovine allergen levels of stationary measurements were somewhat lower than levels in personal measurements (GM ratio 0.47). The GM bovine allergen level in cow stables within the ruminant clinic was twice as high as in examination rooms (GM ratio 2.10, $P < 0.05$). Interestingly, the overall bovine allergen level in dairy barns was four times as high as the level in the cow stable within the ruminant clinic ($P < 0.05$).

The results of bovine allergen levels in airborne-settled dust samples are presented in Table 3. Bovine allergen levels in cow barns within the ruminant clinic were significantly higher than in examination rooms (GM ratio 3.70). Bovine allergen exposure in the canteen was detectable, but far lower than in examination rooms (GM ratio 0.10).

Comparisons between inhalable dust and bovine allergens

Moderate to weak, but significant, Pearson correlations were observed between personal inhalable dust levels and bovine allergen levels ([ruminant clinic: students, $r = 0.50$, $P < 0.0001$; workers, $r = 0.37$, $P = 0.03$] and [dairy barns: workers, $r = 0.64$, $P < 0.0001$]). Correlations for stationary measurements were in the same range (ruminant clinic: $r = 0.48$, $P = 0.002$; and dairy barns: $r = 0.60$, $P < 0.0001$).

Determinants and variability of exposure levels

Results of the mixed-effect models for personal and stationary bovine allergen levels in different barns in relation to potential exposure determinants are presented in Table 4. Looking at mixed-effect models for personal samples, compost appeared to be the strongest significant determinant of personal exposure to bovine allergens (a factor of 6.11) after adjusting for determinants milking by robot and surface size as square meters per cow. This means that using compost bedding in barns leads to more than six times higher personal exposure levels compared with saw dust bedding. Also rubber-filled mattress and rubber mats resulted in higher personal exposure levels than saw dust bedding. In personal

Table 1. Personal inhalable dust and bovine allergen levels for workers and students in the ruminant clinic and workers in dairy barns stratified by job title and type of bedding.

	Dust, mg/m ³					Allergen, µg/m ³					Allergen, µg/mg of dust					
	N	ND	AM	GM	GSD	Range	ND	AM	GM	GSD	Range	ND	AM	GM	GSD	Range
Ruminant clinic																
Students																
Preclinical	25	7	0.52	0.43	1.8	<LOD-1.48	—	4.33	3.16	2.1	0.90-24.8	—	8.94	7.26	2.1	0.93-20.3
Uniform	25	11	0.38	0.33	1.9	<LOD-0.90	—	0.88	0.65	2.3	0.10-2.35	—	2.47	1.96	2.1	0.45-6.72
Differential	14	2	0.48	0.38	2.0	<LOD-1.04	—	3.06	1.42	3.8	0.12-16.0	—	5.35	3.70	3.1	0.17-15.3
Total	64	20	0.46	0.38	1.9	<LOD-1.48	—	2.70	1.43	3.2	0.10-24.8	—	5.63	3.76	2.7	0.17-20.3
Workers																
Daytime	5	—	5.34	2.40	3.6	0.73-20.5	—	1.44	1.14	2.3	0.35-2.48	—	0.68	0.48	3.0	0.08-1.35
Morning	17	—	3.24	1.50	3.7	0.14-20.8	—	2.15	0.93	3.6	0.10-13.6	—	1.19	0.62	3.4	0.05-6.53
Afternoon	10	—	3.58	1.35	3.9	0.37-16.1	—	2.88	1.85	2.7	0.47-8.55	—	2.83	1.37	5.1	0.06-11.0
Total	32	—	3.67	1.56	3.6	0.14-20.8	—	2.27	1.19	3.2	0.10-13.6	—	1.62	0.76	3.9	0.05-11.0
Total	96	20	1.53	0.61	3.1	<LOD-20.8	—	2.56	1.34	3.2	0.10-24.8	—	4.29	2.21	3.9	0.05-20.3
Dairy barns																
Workers																
Compost	15	—	1.60	1.38	1.8	0.41-3.06	—	4.33	2.95	2.3	1.04-16.7	—	2.43	2.14	1.7	1.14-6.39
Sawdust	15	2	0.73	0.51	2.6	<LOD-2.51	—	1.18	0.61	3.2	0.10-5.56	—	1.86	1.13	2.9	0.11-6.26
Rubber filled mattress	11	—	1.33	0.86	2.3	0.32-6.86	—	2.84	1.83	2.4	0.55-11.9	—	2.40	2.12	1.8	0.54-4.31
Rubber mats	21	—	1.35	0.99	2.2	0.28-5.70	—	6.16	1.50	5.1	0.13-46.8	—	2.67	1.51	3.1	0.28-10.7
Total	62	2	1.26	0.89	2.3	LOD-6.86	—	3.92	1.47	3.8	0.10-46.8	—	2.37	1.63	2.5	0.11-10.7
Overall	158	22	1.42	0.71	2.8	>LOD-20.8	—	3.09	1.39	3.4	0.10-46.8	—	3.54	1.96	3.4	0.05-20.3

