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Exposure-Affecting Factors of Dairy Farmers' Exposure to Inhalable Dust and Endotoxin

Ioannis Basinas[1*], Torben Sigsgaard[1], Mogens Erlandsen[2], Nils T. Andersen[1], Hisamitsu Takai[3], Dick Heederik[4], Øyvind Omland[1,5], Hans Kromhout[4] and Vivi Schlünssen[1,6]

1.Department of Public Health, Section for Environment, Occupation and Health, Danish Ramazzini Center, Aarhus University, Bartholins Allé 2, bg 1260, 8000 Aarhus C, Denmark
2.Department of Public Health, Institute of Biostatistics, Aarhus University, Bartholins Allé 2, 8000 Aarhus C, Denmark
3.Department of Engineering, Aarhus University, Finlandsgade 22, 8200 Aarhus N, Denmark
4.Division of Environmental Epidemiology, Institute for Risk Assessment Sciences (IRAS), Utrecht University, PO Box 80178, 3508 TD Utrecht, The Netherlands
5.Department of Occupational Medicine, Danish Ramazzini Center, Aalborg University Hospital, Havrevangen 1, 9000 Aalborg, Denmark
6.Department of Occupational Medicine, Danish Ramazzini Center, Aarhus University Hospital, Nørrebrogade 44, 8000 Aarhus C, Denmark
*Author to whom correspondence should be addressed. Tel: +45-8716-8016; fax: +45-8716-7307; e-mail: ibas@mil.au.dk
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ABSTRACT

Introduction: Studies on determinants of dairy farmers' exposure to dust and endotoxin have been sparse and so far none has addressed the combined effect of tasks and farm characteristics.

Objective: To study whether and how work tasks and specific stable characteristics influence the level of dairy farmers' personal exposure to inhalable dust and endotoxin.

Methods: We applied an observational design involving full-shift repeated personal measurements of inhalable dust and endotoxin exposure among 77 subjects (owners and farm workers) from 26 dairy farms. Performed tasks were self-registered in activity diaries, and information on stable characteristics was collected through personal interviews and walk-through surveys. Associations between exposure, tasks, and stable characteristics were examined in linear mixed-effect models with individual and farm treated as random effects. Separate as well as combined models for tasks and stable characteristics were elaborated.

Results: The 124 personal samples collected had a geometric mean level (geometric standard deviation) of 360 EU m^{-3} (3.8) for endotoxin exposure and of 1.0 mg m^{-3} (2.7) for dust exposure. Identified factors that increased endotoxin exposure included a lower outdoor temperature and use of slope-based or back-flushed slurry systems along with milking, distribution of bedding, and handling of feed and seeds in barns. For dust, exposure was higher when fully automatic (robotic) milking was used and during re-penning of animals, handling of feed and seeds, handling of silos and when distributing bedding. Dust exposure increased also as a result of use of rail feed dispensers in a model without fully automatic milking.

Conclusions: The current exposure to dust and in particular endotoxin among Danish dairy farmers demand effective strategies to reduce their exposure. The present findings suggest that future interventions should focus on feeding and manure handling systems. Use of respirators during handling of feed and

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distribution of bedding should be advised until adequate risk management measures have been established. The expected increased use of fully automatic milking in the future might increase dust exposure of dairy farmers.

KEYWORDS: dairy farmers; determinants; dust; endotoxin; variability

INTRODUCTION

Dairy farming is the second most important branch of livestock farming in Denmark after pig farming, with an annual export value of almost 3 billion USD. Similarly to most western countries, the Danish dairy production is industrialized with cows being kept in confined conditions. The average herd size in Denmark is ~140 milking cows with farms primarily located in the areas of Jutland and Funen (StatBank Denmark, 2011; Landbrug og Fødevarer, 2012).

Similar to workers from pig and poultry stables, workers in modern dairies suffer from increased rates of respiratory symptoms and disorders. Cross-shift and longitudinal decline in lung function, asthma, chronic bronchitis, bronchial hyper-responsiveness, wheeze, and cough are most common (Reynolds *et al.*, 2013). Allergic sensitization against cow dander is also reported (Heutelbeck *et al.*, 2007). Organic dusts and related microbial exposures are the main inducers of these respiratory symptoms; important markers include fungal spores and hyphae, peptidoglycans, glucans, and bacterial endotoxin (Schenker *et al.*, 1998; Douwes *et al.*, 2003; Reynolds *et al.*, 2013). Endotoxin exposure is suggested to play a major role in the respiratory morbidity of livestock farmers (Donham *et al.*, 1995; Reynolds *et al.*, 1996; Vogelzang *et al.*, 1998; Kirychuk *et al.*, 2006; Heederik *et al.*, 2007; Bønløkke *et al.*, 2009; Reynolds *et al.*, 2013), and more recently, it is also conceptualized as an important player in the suggested protective effect of farming exposure against allergic sensitization and allergic asthma (Smit *et al.*, 2008; Basinas *et al.*, 2012a). Dairy farmers are reported to be exposed to organic dust and endotoxin concentrations averaging between 0.8 and 2.4 mg m^{-3} and 220 and 1500 EU m^{-3} respectively, and frequently exceeding the recommended or established exposure limits by several folds to orders of magnitude (Basinas *et al.*, 2013b).

