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Exposure levels, determinants and IgE mediated sensitization to bovine allergens among Danish farmers and non-farmers

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ABSTRACT

Background: Bovine allergens can induce allergic airway diseases. High levels of allergens in dust from stables and homes of dairy farmers have been reported, but sparse knowledge about determinants for bovine allergen levels and associations between exposure level and sensitization is available.
Objective: To investigate levels and determinants of bovine allergen exposure among dairy, pig and mink farmers (bedroom and stable), and among former and never farmers (bedroom), and to assess the prevalence of bovine allergen sensitization in these groups.
Methods: In 2007–2008, 410 settled dust samples were collected in stables and in bedrooms using an electrostatic dust-fall collector over a 14 day period among 54 pig farmers, 27 dairy farmers, 3 mink farmers as well as 71 former and 48 never farmers in Denmark. For farmers sampling was carried out both during summer and winter. Bovine allergen levels ($\mu g/m^2$) were measured using a sandwich ELISA. Determinants for bovine allergen exposure in stables and bedrooms were explored with mixed effect regression analyses. Skin prick test with bovine allergen was performed on 48 pig farmers, 20 dairy farmers, 54 former and 31 never farmers.
Results: Bovine allergen levels varied by five orders of magnitude, as expected with substantially higher levels in stables than bedrooms, especially for dairy farmers. Bovine allergen levels in bedrooms were more than one order of magnitude higher for dairy farmers compared to pig farmers. Former and never farmers had low levels of bovine allergens in their bedroom. Bovine allergen levels during summer appeared to be somewhat higher than during winter.

Increased bovine allergen levels in the bedroom were associated with being a farmer or living on a farm. Mechanical ventilation in the bedroom decreased bovine allergen level, significant for dairy farmers $\beta = -1.4$, $p < 0.04$. No other significant effects of either sampling or residence characteristics were seen. Allergen levels in dairy stables were associated to type of dairy stable, but not to other stable or sampling characteristics. Sensitization to bovine allergens was only found in one pig farmer.
Conclusion: This study confirms high bovine allergen levels in dairy farms, but also suggests sensitization to bovine allergens among Danish farmers to be uncommon. Furthermore the importance of a carrier home effect on allergen load is emphasized. Whether the risk for bovine sensitization is related to the allergen level in the stable or the dwelling remains to be determined.

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Background

Dairy farmers suffer from respiratory disorders, similar to workers from pig and poultry farms. Cross-shift and longitudinal decline in lung function, asthma, chronic bronchitis, bronchial hyperresponsiveness, wheeze and cough are most commonly found (Reynolds et al., 2013). Cattle are a well-known source of allergens, and the population at risk for allergic diseases caused by sensitization to bovine allergens mainly consists of dairy farmers (Heutelbeck et al., 2007). The main sources of bovine allergens are hair and dander (Prahl, 1981). The 20 kDa protein designated Bos d2 is the most important respiratory allergen in bovine antigen extracts and is present in both bovine hair and dander, but several other molecules have been identified as major allergens as well (Rautiainen et al., 1997; Ylönen et al., 1992).

Previous studies among non-selected farming populations, suggest the prevalence of bovine allergen sensitization among dairy farmers to be low, i.e., between 2 and 5% (Iversen and Pedersen, 1990; Kronqvist et al., 1999). Studies among symptomatic farmers though, suggest sensitization to bovine allergens to be a significant risk factor for occupational asthma and rhinoconjunctivitis. In Finland, sensitization to bovine allergens has earlier been reported to be common among Finnish dairy farmers (Rautalahti et al., 1987), and bovine sensitization has been a leading cause of occupational asthma in Finland, accounting for 609 out of 2609 notified cases of occupational asthma between 1989 and 1995 (Karjalainen et al., 2000). In Germany, nearly 500 subjects (9% of all referrals) referred to the Agricultural Institutions for Statutory Accident Insurance and Prevention (Landwirtschaftliche Berufsgenossenschaften) between 1990 and 2002 were found to be sensitized to bovine allergens (Heutelbeck et al., 2007).

