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ENDOTOXIN CONCENTRATION IN MODERN ANIMAL HOUSES IN SOUTHERN BAVARIA

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Abstract: Agricultural work, particularly livestock farming, is considered to be a notable risk factor for occupational diseases. Endotoxin as a major component of organic dust causes adverse health effects of the airways among farmers. Endotoxin concentrations in airborne and settled dust were measured in modern, naturally ventilated animal houses for different species. Median values of airborne inhalable endotoxin ranged from 16.9 EU/m^3 for dairy cattle, 557.9 EU/m^3 for beef cattle, 668.7 EU/m^3 for pigs, 463.2 EU/m^3 for laying hens, to 1,902 EU/m^3 for turkeys. The endotoxin levels in settled dust followed the same pattern as the airborne samples. The concentrations were lower than in previous studies, but the proposed Dutch endotoxin threshold (50 EU/m^3) was exceeded in most cases. Thus, endotoxin levels in modern animal houses still give concern for health and further reduction of endotoxin exposure in animal houses is desirable.

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INTRODUCTION

Agricultural work, particularly livestock farming, is considered to be a notable risk factor for occupational airway diseases. In Germany, animal production is widespread in the rural areas, especially in parts of Lower Saxony, North Rhine Westphalia and Bavaria [1, 27], and appears in facilities with increasing size and specialisation. During their daily work in the animal houses farmers are exposed to a considerable amount of inorganic and organic dust, containing bioaerosols such as fungi, bacteria or their components, as well as gases (e.g. ammonia) and chemicals (e.g. pesticides, disinfectants) [26]. Bioaerosols can adhere to different sizes of organic dust particles, classified as inhalable and respirable dust fraction and settled dust. The major components of organic dust are endotoxins, which are part of the outer membrane of Gram negative bacteria. Their purified derivatives are called lipopolysaccharides (LPS) [19]. Endotoxins can be found ubiquitously on surfaces of animals, plants and soil [18, 19] and at higher concentrations in all occupational environments with exposure to organic dust, including cotton production [15] and intensive farming [24, 33]. In animal houses, the major contributors to endotoxin-contaminated organic dusts are animal faeces and bacteria-contaminated plant materials [26]. Endotoxin is a potent inducer of neutrophilic airway inflammation and thought to be a major risk factor for adverse health effects of the airways among farmers [24]. Due to its proinflammatory properties, high endotoxin exposure is considered to be associated with acute inflammatory processes – the so called ODTS (organic dust toxic syndrome), as well as chronic obstructive pulmonary diseases (COPD) and

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asthma-like syndrome [24, 27, 31]. Such diseases are very common in farmers [20, 24, 25]. Several environmental studies have shown an increased risk for the development of work-related symptoms in animal farmers [7, 25, 26] and high levels of endotoxin concentrations in animal houses and ambient areas were reported [22, 30]. In contrast, there is also evidence that endotoxin exposure in early life (e.g. contact with farm animals) might reduce atopy and prevent the development of asthma and respiratory allergies in children [2, 3, 11, 14, 34]. The effect seems to be sustained until adulthood [17, 28].

In Southern Germany, cattle, pig and poultry husbandry occurs predominantly inside animal houses. Exposure patterns of dust and endotoxin concentrations in these houses may vary over the year due to the cycles of animal production, and are affected by animal species, housing conditions, feeding management, seasonal climate conditions and diurnal activities. In 2005, a pilot project was conducted in Bavaria concerning the analysis of modern, ecologically compatible and economically competitive practices of livestock husbandry, as well as aspects of species appropriate husbandry and environmental sustainability. Different types of modern housing systems for different types of animals, mainly operated with natural ventilation, were investigated with respect to exposure conditions.

The aim of this study was to determine endotoxin concentrations in airborne and settled dust samples taken from naturally ventilated modern livestock buildings for different species (dairy cattle, beef cattle, pigs, laying hens, turkey). In addition, the potential seasonal and day/night variation was assessed.

MATERIALS AND METHODS

Sampling locations. The study included 12 animal houses with livestock husbandry in Southern Germany. Of these animal houses 4 housed dairy cattle, 1 beef cattle (breeding), 3 pigs (fattening), 4 poultry (3 houses for laying hens and 1 house for turkeys). All buildings were modern housing systems (constructed between 2002-2004), mainly operated with natural ventilation and adapted to animal needs and health in order to optimise animal welfare and environmental sustainability. In total, 174 airborne and settled dust samples were taken in the livestock buildings during spring, summer and winter between June 2004 – July 2005. The most important data on the characteristics of the animal houses and on the number of samples are summarised in Tables 1 and 2.