The identification of exposure determinants forms the foundation for any effective exposure control and prevention strategy (Burstyn and Teschke, 1999). Recently, a comprehensive review on the levels and determinants of livestock farmers' exposure to dust and endotoxin was published (Basinas *et al.*, 2013b). The authors concluded that there is an urgent need for establishment of effective exposure control and prevention strategies for workers in livestock stables and suggested that these should be based on knowledge currently available. However, they also stressed that available information on determinants of personal exposure concerning cattle farmers is limited. Similar conclusions were drawn by another recent study that reviewed the health effects of dairy farming (Reynolds *et al.*, 2013). Previous studies on determinants of dust and endotoxin exposure in dairy farmers either included only a few potential determinants in their assessments (Virtanen *et al.*, 1988; Basinas *et al.*, 2012b; Samadi *et al.*, 2012) or focused specifically on workers of large dairies (Nieuwenhuijsen *et al.*, 1999; Garcia *et al.*, 2013) who will tend to have more constant and less intermittent daily tasks compared with workers of smaller farms. In addition, effects of tasks and stable characteristics were always investigated separately, an approach that ignores potential interaction effects. Studies involving both tasks and characteristics are generally sparse and have focused exclusively on pig production workers (Preller *et al.*, 1995; Basinas *et al.*, 2013a).

The present study aims to explore the influence of tasks performed by farmers on personal levels of exposure to dust and endotoxin in connection to the engineering settings and farm practices applied in Danish dairy confinement buildings. To assess relationships between exposure, tasks, and stable characteristics, we established linear mixed-effect models including tasks and farm characteristics that explained variability in dust and endotoxin exposure across farms, workers, and between days. We used repeated personal sampling in subjects from 26 small- and medium-sized (i.e. with <500 milking cows) dairy farms and a strictly observational design to collect information on potential determinants.

MATERIALS AND METHODS

Study design

Details concerning the design, sampling strategy, and the applied measurement and analytical methods in the

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exposure assessment part of the 15th year follow-up of the SUnd Stald (SUS) study have been published elsewhere (Basinas *et al.*, 2012b). In short, the SUS study cohort comprises 1964 students from all agricultural schools of Denmark and 407 randomly selected conscripts, which were used as controls. The baseline investigation occurred between 1992 and 1994. In 2008, a full-scale follow-up was performed, including an in-depth exposure assessment part (Elholm *et al.*, 2010). Information on employment status for a total of 1239 participants was available (66% participation rate). Of those, 423 (34%) were still active and full-time employed in farming, including 91 dairy farmers located in the area of Jutland, Denmark. Of these, 33 dairy farmers from Jutland were randomly selected and for 26 farmers permission was acquired to perform measurements in the farms that they worked.

Sampling: strategy and analytical methods

Two measurement visits in randomly selected working days were established for every farm included and carried out during the years 2008 and 2009. To address seasonality in activity patterns, one visit was performed during summer (1st of May and 1st of October) and one during winter (17th of November and 3rd of April). Seasons were established following an inspection of climatic data from previous years (Basinas *et al.*, 2013a). All workers on the selected farms were invited to participate in the personal measurement series, which covered the whole working period including both indoor and outdoor (field) activities. On average, sampling was performed for 290 min (SD = 83) during summer and for 280 min (SD = 125) during winter. Overall, 77 dairy farm workers and owners—henceforth simply referred to as dairy workers—were monitored for a total of 124 personal dust measurements.

Personal dust monitoring was performed using a portable AirChek XR5000 pump connected to a conical inhalable sampler (CIS; JS Holdings, Stevenage, UK) that contained a 37-mm glass fibre (GFA) filter (Whatman international Ltd, Maidstone, UK). The pumps operated at 3.5 l min^{-1} and two sampling trains per worker were used, with the sampling heads attached near the workers breathing zone, one at each side of the torso. The filter for endotoxin analysis was randomly chosen. Filters, including blank ones, were gravimetrically measured (pre- and post-sampling weighing) in a room with controlled climatic conditions and then extracted in pyrogen-free water (PFW) with 0.05% (v/v) Tween-20. Analysis for the endotoxin content in the extracts was performed in PFW (1:200 dilution) using a quantitative kinetic chromogenic Limulus Amebocyte Lysate (LAL) test (Kinetic-QCL 50-650U kit; Lonza, Walkersville, MD, USA) (Spaan *et al.*, 2008). Samples on the upper outer end of the standard curve were retested in a higher dilution (maximum 1:2000). The limit of detection (LOD) was 0.074 mg per filter for dust and 13.69 EU per filter for endotoxin. Samples with measured dust ($n = 2$) or endotoxin ($n = 1$) concentration below the LOD were assigned a 2/3 value of the corresponding LOD. Measured dust and endotoxin concentrations were expressed as cubic meter of air (m^3).

Collection of data on determinants

Workers were requested to document their performed tasks in structured activity diaries with 30-min interval checklists. Walk-through surveys in every department of the farm were performed to acquire information on farm characteristics, engineering parameters, and the hygienic conditions present on the measurement day. Pre-fixed inspection sheets designed to allow assessment for >120 well-defined characteristics were used, and the list of potential characteristics was further expanded through a post-measurement interview covering further engineering details in relation to the main cow stable. Tables 1 and 2 give an outline of the information on tasks and stable characteristics collected.