Few studies have quantitatively assessed bovine allergens in the environment, and they all found high levels of allergens in cow stables (Berger et al., 2005; Virtanen et al., 1988; Zahradnik et al., 2011). Zahradnik et al. (2011) reported median levels of bovine hair allergen in cow stables to be 51,700 $\mu g/m^2$. In other types of stables bovine hair allergens were much lower, with median concentrations 0.4 and 316 $\mu g/m^2$, and for dwellings between 4.3 and 104 $\mu g/m^2$. Among farmers suffering from asthma caused by bovine allergens Hinze et al showed that concentrations of sIgE towards Bos d2 were correlated with concentrations of Bos d2 in house dust samples (Hinze et al., 1997), but we are not aware of any study that has combined a quantitative evaluation of bovine allergen concentration levels among unselected farmers and non-farmers with data on bovine sensitization and furthermore data on determinants for bovine allergen levels. Such a study is crucial in order to set priorities for prevention of respiratory diseases among dairy workers.

The aims of this study were (a) to investigate levels and determinants of bovine allergens in stables and bedrooms among dairy, pig, and mink farmers, (b) to compare these levels with levels measured in former and never farmers' bedroom and (c) to assess the prevalence of bovine allergen sensitization within the same populations.

Material and methods

Study population

The exposure assessment of the "Sund Stald" (SUS), termed Healthy Stables in English, has been described in detail in a previous publication (Basinas et al., 2012a). In brief, the SUS study was initiated in 1992 with the aim to describe the occurrence of respiratory symptoms in a farming environment and to investigate the effect of farming on the development of allergy, asthma and respiratory disease (Elholm et al., 2010). At the 15 year follow-up study 1239 participants (66% participation rate) were identified of which 423 (34%) were still active and employed full-time in farming. Thirty-three dairy farms, 75 pig farms and 3 mink farms were randomly selected. Twelve farmers declined to participate, and 16 were excluded: due to various reasons -i.e. poor health ($n = 2$), inability to establish contact ($n = 3$), part-time employment as a farmer ($n = 6$), or migration or change of occupation ($n = 5$). The resulting population consisted of 54 pig farmers, 27 dairy farmers and 3 mink farms. All gave their consent for the inclusion of their farms in the measurement study. Finally 71 former farmers (from the farming students population) and 48 never farmers (from the control group) were included in the study population and hereafter called non-farmers.

Passive airborne dust sampling

Settled airborne dust was collected passively over a 14-day period with the electrostatic dust-fall collector (EDC), consisting of a polypropylene folder with 4 electrostatic cloths (ZEEMAN, Alphen aan de Rijn, the Netherlands,), each having a surface exposure area of 0.0209 m^2 (Noss et al., 2008). EDCs is a method of sampling of airborne particles that is inexpensive and reliable; strong correlations ($r = 0.85$) between endotoxin concentrations measured from different EDCs used in parallel in bedrooms have previously been reported (Noss et al., 2008). For the farmers samplers were placed in stables and bedrooms both during summer and winter, whereas for non-farmers only the bedrooms were sampled either during summer or winter.

Stable measurements were collected where the farmer spent most of his working time. Stable measurements were performed on randomly chosen working days during 2008–2009 (Basinas et al., 2012a). Bedroom measurements were initiated at the same time as the stable measurements were carried out.

All participants were instructed to place the EDCs in their bedrooms around 1.5 m above floor level, and to keep a sampling duration of 14 days both in stables and bedrooms. Samplers were returned by regular post in separate pre-stamped envelopes that were provided beforehand.

For each stable measurement relevant sampling characteristics (i.e. date of placement, sampling height, position and distance from ventilators) were registered at the time of sampling initiation, whereas farm characteristics, engineering parameters, and the hygienic conditions present in each stable of the farm were registered during walk-through surveys performed when personal measurements were collected (Basinas et al., 2012a).

For bedroom measurements, the participants filled in a questionnaire addressing sampling characteristics (i.e. duration, position and height) together with information on type of residence (farm with livestock, farm without livestock, other type of housing), bedroom ventilation (active ventilation yes/no), type of bedroom flooring (carpet yes/no), type of wall surface (painted walls, wall paper), position of bedroom (cellar, ground floor or; >ground floor), type of windows (single or double glass) and type of heating (central heating, stove). The questionnaire was returned together with the sampler.

Dust extraction

Dust from one EDC cloth pr. sampler was extracted in 20 ml phosphate buffer saline with 0.05% Tween 20 (PBST) by rotation for 1 h at room temperature. After extraction the cloth was removed, the extract was centrifuged at $3000 \times g$ for 15 min, and the supernatant was stored in aliquots at $-80\,^{\circ}C$ until analysis.