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

Table 2. Stationary inhalable dust and bovine allergen levels in the ruminant clinic and in dairy barns stratified by location and type of bedding.

	N	Dust, mg/m ³				Allergen, µg/m ³				Allergen, µg/mg of dust						
		ND	AM	GM	GSD	Range	ND	AM	GM	GSD	Range	ND	AM	GM	GSD	Range
Ruminant clinic																
Examination rooms	11	6	0.12	0.11	1.4	<LOD-0.22	—	0.21	0.11	2.9	0.03-1.12	—	1.59	0.99	2.7	0.31-5.06
Stables	25	1	0.21	0.19	1.6	<LOD-0.49	—	0.28	0.23	1.9	0.08-0.74	—	1.48	1.21	1.9	0.44-3.68
Total	36	7	0.18	0.15	1.6	<LOD-0.49	—	0.26	0.18	2.4	0.03-1.12	—	1.51	1.14	2.1	0.31-5.06
Dairy barns																
Compost	45	—	0.61	0.47	2.2	0.08-1.59	—	1.32	0.98	2.3	0.16-4.18	—	2.57	2.09	2.0	0.37-7.39
Sawdust	25	5	0.34	0.22	2.5	<LOD-2.35	—	1.05	0.67	2.8	0.12-3.57	—	4.10	2.79	2.5	0.61-13.6
Rubber filled mattress	34	3	0.39	0.26	2.3	<LOD-2.73	—	3.95	1.31	3.9	0.09-35.6	—	7.28	4.93	2.6	0.48-29.6
Rubber mats	27	7	0.28	0.18	2.4	<LOD-1.27	—	2.90	0.76	4.3	0.06-33.5	—	5.66	3.83	2.4	0.68-26.4
Total	131	15	0.43	0.28	2.5	<LOD-2.73	—	2.27	0.93	3.2	0.06-35.6	—	4.72	3.13	2.5	0.37-29.6
Overall	167	22	0.37	0.22	2.4	<LOD-2.73	—	1.84	0.66	3.7	0.03-35.6	—	4.03	2.52	2.6	0.31-29.6

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

samples, milking by robot was significantly associated with lower bovine allergen levels (a factor of 0.35) compared with a traditional parlor. Similarly, increased surface size per cow was associated with slightly lower bovine allergen levels (a factor of 0.91). Looking at mixed-effect models for stationary samples, compost and rubber mats were as the strongest determinants of bovine allergens (a factor of 1.90). Like observed for personal samples, an increased surface size per cow was related to lower bovine allergen levels (a factor of 0.91), while milking by robot was not significantly associated to allergen levels. For personal allergen content no significant determinants were identified, which suggest that no major allergen content differences exist for investigated determinants.

Table 5 presents the estimated within- and between-worker variance components. The between-worker variance, for exposure presented as µg/m³, was moderately reduced (a reduction of 46%) when all potential determinants were included as fixed effects simultaneously in the model. Milking by robot explained a considerable part of exposure variability (22%), followed by bedding types explaining 18%. The within-worker variance was not affected by determinants in the models. Between-worker variances of exposure were lower for allergen content compared with variances in allergen concentrations. When all determinants were included simultaneously in the models, the reduction of between-worker variance was only 22%.

Discussion

This study comprehensively quantified personal and stationary inhalable exposure levels of bovine allergens in a ruminant clinic as well as in several dairy barns. Additionally, airborne settled bovine allergen levels were measured using EDCs. In general, bovine allergens were measurable in all samples collected at different locations, despite levels of dust being non-detectable in some cases. All dust collection methods, including active and passive measurements, showed a wide range of bovine allergen levels. In the ruminant clinic, allergen levels largely differed based upon job titles and locations. Bedding appeared to be a significant determinant of bovine allergen exposure levels in dairy barns, especially compost bedding as the most influential determinant of exposure increase. Milking by robot as well as type of bedding were important exposure determinants explaining in part the variability in exposure.