Airborne endotoxin sampling. For airborne endotoxin measurements, stationary samples of inhalable and respirable dust fractions were collected using personal samplers over the feeding period (day) for 1 hour and during night time for 6 hours. Single day and one night samples were taken in each animal house during spring, summer and winter, respectively (except for 3 animal houses, where measurements were taken in only 2 seasons). Due to time restrictions of the study, no measurements took place in autumn. The personal samplers were placed in the middle of the animal houses about 1.5 m above ground level. Dust samples for endotoxin measurements were collected on glass fibre filters (MN 85/90 BF, 37 mm, Macherey-Nagel), which were manufactured with an organic binder for higher mechanic resistance. The suction pumps were operated at a rate of 3.5 l/min for inhalable dust sampling, and at a rate of 2.0 l/min for respirable dust sampling.

Table 1. Characteristics of the different animal houses.

Animals kept	No. of animals	Size (m)	Ventilation
Cattle			
Dairy cows	110	66 × 25 × 8	ridge v. with large area curtains (climate controlled)
Dairy cows	110	54 × 26 × 10	ridge v. with large area curtains (climate controlled)
Dairy cows	80	46 × 29 × 11	ridge v. with large area curtains (manually operated)
Dairy cows	70	30 × 34 × 5	ridge v. with large area curtains (manually operated)
Beef cattle	140	60 × 20 × 8	ridge v. and space boards (no ventilation control)
Pigs			
Fattening pigs	1,200	64 × 49 × 4	natural v. (partly climate controlled)
Fattening pigs	1,400	100 × 18 × 7	dual v.
Fattening pigs	600	61 × 25 × 7	natural v. (with climate controlled curtains)
Fattening pigs	600	51 × 16 × 5	ridge v. (climate controlled)
Poultry			
Laying hens	2,200	40 × 10 × 5	low pressure v.
Laying hens	3,000	30 × 10 × 5	forced v.
Laying hens	500	9 × 8 × 3	naturally v. with curtains
Turkeys	1,500	60 × 13 × 6	naturally v.

Table 2. Number of animal houses, measurement days and samples.

Type of animal house	Number of animal houses	Measurement days	Airborne dust samples	Settled dust samples
Dairy cattle	4	11	44	11
Beef cattle	1	3	12	3
Pigs	4	9	36	9
Laying hens	3	9	36	8
Turkeys	1	3	12	3

Endotoxin sampling in settled dust. In each animal house, 5-6 samples of settled dust were collected on a spatula from different dry surfaces at a height of 0.5-1.5 m above ground level (e.g. ledges, window sills) on each measurement day. Fine dust was separated from hay and straw with

Table 3. Endotoxin concentrations in airborne samples.

Air samples	Endotoxin concentration (EU/m³)							
	Inhalable				Respirable			
	No. of samples	Minimum	Median	Maximum	No. of samples	Minimum	Median	Maximum
Dairy cattle	22	2.8	16.9	66	22	0.3	3.1	61
Beef cattle	6	124.0	557.9	1,025	6	0.9	10.2	31
Pigs	18	43.2	668.7	7,469	18	1.9	23.1	236
Laying hens	18	21.8	463.2	21,933	18	2.5	62.0	12,282
Turkeys	6	467.1	1,902.0	5,292	6	94.0	362.0	762

a sieve (mesh size 0.5 mm). These samples were combined to 1 representative sample for every measurement day.

Collected airborne dust samples were stored at 6°C and settled dust samples were stored at room temperature. Endotoxin concentrations determined within 1 week after collection in the laboratory of the Institute for Occupational and Environmental Medicine, University of Munich.

Endotoxin analyses. Endotoxin concentrations in airborne dust samples were determined according to the European Guideline EN 14031 using the chromogen-kinetic LAL (Limulus Amoebocyte Lysate) assay (QCL Cambrex). Endotoxin concentrations in settled dust samples were determined according to a previously described method [1, 35]. Briefly, from each sample 100 mg were extracted by rapid shaking with 7 ml endotoxin-free water for 1.5 h. Thereafter, the suspension was diluted 1:100 for settled dust samples. An aliquot of 100 µl was added to a microtitre plate (96 well, Falcon) and assayed with Limulus-Amoebocyte-Lysate (QCL Cambrex). To obtain information about possible enhancement or inhibition reactions of the LAL assay, a replicate of each sample was spiked with an endotoxin standard. A standard calibration curve (0.05-0.5-5-50 EU/ml), a laboratory blank and an internal laboratory standard were included on each plate. As recommended by the manufacturer, optical density at 405 nm was measured by an automatic reader (PowerWave™, MWG Biotech Inc., Mendelhall Oaks Parkway, NC, USA). If spike recovery was below 45%, the suspension was further diluted and the analysis was repeated. The intra-assay variability (EU/mg dust) was less than 10%, the inter-assay variability was lower than 20%. As the LAL assay measures the activitiy of different types of endotoxin, the results are expressed in Endotoxin Units (EU). Endotoxin levels for airborne endotoxin were expressed in Endotoxin Units per 1 m³ (EU/m³) and for endotoxin in settled dust in Endotoxin Units per 1 mg dust (EU/mg). Our assay had a potency of 11 EU/ng against *Escherichia coli* 055:B5.