The outdoor temperature was measured instantly on site with a portable weather station (OBH Nordica A/S, Taastrup, Denmark) with a measurement accuracy of ±1°C, and a DLE40 Laser Rangefinder (Robert Bosch GmbH, Leinfelden-Echterdingen, Germany; accuracy of ±1 mm) was used to measure stable dimensions.

Description of participating farms

Selected dairy farms ($n = 26$) typically comprised four to eight compartments including the main stable—i.e. stable were lactating cows are kept—and secondary compartments like, among others, calve, gestation, and nursery or heifer stables. Secondary compartments involved different housing systems in older buildings compared with the main stables, and workers typically worked both in the main and in several

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Table 1. Tasks performed by all (n = 77) dairy workers participating in the study, as well as for a subgroup of 65 workers of main stables

Covariates	Coding	n (median)[a]	
		Overall[b] (n = 124)	Only main stable[c] (n = 101)
Working tasks, in minutes			
Controlling	Continuous	55 (30)	47 (30)
Milking (including gathering of cows)	Continuous	70 (135)	70 (135)
Locking the cattle in and out (grazing)	Continuous	11 (30)	11 (30)
Loading/unloading animals and moving heifers to the meadow or back	Continuous	18 (30)	11 (25)
Re-penning of animals	Continuous	15 (30)	13 (30)
Inseminating	Continuous	6 (30)	4 (30)
Ear-marking, injecting, or handling sick animals	Continuous	30 (30)	26 (30)
Handling dead animals	Continuous	3 (15)	2 (22.5)
Preparing feed	Continuous	59 (30)	48 (30)
Feeding (manual or mechanical)	Continuous	80 (45)	69 (45)
Preparation and disposal of bedding	Continuous	69 (35)	58 (30)
Removing manure	Continuous	50 (30)	38 (30)
Sweeping and scraping corridors	Continuous	40 (25)	34 (22.5)
Washing/cleaning of milking stables	Continuous	29 (30)	28 (30)
Disinfecting cubicles, pens, or stables	Continuous	6 (25)	2 (30)
Repairing/maintaining animal buildings/feed rooms and stable installations	Continuous	13 (45)	13 (45)
Administrative/office work	Continuous	11 (45)	10 (45)
Repairing/maintaining machinery and equipment (e.g. tractor, harvester etc.)	Continuous	9 (60)	6 (60)
Handling of feeding materials, seeds, grain in the barn	Continuous	7 (22)	7 (22)
Working with silos and drying plants	Continuous	6 (68)	6 (67.5)
Work in the fields (working the soil, sowing, harvesting, fencing etc.)	Continuous	14 (82.5)	9 (75)
Diverse	Continuous	34 (35)	26 (30)

n = number of observations.
[a]Number of positive observations and related median values for continues parameters.
[b]For all farmers included.
[c]Only for workers with ≥30% of animal-related time spent on the main stable.

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Table 2. Outdoor temperature, stable characteristics, and related frequency of occurrence for the 101 measurements[a] from workers of main stables (n = 65)

Covariates	Coding	n (median)[b]
Stable characteristics		
Outdoor temperature, °C	Continuous	101 (12)
Automatic (robotic) milking	Present (1) or absent (0)	16
Surface area (m^2) per cow	Continuous	101 (10.7)
Housing arrangement		
Stable without deep-litter pens	Present (1) or absent (0)	21
Flooring		
Concrete, deep cubicle/litter	Present (1) or absent (0)	19
Concrete with mattresses	Present (1) or absent (0)	60
Concrete and rubber mats	Ref.	22
Alleyways covered by rubber mats	Present (1) or absent (0)	23
Automatic surface scrapper used in alleyways	Present (1) or absent (0)	54
Bedding		
Main bedding, straw[c]	Present (1) or absent (0)	56
Additional bedding, sand[d]	Present (1) or absent (0)	7
Additional bedding, straw[d]	Present (1) or absent (0)	37
Additional bedding, none[d]	Ref.	57
Integrated feed storage/handling area	Present (1) or absent (0)	28
Feeding		
Automatic feeding	Present (1) or absent (0)	13
Feeding by an automatic rail dispenser	Present (1) or absent (0)	11
Grinding of grain performed in the farm	Present (1) or absent (0)	27
Compound feed distributed with silage	Present (1) or absent (0)	11
Insulated roof	Present (1) or absent (0)	27
Ventilation		
Mechanical or mechanically supported	Present (1) or absent (0)	13
Natural without controlling device	Present (1) or absent (0)	23
Natural with controlling device	Ref.	65
Ceiling fans installed	Present (1) or absent (0)	30
Powder disinfectants used	Present (1) or absent (0)	56

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Table 2. *Continued*

Covariates	Coding	*n* (median)[b]
Slurry handling (only endotoxin)		
Slurry system: slope and back flushing	Present (1) or absent (0)	18
Slurry system: scraper based or round flushing	Ref.	83
Pit emptied at least 1 per week	Present (1) or absent (0)	57
Pit supplied or emptied via other departments	Present (1) or absent (0)	9

n = number of observations.
[a]With ≥30% of animal-related time spent on the main stable.
[b]Number (*n*) of positive observations and related median values for continues parameters.
[c]Refers to the material used in cubicles or stalls.
[d]Refers to the material used on deep-litter pens, when different from main.

of the secondary farm compartments. On average, each farm housed 155 milking cows (range: 42–530), mostly in free-stall stables sized between 392 and 4200 m^2. Natural ventilation through openings in the walls was most common. The majority (*n* = 19) of farms milked their cows in herringbone and rotary parlours, whereas pipe milking was used in two farms with main stables bearing a tie-up design. Fully automatic milking (also known as robotic milking) was performed in the remaining five farms, and two additional farms, previously milking in parlours, installed the system during the course of the study. A more detailed description of the selected farms can be found in an earlier publication (Basinas *et al.*, 2012b).