Personal exposure levels to bovine allergens among workers measured in the ruminant clinic (GM 1.19 µg/m³) and dairy barns (GM 1.47 µg/m³) were roughly two to

Table 3. Airborne settled bovine allergen levels ($\mu\text{g}/\text{m}^2$) in the ruminant clinic stratified by location.

	N	ND	AM	GM	GSD	Range
Canteen	2	—	5.40×10	5.40×10	1.0	5.35×10^4 to 5.44×10
Examination rooms	3	—	5.70×10^2	5.68×10^2	1.1	5.09×10^3 to 6.45×10^2
Stables	6	—	5.09×10^3	2.10×10^3	9.2	2.82×10^4 to 1.17×10^4
Total	11	—	2.94×10^3	7.55×10^3	8.3	2.82×10^4 to 1.12×10^4

N, number of measurements; ND, number of non-detectable measurements; AM, arithmetic mean; GM, geometric mean; GSD, geometric standard deviation.

Table 4. Parameter estimates in the final mixed effects model of the log-transformed personal and stationary exposure to bovine allergens in dairy barns in relation with exposure determinants.

Determinant of exposure	Allergen, $\mu\text{g}/\text{m}^3$				Allergen, $\mu\text{g}/\text{mg}$ of dust				
	B	SE	P value	FEC	B	SE	P value	FEC	
Personal samples	Intercept ^a								
	Type of bedding								
	Compost	1.81	0.57	0.003	6.11	—	—	—	—
	Rubber-filled mattress	0.69	0.61	0.267	1.99	—	—	—	—
	Rubber mats	0.66	0.53	0.223	1.93	—	—	—	—
	Sawdust	Ref	Ref	Ref	Ref	—	—	—	—
	Milking by robot (Yes vs. no)	-1.06	0.45	0.024	0.35	—	—	—	—
	Surface size per cow	-0.10	0.05	0.035	0.91	—	—	—	—
Stationary samples	Intercept ^a								
	Type of bedding								
	Compost	0.64	0.30	0.038	1.90	-0.45	0.23	0.048	0.64
	Rubber-filled mattress	-0.28	0.32	0.369	0.76	-0.39	0.24	0.105	0.68
	Rubber mats	0.66	0.29	0.027	1.96	0.30	0.22	0.172	1.35
	Sawdust	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
	Milking by robot (Yes vs. no)	—	—	—	—	—	—	—	—
	Surface size per cow	-0.10	0.03	0.002	0.90	0.05	0.02	0.043	1.05

β , regression coefficient; SE, standard error; FEC, factor of exposure change.

^aThe intercept presents the exposure level for workers working in barns using sawdust after adjusting milking by robot and surface size per cow.

three times higher as the levels (mean $0.46 \mu\text{g}/\text{m}^3$) previously reported in Finish cowsheds (Virtanen *et al.*, 1988a). Similarly, the stationary exposure levels of bovine allergens measured in dairy barns (GM $0.93 \mu\text{g}/\text{m}^3$) were also somewhat higher than the levels (means between 0.35 and $0.73 \mu\text{g}/\text{m}^3$) reported in the Finish study, while the observed levels in the cow barns within the ruminant clinic (GM $0.23 \mu\text{g}/\text{m}^3$) appeared to be relatively low (Virtanen *et al.*, 1988a). Comparison of our results with Finish study needs to be made with care, as dust sampling methods are different (inhalable versus total dust fraction). More importantly, different allergen assays were applied, and a comparison between the assays is not available. Systematic differences between allergen assays have been reported before for other inhalable occupational allergens (Hollander *et al.*, 1999; Bogdanovic *et al.*, 2006).

Within the ruminant clinic, levels of bovine allergens for workers in the afternoon shift were notably higher than during the morning shift. Similar data are not available from other studies on bovine allergen exposures. Higher levels of bovine allergens during the afternoon shift contrast to previous findings on endotoxin exposure levels from the same samples (Samadi *et al.*, 2011). This suggests that determinants of exposure for bovine allergens and endotoxin are not similar. This difference is probably to be a result of different tasks performed during work-shifts. Tasks performed by ruminant clinic workers in the afternoon shift were more observational resulting in walking close to cows, suggesting that more time spent in close contact to cows could be important determinants of cow allergen exposure.

Among students' job titles, highest levels of bovine allergens were observed in preclinical students.