Table 1
Concentrations (in μg/m²) of bovine allergens measured with electrostatic dust-fall collectors (EDCs) within different types of stables and within bedrooms of farmers and non-farmers from Denmark.

Type of participant	Stables						Bedrooms					
	k	*n*	*n* < LOD (%)	GM (μg/m²)	GSD	Range	*k*	*n*	*n* < LOD (%)	GM (μg/m²)	GSD	Range
Farmer, dairy												
Summer	20	20	0 (0)	45446	2.43	9544–195662	24	24	0 (0)	9.78	1.96	1.42–66.79
Winter	24	24	0 (0)	37107	2.12	1741–203831	26	26	0 (0)	7.18	3.95	0.84–122
Overall	25	44	0 (0)	40747	2.24	1741–203831	27[b]	50	0 (0)	8.34	2.96	0.84–122
Farmer, pig												
Summer	45	45	2 (4)	1.86[a]	3.63[a]	0.21–24.08	47	47	11 (23)	0.54[a]	5.03[a]	0.21–10.35
Winter	47	47	1 (2)	1.41[a]	2.61[a]	0.36–57.21	53	53	15 (28)	0.50[a]	4.64[a]	0.19–7.09
Overall	52	92	3 (3)	1.62[a]	3.13[a]	0.21–57.21	54[b]	100	26 (26)	0.52[a]	4.77[a]	0.19–10.35
Farmer, mink												
Summer	0	0	0 (0)	–	–	–	0	0	0 (0)	–	–	–
Winter	2	3	0 (0)	1.82	1.42	1.25–2.49	2	2	1 (50)	–	–	0.44
Overall	2	3	0 (0)	1.82	1.42	1.25–2.49	2	2	1 (50)	–	–	0.44
Non-farmer												
Summer	–	–	–	–	–	–	69	69	42 (61)	0.11[a]	5.91[a]	0.16–10.58
Winter	–	–	–	–	–	–	50	50	34 (68)	0.09[a]	3.21[a]	0.15–0.93
Overall	–	–	–	–	–	–	119	119	76 (64)	0.09[a]	5.13[a]	0.15–10.58

k = Number of participants; *n* = number of measurements; LOD = limit of detection; GM = geometric mean; GSD = geometric standard deviation; Range = range of measured concentrations.

[a] Estimated with maximum likelihood estimation (MLE) using non-linear regression models in the SAS NLMIXED procedure When repeated measurements were present the subjects were treated as a random effect.

[b] At one farm 2 farmers participated.

Bovine allergen analysis

Bovine hair allergen concentrations in the EDC extracts were determined with a Sandwich ELISA as described by Zahradnik et al. (2011). In brief, microtiter plates were coated with 100 μl/-well rabbit anti-cow hair polyclonal antibodies, and then blocked with 200 μl of 1% gelatin in PBST. Standards, assay controls and samples were diluted in the wells and then plates were incubated for 1 h in 22 °C. A standard 8-point curve was obtained from a commercial cow hair extract (Allergon, Ångelholm, Sweden) with concentrations ranging from 0.08 to 10 ng/ml. The bound proteins were incubated for 1 h with biotinylated anti-cow hair antibodies, followed by streptavidin-peroxidase conjugate (Poly-HRP80-SA, Fitzgerald, Concord, MA, USA) and finally ABTS substrate (Sigma-Aldrich, Steinheim, Germany). The absorbance was read at 414 nm. The lower limit of detection (LOD) was the concentration corresponding to the minimal value of the curve fit function plus the 6-fold standard deviation of the zero standard. The average LOD was 0.095 ng/ml which corresponds to a level of 0.18 μg/m². As the LOD varied by approx. 20% depending on test day the average of 25 plates was used).

Bovine sensitization

Skin prick test (SPT) with bovine dander allergen (ALK-Abello®) was performed among 48 pig farmers, 20 dairy farmers, 54 former and 31 never farmers. A positive SPT was defined as at least 3 mm in mean diameter provided a negative control.