Data analysis

Dust and endotoxin exposure concentrations approximate logarithmic distributions and thereby data were log-transformed and are summarized as geometric means (GM) with their geometric standard deviations.

Most animal-related work was performed in the main stable (median: 86%, range: 0–100%). A restricted population sample—hereafter called measurements from main stable workers—comprising measurements with minimum 30% of the animal-related time spent on main stable (*n* = 101) was therefore used to simplify the analysis for determinants and to reduce potential bias from work in secondary stables. Six measurements with no time spent inside stables as well as another fifteen with >70% of the animal related time on secondary stables were thus excluded.

The effect of tasks and stable characteristics on the log-transformed exposure concentrations was examined by linear mixed-effect models separately for dust and endotoxin exposure. The random effects (variance components) reflected the hierarchical sampling scheme with farms and workers within farms and the fixed effects to the potential determinants. Analysis was performed with the MIXED PROCEDURE of SAS v.9.3. Fixed covariates with less than five observations were *a priori* excluded from the modelling process. Inspired by our previous analysis for determinants among pig farmers (Basinas *et al.*, 2013a), we elaborated first multivariate models separately for tasks and stable characteristics. Univariate models were followed by a classical forward stepwise selection process starting with the covariate showing the most significant effect on the exposure. Model expansion occurred only with covariates showing a $P \leq 0.05$. The derived final models with tasks and stable characteristics were then merged into one model to allow assessment of combined effects.

To increase statistical power for the assessment of effects of non-animal-related and field working activities, models for tasks were also fitted using all 124 measurements available.

In all models, estimation of variance components was based on the restricted maximum likelihood method. Model diagnostics included standard residual probability plots and scatter plots of residuals versus predicted values. Stable characteristics were included in the models as indicator variables (present versus absent), except for the outdoor temperature and the surface area per cow, which were entered as continuous variables (Table 2). For tasks, the actual time spent in minutes by the workers on the tasks was used

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(Table 1). Correlations between tasks and farm characteristics were examined prior to the modelling process by computing Spearman correlation coefficients.

RESULTS

Farm characteristics and tasks included in the database are given in Tables 1 and 2. Frequencies of occurrence are given with median population values for continuous parameters.

Table 3 summarizes measured inhalable dust and endotoxin concentrations for the whole population and main stable workers separately. Workers were exposed to an overall GM level of 1.0 mg m^{-3} inhalable dust and 360 EU m^{-3} inhalable endotoxin. No systematic differences existed in measured concentrations between the whole population and main stable workers.

The final multivariate models on the effects of tasks for the subsample of main stable workers are presented in Table 4. The model for dust comprised six tasks explaining 42% of the between-workers and 27% of the total exposure variability; important tasks included the re-penning of animals, washing of milking stables, reparation of installations, and feed barn or silo work. Preparation and spread of bedding, milking, and the handling of feed in barns were included in the model for endotoxin, which explained 32% of the between-workers and 31% of the total variability in exposure. The latter was the task with the strongest effect with the population median performance of 22 min resulting in a factor 1.77 increase in endotoxin exposure. The task with the strongest influence on both endotoxin and dust exposure though was preparation and handling of bedding, explaining 25 and 6% of variability in exposure respectively after adjustment for other tasks.

Multivariate analysis using all measurements available (n = 124) resulted in model parameters and size effects similar to the restricted main stable sample, except for milking which was not included in the final model for endotoxin when all available measurements were included (not shown). The explained total variability by these models was 20% for dust and 25% for endotoxin exposure.

An increase in outdoor temperature by 10°C was associated with a 12% decrease in levels of personal endotoxin exposure (Table 5). Stables with slope or back-flush based slurry handling were related to ≥3-fold increase in endotoxin exposure when compared with stables where pit stirring was performed—i.e. scraper based or round flushing. For exposure to dust, the final model comprised use of automatic scrappers in alleyways that, when present, reduced the workers exposure by 45% and robotic milking which,

Table 3. Basic measurement attributes and concentrations of inhalable dust (mg m^{-3}) and endotoxin (EU m^{-3}) exposure measured through personal sampling in Danish cattle farmers

Period	*n*	*f*	*k*	Dust			Endotoxin			*r*
				AM	GM (GSD)	Range	AM	GM (GSD)	Range	
All measurements										
Overall	124	26	77	1.6	1.0 (2.7)	<LOD–9.8	760	360 (3.8)	<LOD–5900	0.63*
Summer	62	26	62	1.5	0.9 (2.5)	0.2–9.8	510	290 (3.2)	18–3400	0.64*
Winter	62	26	62	1.8	1.1 (2.9)	<LOD–9.4	1010	450 (4.0)	<LOD–5900	0.61*
Only main stable measurements										
Overall	101	26	65	1.5	1.0 (2.7)	<LOD–9.8	750	350 (3.6)	<LOD–5900	0.67*
Summer	50	26	50	1.4	0.9 (2.4)	0.2–9.8	480	290 (3.0)	30–2900	0.62*
Winter	51	26	51	1.6	1.0 (3.0)	<LOD–9.4	1020	430 (4.2)	<LOD–5900	0.70*

n = number of measurements; *f* = number of farms; *k* = number of workers; *r* = Pearson correlations between measured dust and endotoxin concentrations; AM = arithmetic mean; GM = geometric mean; GSD = geometrical standard deviation.
*P < 0.0001.