Table 5. Variance components and confidence intervals (95% CI) of the log-transformed personal exposure to bovine allergens in dairy barns.

Allergen levels	Determinants	σ_{bw}^2 (95% CI)	σ_{ww}^2 (95% CI)	Reduction in σ_{bw}^{2A}	Reduction in σ_{ww}^{2B}
$\mu\text{g m}^{-3}$	Random effect model only	0.94 (0.51–2.31)	0.79 (0.53–1.32)	—	—
	Bedding type	0.77 (0.39–2.19)	0.79 (0.53–1.32)	18	0
	Milking by robot	0.73 (0.37–2.07)	0.79 (0.53–1.33)	22	0
	Surface size per cow	0.98 (0.53–2.43)	0.78 (0.52–1.31)	-4	1
	Bedding type + milking by robot	0.61 (0.28–2.07)	0.80 (0.53–1.34)	35	-1
	Bedding type + milking by robot + surface size per cow	0.51 (0.23–1.97)	0.78 (0.51–1.32)	46	1
	Random effect model only	0.18 (0.06–1.63)	0.67 (0.45–1.10)	—	—
	Bedding type	0.16 (0.05–2.16)	0.67 (0.45–1.10)	11	0
$\mu\text{g per mg of dust}$	Milking by robot	0.16 (0.05–2.35)	0.68 (0.46–1.12)	11	-1
	Surface size per cow	0.19 (0.07–1.62)	0.68 (0.46–1.11)	-5	-1
	Bedding type + milking by robot	0.16 (0.05–2.70)	0.68 (0.46–1.12)	11	-1
	Bedding type + milking by robot + surface size per cow	0.14 (0.04–4.31)	0.69 (0.46–1.14)	22	-3

σ_{ww}^2 , between-worker variance; σ_{bw}^2 , within-worker variance; CI, confidence interval.

^A $= [(\sigma_{ww}^2 \text{ empty model} - \sigma_{ww}^2 \text{ full model}) / \sigma_{ww}^2 \text{ empty model}] \times 100$.

^B $= [(\sigma_{bw}^2 \text{ empty model} - \sigma_{bw}^2 \text{ full model}) / \sigma_{bw}^2 \text{ empty model}] \times 100$.

Surprisingly, exposure for this group of students was almost three times higher than exposure of workers within the ruminant clinic. Comparison with other studies is not possible. Nonetheless, preclinical students had relatively much more close contact to cows compared with the other students and also workers in the clinic, because preclinical students were measured during their practical clinically examining the cows. The doors of teaching rooms are often closed causing less ventilation. Furthermore, the sampling duration was much shorter for these preclinical students compared with the other students, resulting in only high-exposure task measurements. So all these likely lead to higher bovine allergen exposures. In the Netherlands generally only small number of farmers are working at the dairy farm, similar so, only small number of workers in the clinic are animal caretakers, further distinction in job titles within the farm workers and animal clinic workers is therefore not possible.

We found significant correlations between the levels of bovine allergens and inhalable dust levels, as previously observed in a companion animal setting for cat and dog allergens and inhalable dust (Samadi *et al.*, 2010). The type of bedding material applied in dairy barns affected the levels of both personal and stationary bovine allergen exposures. These differences in levels due to bedding materials are comparable with those levels of laboratory animal allergens in relation to bedding materials applied in laboratory animal facilities (Kaliste *et al.*, 2004). When looking at bovine allergen content based on bedding type, the patterns of allergen in $\mu\text{g}/\text{mg}$ of dust were different than observed for allergen levels as $\mu\text{g}/\text{m}^3$. The allergen content (in $\mu\text{g}/\text{mg}$ of dust) in personal samples for workers in dairy barns was in the same range for all bedding types. This suggests that bedding materials can influence the allergen exposure levels, possibly through dust formation including allergens, rather than through altered allergen content.

Slightly higher personal exposure levels of bovine allergens compared with those levels obtained by stationary measurements is in concordance with results of previous studies for other agents (Berger *et al.*, 2005; Samadi *et al.*, 2009, 2010, 2011, 2012b). This disparity can be elucidated by the fact that stationary measurements generally are performed at larger distances from the source. However, the results of stationary exposure monitoring for bovine allergens might be appropriate, for instance, to study effectiveness of measures diminishing exposure.