Statistical methods

All data were analyzed with SAS version 9.3 for windows. Visual inspection of the data revealed separate log-normal distributions for concentrations among dairy stables, pig stables and bedrooms; therefore data were log-transformed and summarized by their geometric mean (GM) and their geometric standard deviation (GSD). When values below the limit of detection (LOD) were present GMs and GSDs were imputed using maximum likelihood estimation (MLE) approach by the NLMIXED procedure (Jin et al., 2011). When relevant the subject id was treated as a random effect to account for the presence of correlations between repeated measurements of exposure. To provide information on factors potentially affecting bovine allergen exposure levels in bedrooms the models were expanded with fixed effects—i.e. sampling duration, season, sampling height, farm living (farm with livestock, farm without livestock, non-farm), floor type, ventilation and farmer status. Pooled and stratified analysis across bedrooms of dairy farmers, pig or mink farmers and non-farmers was performed.

Similar models for measurements inside cow stables were built. Due to no measurements below LOD, potential determinants of bovine allergen exposure in cow stables were explored in classical linear mixed effect regression (PROC MIXED) using a restricted maximum likelihood estimation (REML). A variance compound symmetry covariance structure was assumed.

The SUS study was approved by the ethics committee of Aarhus County (AA-19912197) and the Danish Data Protection Agency. The SUS follow-up study was also approved by the ethics committee of Aarhus County (AA-20070074) and the Danish Data Protection Agency. Informed written consent was obtained from all participants.

Results

Altogether 419 passive dust samples were collected. Of these, nine were excluded from the final analysis due to missing information about sampling duration ($n = 3$), duplicate bedroom winter measurement ($n = 5$) and one extreme value for a pig stable (>8638 μg/m²). Of the remaining 410 measurements 106 were below LOD, 3 (2%) among stable measurements and 103 (38%) among bedroom measurements.

In Table 1 concentrations of bovine allergen are presented within the different types of stables and within bedrooms of farmers and non-farmers. The distribution of measurements below LOD is also displayed.

The bovine allergen levels were substantially higher in stables than bedrooms, especially for dairy farmers, with a GM (GSD) of 40,746 μg/m² (2.24) in dairy stables and 8.34 μg/m² (2.96) in bedrooms. Bovine allergen level from dairy farmers were four orders of magnitude higher compared to levels in stables from pig and mink farmers, but also bedroom concentrations were on average 15 fold higher for dairy farmers compared to pig farmers. Non-farmers had low levels of bovine allergen in their bedroom (GM = 0.09 μg/m^2), and no difference was observed for former and never farmers (results not shown). In general bovine allergen levels were slightly higher in summer than winter, statistically significant for dairy stables. Only 3 measurements in stables (all pig) were below LOD, and all measurements in bedrooms from dairy farmers were all above LOD, whereas the proportion of measurements below LOD for bedrooms of pig farmers and non-farmers were 26% and 64%, respectively. Only two of the farmers categorized as pig-farmers were concurrently involved in both pig and cattle activities. No extraordinary high values were observed in samples from their stables ($n = 3$) and bedrooms ($n = 4$) and exclusion of these samples hardly affected the given estimates (not shown).

In Table 2, concentrations of bovine allergen in bedrooms of different types of housing are presented. Living on a farm with livestock (GM = 1.18 μg/m^2) and without livestock (GM = 0.35 μg/m^2) were associated with higher levels of bovine allergen in the bedroom compared to a non-farm residence (GM = 0.11 μg/m^2), $p < 0.0001$, irrespective of farmer status. Dairy farmers had the highest levels of bovine allergen in their bedroom compared to both pig/mink farmers and non-farmers, irrespective of type of housing, $p < 0.001$.

Table 3 summarizes mutually adjusted results investigating the impact of sampling and residence characteristics on bedroom bovine allergen. In the overall model, both being a dairy farmer and being a pig/mink farmer were associated with increased bedroom bovine allergen levels (dairy farmer $\beta = 4.25$; pig/mink farmer $\beta = 1.42$, $p < 0001$). Stratified analyses showed ventilation in the bedroom decreased bovine allergen level, significant for dairy farmers $\beta = -1.41$, $p < 0.04$. No other significant effects of either sampling or residence characteristics were seen. For non-farmers, living on a farm with livestock was associated with increased bovine allergens, ($\beta = 1.43$, $p < 0.02$). Borderline higher levels were observed in summer compared to winter ($\beta = 0.61$, $p = 0.07$).