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Table 4. Results from mixed-effect linear regression describing the effect of tasks, per minute of performance, on the log-transformed personal level of exposure to inhalable dust ($mg\ m^{-3}$) and endotoxin ($EU\ m^{-3}$) exposure among Danish cattle farmers. A total of 101 personal measurements performed in 65 main stable workers from 26 dairy farms are used

	n	Duration in minutes, median (range)[a]	Dust			Endotoxin		
			β (*e*)	Change factor[b]	*P*	β (*e*)	Change factor[b]	*P*
Model with tasks								
Intercept (background)			−0.27 (0.15)	0.76	0.09	5.0 (0.20)	148	<0.0001
Milking (including gathering of cows)	70	135 (25–315)				0.0026 (0.0012)	1.42	0.04
Re-penning of animals	13	30 (15–60)	0.018 (0.0061)	1.72	0.006			
Preparation and disposal of bedding	58	30 (8–150)	0.0081 (0.0024)	1.28	0.002	0.019 (0.0031)	1.77	<0.0001
Washing/cleaning of milking stables	28	30 (8–150)	−0.0086 (0.0041)	0.77	0.04			
Repairing/maintaining animal buildings/feed rooms and stable installations	13	45 (15–450)	−0.0025 (0.0012)	0.89	0.05			
Handling of feeding materials, seeds, grain in the barn	7	22 (10–135)	0.013 (0.0052)	1.33	0.02	0.026 (0.0067)	1.77	0.0006
Working with silos and drying plants	6	67.5 (6–120)	0.011 (0.0038)	2.10	0.01			
${}_{bf}\sigma^2$ (naive estimate)			0.20 (0.12)			0 (0)		
${}_{bw}\sigma^2$ (naive estimate)			0.21 (0.36)			0.63 (0.90)		
${}_{ww}\sigma^2$ (naive estimate)			0.32 (0.52)			0.50 (0.74)		

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Table 4. *Continued*

	n	**Duration in minutes, median (range)**[a]	**Dust**			**Endotoxin**		
			β (*e*)	**Change factor**[b]	***P***	**β (*e*)**	**Change factor**[b]	***P***
Explained variability[c]								
Between-farm			0%			0%		
Between-worker			42%			30%		
Within-worker			38%			32%		
Total			27%			31%		

Naive estimates are variance components estimated from a model without fixed effects. *n* = number of observations; β = regression coefficient for log-transformed exposure data; *e* = standard error; ${}_{bf}\sigma^2$ = between-farm variance; ${}_{bw}\sigma^2$ = between-worker (within-farms) variance; ${}_{ww}\sigma^2$ = within-worker (day-to-day) variance.

[a]Only for positive values.

[b]Factor for change in exposure on the original scale for the median time (in minutes) spent on the task by the farmers; estimated as exp (β × median time).

[c]% of explained variability = [1 − (ratio of corresponding variance from model with and without fixed effects)] × 100.

in contrast, increased workers exposure to dust by 144%. The proportion of the total exposure variability explained by these models was 15%, allocated mainly on the between-farm and between-worker components for both dust and endotoxin exposure.

The model for dust was re-fitted without the effect of the milking method. The exclusion resulted in a model consisting of automatic scrappers in alleyways (β = −0.60; *P* = 0.007) and feeding by an automatic rail dispenser (β = 0.90; *P* = 0.01). The explained total variability by this model amounted to 13% comprising all of the between-farms variability and 9% of the between-worker variability.

As shown in Table 6, combining the models for tasks and stable characteristics did not considerably alter the estimated effect size for most of the factors included. However, washing of milking stables was no longer significantly associated to the exposure. Handling feed in barns remained the task with the strongest effect on both dust and endotoxin exposure, while the effects of slurry handling systems and robotic milking also remained sound. The proportion of the overall variability in exposure explained by these models was 36% for dust exposure and 41% for endotoxin exposure. For both agents, the explained variability was almost equally distributed between the temporal within- (day-to-day) and personal between-worker components.

DISCUSSION

This article describes the results of a study on the determinants of dairy farmers' exposure to inhalable dust and endotoxin. Mixed-effect linear regression was used to establish empirical models including effects of tasks and stable characteristics that explained variability in exposure between farms, workers, and days. To our knowledge, no study among dairy workers has previously described the combined effects of tasks and stable characteristics on the personal exposure to dust or endotoxin.