In this study, bovine allergen was measurable in passive airborne dust samples collected with EDCs within the ruminant clinic including cow stables, examination

rooms and canteen. The allergen levels varied about 500-fold between different EDC samples collected in cow stables, which is in accordance with previous findings of wide range of bovine allergen levels obtained by EDCs in German and Danish farms (Zahradnik *et al.*, 2011; Schlunssen *et al.*, 2015). This wide range of EDC levels could be matched to similar wide ranges of bovine allergen levels observed in our personal and stationary measurements. The convenience of EDC settled airborne dust sampling, as an alternative technique versus personal and stationary exposure monitoring, is an apparent advantage for application at large scale. Exposure assessments in agricultural environmental settings such as animal farms are costly and time consuming since such settings spread over a large area and a limited number of workers within each farm are involved. So using EDC might be an appropriate method where the collection of personal or stationary exposure monitoring may be a problem. The detection of low levels of bovine allergens in the canteen are in accordance with the previously reported detection of low levels of cat and dog allergens in a similar setting; the canteen of a companion animal hospital (Samadi *et al.*, 2010). This finding was parallel to results of two other studies, which reported detection of bovine allergens in dwellings of dairy farmers (Zahradnik *et al.*, 2011; Schlunssen *et al.*, 2015) and both studies proposed that bovine allergens are probably spread from the workplace by means of the clothes and hair of farmers. The presence of bovine allergens in canteen is most likely attributed to allergen transfer from the work environments through the clothes and hair of individuals. As yet, it is not known whether those low levels of exposure possess a health risk, as dose-response relationships for bovine allergen exposure and allergic outcomes are lacking. However, sensitization of family members to occupational allergens through household exposure has been reported (Liccardi *et al.*, 2011).

Insight in exposure variation and their determinants is important. Between-worker variance in bovine allergen exposure was increased compared to the within-worker variance for measurements in dairy barns, indicating that between-worker variability in average exposure levels was higher than the day-to-day differences. The same holds for endotoxin and $\beta(1\rightarrow3)$ -glucan exposures in the same dairy barns (Samadi *et al.*, 2009). With only three determinants: bedding material used, cow surface area and presence/absence of a milking robot, 46% of total between-worker variability of bovine allergen exposures could be explained. The explained variance is considerably higher than the 12% explained variability reported by Schlunssen *et al.* in a Danish study using EDC samples despite inclusion of many other suspected

determinants such as type of stable, animal density and ventilation (Schlunssen *et al.*, 2015). However, this comparison should be made with caution since the Danish study included random samples of dairy barns while we included specific samples with known differences in bedding type and milking by robot. Milking by robot versus traditional parlor explained 22% of between-worker variability. We previously reported that milking by robot was a determinant of elevated dust exposure (Samadi *et al.*, 2012b) as most likely workers spent more time on other dusty tasks. Paradoxically to dust, the levels of bovine allergens were negatively associated with the application of milking by robot, indicating that dust does only partly act as a substrate for bovine allergens perhaps due to less contact to cows. The increased levels of bovine allergens for bedding in personal and stationary models suggest the importance of bedding as a source. Given the personal samples were more affected than stationary samples suggest that other unexplored determinants such as type of tasks performed may be important as well. The within-worker variance could not be explained following inclusion of exposure determinants in the models, as the included determinants did not vary over time.

There is evidence that dairy farmers may get sensitized to bovine allergens (Rautalahti *et al.*, 1987; Virtanen *et al.*, 1988b; Ylonen *et al.*, 1992; Doekes, 2000; Spiewak, 2001), suggesting that exposure to bovine allergens can be considered a risk factor for sensitization although conflicting results have been reported as well (Schlunssen *et al.*, 2015). In general no dose-response relationships are known. As the majority of dairy farmers often initiates working during childhood and usually continues working after 65 years of age, cumulative exposure to bovine allergen among dairy farmers could possibly be higher than the normal years of employment. The collected exposure data from this study could be a first step to explore the incidence/prevalence of sensitization and subsequent allergic airway diseases associated with the levels of bovine allergen exposure.

Conclusion

This study provides insights in exposure levels of bovine allergens using active and passive airborne dust measurements. The findings showed that workers and students in veterinary medicine were likely exposed to a wide range of bovine allergen levels. Exposure levels varied dependent on job titles, being highest among preclinical students who had the closest contact with the cows. Milking by robot or in a conventional parlor and type of

bedding material appeared to be significant determinants of exposure in dairy barns. The exposure data collected in this study can be a first step to take investigation of exposure–response relations between bovine allergen exposure and development of bovine sensitization and/or bovine allergen-induced asthma a step forward.

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