The impact of cow stable characteristics on bovine allergen levels was assessed. Multivariate analysis with data from 25 dairy stables (Table 4) showed borderline lower bovine allergen levels in stables housing mainly heifers and/or calves ($\beta = -1.14$, $p = 0.08$) compared with levels in main stables with lactating cows. Higher levels were observed in summer compared to winter ($\beta = 0.55$, $p = 0.04$). Area per cattle head, disinfection applied or the presence of a mechanical ventilation system did not affect the bovine allergen level in the cow stable.

Among 48 pig farmers, 20 dairy farmers, 54 former and 31 never farmers only one pig farmer (former dairy farmer) had a positive SPT for bovine allergen. He was not sensitized to other common airborne allergens (house dust mites, grass, birch, cat, dog, molds, and storage mites).

Discussion

Levels and determinants of bovine allergen

We found, that bovine allergen levels varied more than five orders of magnitude, with, as expected, substantially higher levels in stables than bedrooms, especially for dairy farm stables. Interestingly, bovine allergen bedroom levels were more than one order of magnitude higher for dairy farmers compared to pig farmers, and levels during summer appear to be somewhat higher than in winter. Former and never farmers had low levels of bovine allergens in their bedroom.

Table 2
Concentrations (in μg/m^2) of bovine allergen measured with electrostatic dust-fall collectors (EDCs) in bedrooms of Danish farmers and non-farmers, stratified by farm living.

Type of participant	Farm living																	
	Farm with livestock						Farm without livestock						Non-farm house					
	k	n	n < LOD (%)	GM[a]	GSD[a]	Range	k	n	n < LOD (%)	GM[a]	GSD[a]	Range	k	n	n < LOD (%)	GM[a]	GSD[a]	Range
Dairy farmer	21	37	0(0)	9.01	2.55	1.50–122.21	5	7	0(0)	7.92	2.77	1.89–21.24	4	6	0(0)	5.29	5.16	0.84–63.34
Pig or mink farmer	30	53	16(30)	0.38	4.67	0.19–7.08	14	27	4(15)	1.07	3.94	0.28–10.35	12	22	6(27)	0.41	4.67	0.24–6.35
Non-farmer	8	8	2(25)	0.38	7.4	0.16–9.02	24	24	16(67)	0.09	4.98	0.15–2.51	87	87	58(67)	0.09	4.14	0.18–10.58
Overall	59	98	18(18)	1.18	7.77	0.16–9.02	43	58	20(35)	0.35	8.71	0.15–21.24	103	115	65(57)	0.11	7.05	0.18–63.34

k = Number of participants; n = number of measurements; LOD = limit of detection; GM = geometric mean; GSD = geometric standard deviation; Range = range of measured concentrations.
[a] In presence of censoring, GM and GSD is imputed with maximum likelihood estimation (MLE) using non-linear regression models in the SAS NLMIXED procedure. When repeated measurements were present the subjects were treated as a random effect.

Table 3
Regression analysis results describing the effect of sampling, spatial and personal characteristics on the log-transformed bovine allergen concentrations (in μg/m^2) measured in 83 bedrooms of farmers and 119 bedrooms of non-farmers.

Covariate	Overall (n = 269)[a]				Dairy farmer (n = 50)[a]				Pig or mink farmer (n = 102)[a]				Non-farmer (n = 119)[b]			
	n	*β*	*se*	*p*	*n*	*β*	*se*	*p*	*n*	*β*	*se*	*p*	*n*	*β*	*se*	*p*
Intercept		−2.24	0.53	<0.0001		1.32	0.77	0.1		−0.61	0.77	0.4		−2.41	1.11	0.03
Farm status																
Dairy farmer	50	4.25	0.33	<0.0001		–				–				–		
Pig or mink farmer	102	1.42	0.27	<0.0001		–				–				–		
Non-farmer	119	Ref				–				–				–		
Sampling characteristics																
Season (summer; winter = ref)	140	0.28	0.16	0.09	24	0.15	0.18	0.4	47	0.11	0.27	0.7	69	0.61	0.34	0.07
Height of sampling (cm)	165	−0.002	0.003	0.5	160	0.0003	0.0004	0.6	150	−0.002	0.004	0.6	180	−0.002	0.006	0.7
Farm living																
Farm with livestock	98	0.21	0.28	0.5	37	0.45	0.45	0.3	53	−0.17	0.45	0.7	8	1.43	0.62	0.02
Farm without livestock	58	0.44	0.27	0.1	7	0.11	0.52	0.8	27	0.90	0.49	0.07	24	0.14	0.43	0.7
Non-farm house	115	Ref			6	Ref			22	Ref			87	Ref		
Bedroom characteristics																
Situated above ground floor (yes; no = ref)	121	−0.04	0.20	0.8	30	−0.03	0.34	0.9	47	0.13	0.34	0.7	44	−0.11	0.36	0.8
Carpet floor(yes; no = = ref)	125	0.19	0.20	0.3	27	0.11	0.33	0.7	56	0.02	0.34	0.9	42	0.19	0.35	0.6
Ventilated mechanically (yes; no = ref)	8	−1.22	0.65	0.07	3	−1.41	0.66	0.04		–				–		
Between variance (naïve model)		0.71 (3.92)				0.61 (0.57)				0.56 (0.80)				–		
Within variance (naïve model)		1.19 (1.33)				0.36 (0.48)				1.64 (1.63)				–		
Total variability explained		64%				8%				10%				58%[c]		