For simplicity, our analysis was restricted to farm characteristics associated only with the main stable; all measurements performed >70% of total time in stables within secondary stables were excluded. This was justified by the small portion of time farmers spent within secondary compartments, accounting on average for ≤8% of the total working time. Considerable bias due to the inclusion of measurements with time spent on secondary stables is unlikely though, and similar model estimates were found in a subsample

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Table 5. Linear mixed-effect regression results describing the effect of stable characteristics on the log-transformed personal level of exposure to inhalable dust (mg m^{-3}) and endotoxin (EU m^{-3}) exposure among Danish cattle farmers. Results are based on 101 personal measurements of work performed primarily in the main cow stables

	n	Dust			Endotoxin		
		β (e)	Change factor[a]	*P*	β (e)	Change factor[a]	*P*
Model with stable characteristics							
Intercept (background)		0.14 (0.15)	1.15	0.4	6.0 (0.20)	403	<0.0001
Automatic (robotic) milking (0/1)	16	0.89 (0.28)	2.44	0.003			
Use of automatic scrappers in walk-alleys (0/1)	54	−0.60 (0.21)	0.55	0.006			
Outdoor temperature, °C (median: 12 range: −1 to 26)	101				−0.025 (0.011)	0.78	0.03
Use of a slope or back-flush based slurry system (0/1)	29				1.22 (0.36)	3.39	0.002
${}_{bf}\sigma^2$ (naive estimate)		0 (0.12)			0 (0)		
${}_{bw}\sigma^2$ (naive estimate)		0.34 (0.36)			0.71 (0.90)		
${}_{ww}\sigma^2$ (naive estimate)		0.51 (0.52)			0.69 (0.74)		
Explained variability[b]							
Between-farm		100%			0%		
Between-worker		6%			21%		
Within-worker		2%			7%		
Total		15%			15%		

Naive estimates are variance components estimated from a model without fixed effects. n = number of observations; β = regression coefficient for log-transformed exposure data; e = standard error; ${}_{bf}\sigma^2$ = between-farm variance; ${}_{bw}\sigma^2$ = between-worker (within-farms) variance; ${}_{ww}\sigma^2$ = within-worker (day-to-day) variance.

[a]Factor for change in exposure on presence versus absence of a characteristics or for 10°C change in temperature estimated as the exp(β) for indicator variables and as exp(β × change in units) for continuous variables.

[b]% of explained variability = [1 − (ratio of corresponding variance from model with and without fixed effects)] × 100.

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Table 6. Results from linear mixed-effect models describing the combined effect of tasks and stable characteristics on the log-transformed personal level of inhalable dust (mg m^{-3}) and endotoxin (EU m^{-3}) exposure among Danish cattle farmers. Results are based on 101 personal measurements of work performed primarily in the main cow stables

	n	Dust			Endotoxin		
		β (*e*)	Change factor[a]	*P*	*β* (*e*)	Change factor[a]	*P*
Model with tasks and stable characteristics							
Intercept (background)		−0.11 (0.17)	0.90	0.5	5.1 (0.22)	164	<0.0001
Tasks, min							
Milking (including gathering of cows)	70				0.003 (0.0012)[b]	1.20[c]	0.02
Re-penning of animals	13	0.018 (0.0061)[b]	1.72[c]	0.005			
Preparation and disposal of bedding	58	0.0068 (0.0025)[b]	1.23[c]	0.01	0.017 (0.0029)[b]	1.67[c]	<0.0001
Washing/cleaning of milking stables	28	−0.0069 (0.0040)[b]	0.81[c]	0.1			
Repairing/maintaining animal buildings/feed rooms and stable installations	13	−0.0026 (0.0012)[b]	0.89[c]	0.04			
Handling of feeding materials, seeds, grain in the barn	7	0.012 (0.0052)[b]	1.30[c]	0.03	0.026 (0.0063)[b]	1.77[c]	0.0003
Working with silos and drying plants	6	0.011 (0.0038)[b]	2.10[c]	0.006			
Stable characteristics							
Outdoor temperature, °C	101				−0.021 (0.0095)[b]	0.81[d]	0.03
Automatic (robotic) milking (0/1)	16	0.62 (0.27)	1.86	0.03			
Use of automatic scrappers in walk alleys (0/1)	54	−0.51 (0.21)	0.60	0.02			
Use of a slope or back-flush based slurry system (0/1)	18				1.01 (0.30)	2.75	0.002

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Table 6. ***Continued***

	n	Dust			Endotoxin		
		β (*e*)	Change factor[a]	*P*	*β* (*e*)	Change factor[a]	*P*
$_{bf}\sigma^2$ (naive estimate)		0.10 (0.12)			0 (0)		
$_{bw}\sigma^2$ (naive estimate)		0.22 (0.36)			0.50 (0.90)		
$_{ww}\sigma^2$ (naive estimate)		0.32 (0.52)			0.47 (0.74)		
Explained variability[e]							
Between-farm		17%			0%		
Between-worker		39%			44%		
Within-worker		38%			36%		
Total		36%			41%		

Naive estimates are variance components estimated from a model without fixed effects. *n* = number of observations; *β* = regression coefficient for log-transformed exposure data; *e* = standard error; $_{bf}\sigma^2$ = between-farm variance; $_{bw}\sigma^2$ = between-worker (within-farms) variance; $_{ww}\sigma^2$ = within-worker (day-to-day) variance.
[a]Factor for change [exp(β)] in exposure on presence versus absence of a characteristic unless otherwise stated.
[b]Per 1 unit change.
[c]Factor for change in exposure on the original scale for the median time (in minutes) spend on the task by the farmers, estimated as exp(β × median time).
[d]Factor for change in exposure for 10°C change in outdoor temperature estimated as exp(β × 10).
[e]% of explained variability = [1 − (ratio of corresponding variance from model with and without fixed effects)] × 100.

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of 82 measurements with minimum 70% of the time spent in the main stable (not shown). Furthermore, estimates for the combined models (Table 6) were similar in analysis without the measurements ($n = 4$) from tie-up stables (not shown). Thus, the presence of these measurements in our sample is also unlikely to have substantially influence the results.