Statistical analysis. Statistical calculations were performed with the software packages Winstat and SPSS for Windows. Since data were not normally distributed, the minimum, maximum and median values are presented. From 3 farms (1 dairy cattle and 2 pig farms) only airborne endotoxin results for 2 seasons were available. These farms were excluded from interpretation of the seasonal variation of endotoxin concentration. For endotoxin in settled dust the result of 1 measurement day in a laying hen house has been omitted.

RESULTS

Airborne endotoxin. Endotoxin levels in inhalable and respirable dust fractions from different animal houses are shown in Table 3. As expected, the endotoxin concentrations in the inhalable dust fraction varied largely between 2.8 EU/m³ in a dairy cattle house at night during winter sampling, and 21,933 EU/m³ in a building for laying hens during the day during winter sampling. The endotoxin concentration in respirable dust was also lowest in a dairy cattle house (0.3 EU/m³) and highest in the building of a laying hen farm (12,282 EU/m³). The highest median concentrations were found in the turkey house, both for the inhalable dust fraction (1,902.0 EU/m³) and the respirable dust fraction (362.0 EU/m³), whereas the maximum values were measured in the laying hens houses. In comparison to the other livestock species, the endotoxin levels in inhalable dust were very low in cattle houses for beef cattle (median 557.9 EU/m³, range 124.0-1,025 EU/m³), and in particular in houses keeping dairy cattle (median 16.9 EU/m³, range 2.8-66 EU/m³). The endotoxin levels in the inhalable fraction exceeded the concentrations in the respirable fraction. The (median) endotoxin concentration in the respirable fraction comprised between 3-20% of endotoxin concentrations in the inhalable fraction, although there were minor differences in this percentage between livestock species (dairy cattle 16%, beef cattle 3%, pigs 3%, hens 11%, turkeys 20%) and between day and night and the seasons.

Diurnal effects. As shown in Table 4, the concentrations of endotoxin were slightly higher during the day than at night. Daytime concentrations (1 h during feeding) ranged from 7-21,933 EU/m³ for the inhalable dust fraction and from 2-762 EU/m³ for the respirable dust fraction. The highest median concentration of inhalable endotoxin during the day (feeding time) was seen in the laying hen houses (3,389 EU/m³), followed by the turkey houses

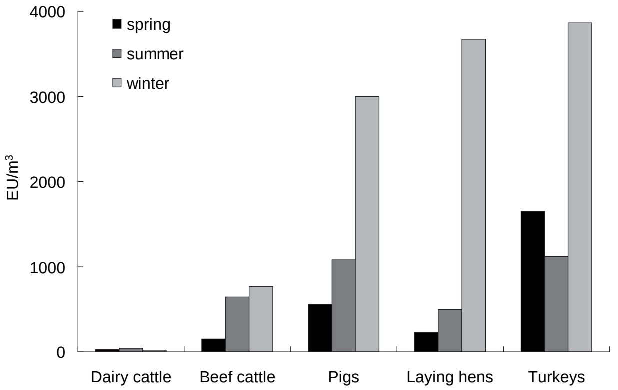

Figure 1. Median endotoxin concentrations of the inhalable dust fraction in different seasons.

(2,031 EU/m³). The endotoxin exposure was far lower in pigs houses (585 EU/m³) and in the cattle barn for beef cattle (516 EU/m³), and with a big difference in the cattle barns for dairy cattle (20 EU/m³). During night (6 h) the concentrations varied between 3-10,546 EU/m³ for inhalable dust and between 1-12,282 EU/m³ for respirable dust. The maximum exposures at night were again found in houses for laying hens, both for the inhalable and the respirable fraction. In the turkey house and in the buildings for laying hens, the median inhalable endotoxin concentration increased significantly during the feeding time by day compared to nighttime, in the hen houses up to a factor of 14. In contrast, the median concentrations in the beef cattle and pig houses during nighttime exceeded the daytime values only slightly in the case of beef cattle, and more strongly in the case of pigs (Tab. 4). Especially 1 pig farm showed particularly high median nighttime endotoxin concentrations (inhalable fraction) in all seasons, but the endotoxin levels of the other pig farms were also considerable during the nighttime.