n = Number of measurements; β = regression coefficient; *se* = standard error; p = p-value.
[a] Mixed models build in proc lnmixed with subject id as a random effect.
[b] Model build in proc lnmixed without a random effect; – = not in the model or not estimated.
[c] Estimated by the adjusted coefficient of determination (R^2).

Table 4
Linear mixed effect regression model results describing the effect of sampling, and stable characteristics on the log-transformed bovine allergen concentrations (μg/m^2) measured in 25 Danish dairy farms.

Covariate	Overall (n = 44)			
	n	β	se	p
Intercept		10.96	1.50	<0.0001
Sampling characteristics				
Season (summer/winter = ref)	20	0.55	0.25	0.04
Height of sampling (cm)	260	0.0003	0.005	0.9
Stable characteristics				
Type of stable and location (1/0)				
Secondary stable (Calve/young stock)	7	−1.14	0.59	0.08
Main stable (dairy cows), Milking area	7	−0.75	0.48	0.1
Main stable (dairy cows), Tending area	30	Ref		
Integrated stable[a] (0/1)	18	−0.35	0.37	0.4
Area per cattle head (m^2)	10.8	−0.031	0.026	0.2
Disinfection applied (1/0 = ref)	25	0.03	0.34	0.9
Mechanical main ventilation system (1/0 = ref)	4	0.35	0.63	0.6
Between variance (naïve model)		0.36 (0.42)		
Within variance (naïve model)		0.60 (0.68)		
Total variability explained		12%		

n = Number of measurements; β = regression coefficient; se = standard error; p = p-value.
[a] Integrated stable refers to a stable that includes facilities for housing animals from different stages of the production.

A couple of earlier studies have assessed levels of bovine allergens in the environment. Recently Zahradnik et al. (2011) used the EDC (Noss et al., 2008) and the same allergen Sandwich ELISA as the one used in the present study and found similar median levels of bovine hair allergen in cow stables, namely 51,700 μg/m^2 (n = 37). Allergen levels in other types of stables ranged between 0.4 (poultry) and 316 μg/m^2 (goat). In pig stables the median level was 1.5 μg/m^2 and in dwellings the median reported concentration for bedrooms of dairy farmers was 12 μg/m^2; both comparable to the results found in this study (GMs of 1.6 and 8 μg/m^2, respectively). In a German study among 45 dairy farmers with occupational asthma or rhinitis due to bovine dander, levels of Bos d2 in settled dust from cattle stables (n = 36) were reported to be high averaging at 20,400 μg/g (Berger et al., 2005). Two orders of magnitude lower levels (300 μg/g) were reported in the living rooms and mattresses in the homes of these farmers. These levels are not comparable to the per m^2 concentration used in this study, but the difference between stable and home seem to be substantially less than the four orders of magnitude we observed. This might be explained by smaller farms and shorter distance between stable and home (Hinze et al., 1997), or the difference between settling dust and reservoir dust (mattresses and floors). The sampling time of the EDC is limited to 2 weeks. In reservoir dust, allergens can accumulate over a longer period of time.

The assay used in this study is comparable to the traditional method used by Berger et al., (2005) and Hinze et al. (1997). Zahradnik et al., (2011) used several samples from stables, living rooms and mattresses of cattle farmers that have been previously tested for Bos d2 with Rocket immunelectrophoresis, and the two methods correlated very strongly (r^2 = 0.974, p < 0.0001).