In the strategy of model making, separate models for tasks and stable characteristics were elaborated, which were then merged into one model to allow the assessment of the combined effects of tasks and stable characteristics. To examine whether derived models were dependent of the applied model building strategy, we also started from the established model with stable characteristics (Table 5) and elaborated further models by adding tasks sequentially, based on their level of significance. This resulted in a model for endotoxin exposure identical to the one presented (Table 6), but for dust 'washing of milking stables' was not included in the final model. However, size effects for remaining factors (not shown) were similar to the ones presented in Table 6, and thus such a dependency for our models seems unlikely.

In the model building, we included a substantial number of potential determinants, and the relatively small measurement sample may have limited our findings. Our combined models for tasks and characteristics though, explained between 35–41% of the total variability in exposure (Table 6); a result similar to previous studies among farmers (Preller *et al.*, 1995; Basinas *et al.*, 2013a). The remaining unexplained variability can generally be attributed to factors that remained unaccounted for (e.g. workers' behaviour, missing tasks or missing characteristics). For example, use of sand bedding in main areas and performance of fur clipping were excluded from the modelling process due to few observations. Personal dust and endotoxin levels of dairy workers from the Netherlands were recently shown to vary significantly depending on the type of the applied bedding material (Samadi *et al.*, 2012), and fur treatment can also be suspected of influencing personal exposure (Virtanen *et al.*, 1988). Furthermore, work style was not incorporated in our study and therefore a substantial part of the between-worker variability could not be accounted for.

Overall, the exposure concentrations of inhalable dust (GM = 1 mg m^{-3}) and endotoxin (GM = 360 EU m^{-3}) in this study are in good agreement with those previously reported in workers of small and medium sized farms from the Netherlands (range of GMs: 0.89–1.5 mg m^{-3} and 220–560 EU m^{-3} for inhalable dust and endotoxin, respectively), where similar sampling and analytical methods were used (Spaan *et al.*, 2006; Smit *et al.*, 2008; Samadi *et al.*, 2012). Comparable exposure levels (GM = 0.82 mg m^{-3} for dust and 334 EU m^{-3}) have also been reported in workers from large (>1000 cows) Californian dairies (Garcia *et al.*, 2013), despite that the recombinant Factor C (rFC) assay was used for endotoxin determination in this study. Though this probably did not lead to biased results as for the assessment of endotoxin exposure in livestock environments, the rFC and the kinetic chromogenic LAL assay are suggested to be highly comparable (Thorne *et al.*, 2010). Nevertheless, differences in climate and production systems between countries and time periods exist and should always be considered when interpreting exposure findings. In general, the exposure levels found are lower than those reported among pig and poultry farmers (Basinas *et al.*, 2013b), but inhalable endotoxin concentrations measured are well above the suggested threshold limit value for occupational endotoxin exposure of 90 EU m^{-3} (DECOS, 2010). Similarly, 36% of the dust measurements in our study were above the health-based occupational exposure limit of 1.5 mg m^{-3} of inhalable grain dust, which, after a formal standard setting process, was recently recommended by the National Health Council of the Netherlands (DECOS, 2011).

Our empirical model results indicate that automatic milking and manure handling methods are strongly associated with personal inhalable dust and endotoxin exposure. Workers on farms with milking robots were on average exposed to 2-fold ($\beta = 0.89$, $P = 0.003$) higher levels of dust exposure when compared with workers on farms with parlour or pipe milking. This is comparable to previously reported effects ($\beta = 0.80$, $P = 0.004$) among workers from Dutch dairies. However, it is unlikely that milking robots *per se* increase the personal levels of exposure to dust. The milking method determines working schedules within a dairy and it may also affect the ratio of tended animals per worker; when two of our study farms changed from parlour to automatic milking, it resulted in fewer employees attending an unchanged number of animals (not shown). Evidently, workers in stables with parlour or pipe milking in our study spent on average more time (120 versus 37.5 min) on milking and related activities compared to workers

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in stables with milking robots. In contrast, the latter workers tended to be more frequently involved (χ^2 test, $P < 0.1$) in more dusty tasks like reparations (31 versus 9%), handling of silos (19 versus 4%), distribution of bedding (75 versus 50%), and inspection (81 versus 40%). Such differences in activity patterns can likely explain the effect of fully automatic milking; however, applied engineering parameters such as the distribution of compound feed by the robots or feeding by rail dispensers may also play a role. In fact, rail feed dispensers in our study correlated strongly ($r = 0.6$, $P < 0.0001$) with robotic milking and a model without robotic milking comprised of automatic scrappers in alleyways and feeding by rail dispensers, the latter showing an effect comparable to robotic milking even after adjustment for working tasks (not shown). Nevertheless, milking robots are progressively adopted by dairy farms worldwide. The demonstrated relationship between automatic milking and inhalable dust exposure thereby implies that hazardous exposures to inhalable dust might increase respiratory morbidity among dairy farmers in the future. This finding demands for more effective strategies to prevent exposure for these workers.

Automatic scrapers in walk-alleys were related to a 40% reduction in the personal levels of dust exposure (Table 6). Frequent scraping of walk-alleys will generally remove surface manure as well as bedding residues limiting thereby their potential for re-suspension. In addition, as a consequence of the lesser availability of surface manure accumulation of faeces dried on the skin of animals, another potential source of dust (Takai *et al.*, 1998; Banhazi *et al.*, 2008) will be reduced.