Among 18 Finnish cow stables and 31 farmers Virtanen et al reported airborne levels of bovine allergens with a mean (SD) of 460 (300) ng/m^3 in personal total dust measurements (n = 30), and with a mean between 350 and 730 (740–2200) ng/m^3 in stationary measurements (n = 36) (Virtanen et al., 1988). These airborne measurements are not comparable to the settled dust measurements in this study, and furthermore different techniques for quantification of bovine allergens were used.

The higher levels of bovine allergen in the farmers bedroom compared to non-farmers and the increased bedroom level for participants living on a farm indicate that both environmental and occupational contamination through personal behavior and/or clothing actually takes place. Among German cattle farmers concentrations of Bos d2 in the living room and mattress dust were found to be significantly higher for those who worked in stables compared to those who had no contact to stables or were indirectly exposed through family members (Berger et al., 2005). In addition, Zahradnik et al. (2011) found different levels of bovine allergens within the dwellings of dairy farmers. Median levels of allergens were lower in living rooms (4 μg/m^2), bedrooms (12 μg/m^2), and kitchens (19 μg/m^2) and higher in home office (63 μg/m^2) and changing rooms (105 μg/m^2), suggesting that bovine allergens are spread from the workplace via the clothes and hair of farmers. Similarly, transport of mouse, cat, wheat allergen and fungal a-amylase from work to home through clothes, shoes and human hair has previously been reported (Krop et al., 2007; De Lucca et al., 2000; Karlsson and Renström, 2005; Vissers et al., 2001). Also for microorganisms a transfer between stable and home is suggested. Normand et al showed that children living on farms were exposed to many microorganisms and that numerous fungal and bacterial taxa found in farm dwellings were also present in animal sheds and stables (Normand et al., 2011).

Generally, bovine allergen levels appear to be somewhat higher during summer than winter, which is opposite of what has been found for dust and endotoxin among Danish farmers from the SUS study (Basinas et al., 2012a). Summer and winter is defined in the same way for active sampling and EDC sampling, as they were initiated the same day. This difference is unlikely to be a result of differences in numbers of housed animals across seasons; there were no differences in stable area per cattle head values between summer (median: 11 m^2/head) and winter seasons (median: 10.5 m^2/head), and the transfer of animals in and out of stables is n general not dependent of season. One possible explanation though could relate to the shedding hair effects in cattle, when they change from winter to summer fur. The ventilation generally is suggested to have smaller effects in cattle stables than pig stables (Basinas et al., 2013; Takai et al., 1998).

Increased bovine allergen levels in the bedroom were associated with being a farmer or living on a farm, but not with sampling or bedroom characteristics, except for mechanical ventilation in the bedroom which seems to decrease the allergen level, which has not been reported by others as far as we are aware of. On the contrary mechanical ventilation has earlier been associated to higher exposure and more pronounced health effects for mold spores (Meyer et al., 2011). Hinze et al. (1997) found that carpets in bedrooms as well in corridors and living rooms were associated to higher levels of Bos d2 in settled house dust, which was not confirmed by our study.

Bovine allergen levels in dairy farming were associated to type of stable with a decreased level in stables housing mainly heifers or calves compared to main stables with dairy cows. This may be explained by differences in age of animals as well as in activity patterns; i.e. more working activities occur in main stables that house more animals (median of 188 vs. 58 animals in main and secondary stables, respectively). Previous studies on determinants for dust and endotoxin exposure show conflicting results on the association between density of cattle in the stable and the level of exposure (Basinas et al., 2014; Samadi et al., 2012; Virtanen et al., 1988).

The variation in sample and stable characteristics only explained 12% of the total variability of bovine allergens in cow stables, despite the inclusion of many suspected determinants like animal density, type of stable and ventilation (Table 4). Likewise we could only explain 8–10% of the total variability of bovine allergen in the farmer's bedrooms, whereas for non-farmers it was possible to explain 58%, largely due to presence or absence of a farm residence with livestock (Table 3). This confirms, not surprisingly, that animal contact is the main driver for exposure and variability of bovine allergens; but it also underlines that we were not able to explain what drives most of the variation in bedroom bovine allergen exposure among farmers. We did not collect information about behavior during or after farm work, e.g. having lunch breaks or changing cloths in the house, or showering before entry, which might be of importance for the in-house exposure levels (Vissers et al., 2001). Our results do not give rise to a firm advice regarding interior design of the bedroom to decrease the risk for exposure.