We observed a 3-fold increase in levels of endotoxin exposure for workers in stables with a natural slope or back-flush based slurry system compared to workers in stables with round or scraper based systems. Endotoxin present in faeces and manure most likely originates from Gram-negative gut bacteria (Wang *et al.*, 1996; Zucker *et al.*, 2000). The absence of regular stirring in slope or back-flush systems could result in increased growth of anaerobic bacteria resulting in higher endotoxin concentrations, but this requires further study. Stratified analyses by system type showed no differences in percentage of slatted floor coverage between strata, but frequent manure flushing (≥1 time per week) was more prevalent among round or scrape based systems compared with those of a natural slope or back-flush design (61 versus 33%; χ^2 test, $P < 0.05$). Previously, ambient endotoxin concentrations in dairy stables have been shown to decrease with an increased frequency of manure flushing (Garcia *et al.*, 2012), indicating that the effect of frequent manure removal also can be important. Further research, preferably in studies with an interventional design, is warranted to validate these findings.

The on average 19% lower endotoxin levels per 10°C increase in outdoor temperature is most likely explained by increased ventilation in the summer period, either by broadening the openings in the curtain covered walls of the natural ventilated buildings, or through an increased ventilation rate in buildings with mechanical or mechanically supported ventilation. Similar, slightly stronger effects of temperature (up to 30%) have been described in previous studies among pig stable workers (Preller *et al.*, 1995; Basinas *et al.*, 2013a). Seasonal effects related to changes in numbers of housed animals due to grazing management issues seem unlikely. There was no significant difference in area size per housed animal across seasons (median of 10.7 m^2 per animal and 10.9 m^2 per animal for summer and winter respectively; Mann–Whitney *U* test, $P = 0.5$).

Considering task effects, preparation and distribution of bedding and handling of feed and seeds in barns were both strong determinants of dust and endotoxin exposure. Time spent on preparation and handling of feed and seeds was associated to increased levels of dust and endotoxin exposure also in our previous analysis among pig farmers (Basinas *et al.*, 2013a). These results are further supported by the high levels of exposure being reported in farmers performing indoor handling or storage of crops (Melbostad and Eduard, 2001; Mołocznik, 2002; Halstensen *et al.*, 2007). In addition, in a study of workers from large (>1000 cows) Californian diaries, time spent re-bedding was associated with significantly higher inhalable dust and endotoxin concentrations compared to time spent milking or conducting other tasks (Garcia *et al.*, 2013). Likewise, Davidson *et al.* (2012), in another study among US dairy workers, reported individual inhalable dust levels during re-bedding as high as 6.81 mg m^{-3}. In this study though, endotoxin exposure concentrations were greatest among parlour milkers (GM = 1037 EU m^{-3}). This finding supports the positive association between non-automated milking and endotoxin exposure that we found. However, it has to

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be noted that production differs between large US dairies and the small- and medium-sized farms included in our study: generally, stable designs in the USA are less enclosed, milking areas are separated from the stables and workers are much more specialized.

The protective effect of parlour and robot washing with a fire-hose on inhalable dust that we found could be a result of lower dust re-suspension due to increased air humidity and binding of dust on walls and floors. Dust reduction techniques based on spraying of animals and surfaces with oil and/or water have been established on the basis of the above principal (Takai and Pedersen, 2000), and washing of parlours has been shown to associate with reduced levels of dust exposure in milkers (Choudhry *et al.*, 2012).

Movement of animals in our study was associated with increased personal exposure to inhalable dust, but only when performed between enclosed farm areas—i.e. re-penning. This is in accordance with previous results among Danish (Basinas *et al.*, 2013a) and Dutch (Preller *et al.*, 1995) pig farmers. Re-penning of cows involves fewer animals and is generally less action afflicted than re-penning of pigs. Tail movements during the process can re-suspend dust from the animal surface potentially increasing the exposure of workers who follow the cows in close proximity or guiding them by the side. Among US dairy farmers though, Garcia *et al.* (2013) found no significant differences between levels of dust exposure of workers moving animals across farm locations and the stationary milkers. In contrast to our study, the working environment—i.e. re-penning, grazing, moving animals between stables and meadows—was not taken into account during the assessment of the specific task.

The contribution of tasks on the total variance reduction was almost twice the size of stable characteristics, indicating tasks to be more important than farm characteristics in determining the personal level of exposure to dust or endotoxin. This is an important observation for exposure classification in future epidemiological studies, but for prevention, stable characteristics are probably of most interest.

CONCLUSIONS

In conclusion, the present study investigated whether and how tasks and specific stable characteristics can influence the personal exposure level of inhalable dust and endotoxin among dairy farmers in Denmark. Its results suggest milking, feeding, and manure handling methods including use of rail feed dispensers and surface manure scrapers as well as non-stirring slurry pit handling to strongly affect the level of dairy farmers exposure to dust and endotoxin. Tasks related to feed and seed handling in barns, performance of milking, and re-penning of animals are strong predictors of the exposure, but most influential is handling and distribution of bedding. These findings provide an important insight on determinants of dairy farmers' exposure to dust and endotoxin. Further studies will be needed to examine whether intervention strategies on the basis of the identified stable characteristics can effectively reduce dust and endotoxin exposure in these workers.

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