External validity of the study

The selection of farms in our study is likely to be representative for Danish farmers. The distribution of farms in Denmark in our initial sampling was similar to the one reported by the Danish authorities, with more than 85% of the farms located in the areas of Jutland and Funen (Statistics Denmark, 2010). A formal analysis showed that selected farms did not differ in size from farms in the initial sample strata (data not shown).

Bovine allergen sensitization

In our population of 20 dairy farmers none were sensitized to bovine allergen. We are aware that our dairy farmer population is small and might be prone to selection, i.e. former cow farmers might have left the population due to symptoms caused by bovine allergy. In fact we found one pig farmer, but formerly dairy farmer, to be sensitized to bovine allergen. Earlier studies have been inconsistent. At baseline in the SUS study 3% male farmers and 1% rural male controls was sensitized to bovine allergens measured by SPT (Sigsgaard et al., 2004). Iversen and Pedersen (1990) in a study among 127 pig and 60 dairy farmers found only 3 (5%) dairy farmers to be sensitized to bovine allergens measured by SPT and 1 (2%) measured by RAST. There was no significant difference between dairy and pig farmers. Kronquist et al. (1999) found bovine sensitization measured by SPT or RAST to be associated to asthma and rhino-conjunctivitis – between 8 and 18% sensitized compared to 1–2% among non-symptomatic subjects – in a survey among 461 Swedish small scale farmers. On the other hand a study comprising data from Danish and Dutch farmers found sIgE sensitization towards bovine allergens to be common, around 25%, but without any association to respiratory symptoms among the participants (Doekes et al., 2000).

In Finland, sensitization to bovine allergens has earlier been reported to be common among dairy farmers. In a random sample of non-smoking Finnish farmers Rautalahti et al. (1987) found 14% to be sensitized to bovine epithelium compared to 1.2% among urban teachers. Associations between bovine sensitization and symptoms among Finnish farmers have been reported (Terho et al., 1987), and bovine sensitization has been a leading cause of occupational asthma in Finland (Karjalainen et al., 2000). There is no obvious answer to the large discrepancies observed in the literature. It has been suggested that a difference in bovine allergen exposure level could serve as an explanation, but until now rather high levels of bovine allergens in cow stables and stables have been documented across all studies. It has also been suggested that a different working behavior (and thereby a higher personal exposure) could explain the discordant findings – e.g. earlier Finnish farmers brushed there cows on a regular basis. One may suspect a very high personal exposure during this task, but the only study that investigated the issue with personal sampling did not report any differences in bovine allergen levels between "brushing" and "non-brushing" farmers (Virtanen et al., 1988).

Taken together though, the occurrence of clinically relevant bovine sensitization among dairy farmers, in general, seems to be quite limited suggesting bovine allergens to be at most a modest risk factor for occupational sensitization. This is in line with what has been found for pig and poultry farmers, where endotoxin exposure is regarded the key exposure of relevance for the health effects (Omland, 2002; Doekes et al., 1997). A couple of studies, including the SUS study, have suggested a protective effect of farming exposure on sensitization, also among dairy farmers (Portengen et al., 2002; Elholm et al., 2013; Basinas et al., 2012b; Smit et al., 2010)

From studies among farmers with bovine sensitization and allergic disease the need for action (secondary and tertiary prevention) is obvious; the study by Hinze et al. (1997) suggest avoidance of farming to decrease both symptoms severity and sIgE level for Bos d2 compared to farmers who continue to work as a farmer.

Concluding remarks

This study confirms high bovine allergen levels on dairy farms, but also suggests sensitization towards bovine allergens to be uncommon. Whether this is related to a simultaneous endotoxin exposure has to be explored in future studies. Bovine allergen exposure is primarily an issue for cow farmers, especially during work but also, to some extent, in their private dwelling, suggesting that allergens are transferred from the stable to the dwellings.

Bovine allergen exposure can, to a limited extent, also take place among non-farmers living on a farm with livestock, whether this is due to a carrier effect or through other exposure ways is not known.

Despite the apparent low bovine sensitization in this study bovine allergy is a recognized problem among dairy farmers with occupational asthma. Whether the risk for bovine sensitization among dairy farmers is in fact related to the allergen level in the stable or the dwelling remains to be determined.

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Conflict of interest statement

The authors declare no conflict of interest.

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