Seasonal effects. The endotoxin concentrations in the inhalable airborne dust fraction in the different animal houses showed highest values in winter samples and lowest concentrations in spring (Fig. 1). Again, the poultry buildings presented the highest concentrations compared to the other animal houses over all seasons, with the turkey hen house at the peak. As mentioned above, endotoxin concentrations in the dairy cattle buildings are far below the other values. Again, the concentration levels for endotoxin in respirable dust were generally below the values from the inhalable fraction. As the respirable dust fraction constituted a part of the inhalable fraction, the seasonal variations in endotoxin concentration are less clearly visible in the respirable fraction, compared to the inhalable fraction, due to the small contribution of respirable dust to the entire collected airborne dust. Median values were about 3% of the median endotoxin level in the inhalable fraction for cattle (breeding cattle) and pigs, and higher for poultry houses (laying hens and turkey hens) with 11% and 20%, respectively. While the portion of endotoxin in respirable dust from inhalable dust in the poultry houses remains approximately the same over the seasons (about 10% in the laying hen houses and about 20% in the turkey house), no trends could be found for the other livestock buildings. The ratio of endotoxin from respirable dust to inhalable dust in dairy cattle houses resulted in 16%, but this information is restricted due to the low (median) endotoxin levels in the inhalable fraction.

Settled endotoxin. The median endotoxin levels in settled dust for the different kinds of species are presented in Table 5. The median endotoxin concentrations, and also the minimal and maximal values, followed the pattern of the airborne inhalable endotoxin fraction. Again, the endotoxin loads in settled dust from surfaces at human breathing height were higher from the poultry houses than those

Table 4. Endotoxin concentrations in airborne day and night samples.

	Day and night endotoxin concentration (EU/m³)							
	Inhalable				Respirable			
Air samples	No. of samples	Minimum	Median	Maximum	No. of samples	Minimum	Median	Maximum
Day								
Dairy cattle	11	7	20	66	11	2	8	61
Beef cattle	3	124	516	689	3	7	14	31
Pigs	9	43	585	7,469	9	2	43	171
Laying hens	9	100	3,389	21,933	9	3	280	533
Turkeys	3	1,773	2,031	5,292	3	94	319	762
Night								
Dairy cattle	11	3	8	55	11	1	1	11
Beef cattle	3	181	600	1,025	3	1	2	29
Pigs	9	112	1,329	4,873	9	6	19	236
Laying hens	9	22	246	10,546	9	3	39	12,282
Turkeys	3	467	1,271	2,440	3	152	405	577

Table 5. Endotoxin concentrations in settled dust samples.

Settled dust	Endotoxin concentration (EU/mg)			
	No. of samples	Minimum	Median	Maximum
Dairy cattle	11	51	365	1032
Beef cattle	3	95	1079	1114
Pigs	9	147	778	2288
Laying hens	8	137	754	11169
Turkeys	3	502	2100	4408

from the other animal houses. Nevertheless, even in houses for pigs and beef cattle considerable amounts of endotoxin were measured in settled dust. A seasonal effect for endotoxin in settled dust could not be evaluated since only 1 sample was collected for each season.

DISCUSSION

Airborne endotoxin. Our measurements showed that high endotoxin concentrations could even occur in modern animal houses and vary largely due to animal types and seasons. Considerable endotoxin levels were found both in inhalable and respirable fraction of airborne dust as well as in settled dust. The concentration of the inhalable endotoxin fraction ranged between 2.8 EU/m³ for dairy cattle and 21,933 EU/m³ in a poultry house for laying hens. The corresponding endotoxin concentration in the respirable fraction followed nearly the same pattern and was usually only 3-20% of the inhalable fraction. Compared to the proposed Dutch endotoxin threshold of 50 EU/m³ [9] it is obvious that this limit value could only be met in dairy cattle houses. Von Mutius *et al.* [34] also observed a significantly higher endotoxin concentration in the inhalable fraction (geometric mean 649 EU/m³) of animal houses than in the respirable fraction (geometric mean 7 EU/m³). The big concentration differences between endotoxin in inhalable and respirable fraction indicate that endotoxin is bound to larger particles in the animal houses [1]. Compared with other studies, our results for endotoxin concentrations in cattle [30], pig [12, 30] and poultry [30] houses are somewhat lower or approximately in the same range. However, comparison of our endotoxin concentrations with results obtained in other studies has to be approached with caution, because endotoxin analyses may differ from laboratory to laboratory and sampling method and time vary. This has been demonstrated shown in various studies evaluating results of different laboratories and analytical methods. In 2 laboratory comparison studies concerning endotoxin assays, Chun *et al.* [6] showed high variations. Zucker *et al.* [36] compared 2 different sampling methods for endotoxin and found that the suitability of each method depended on the level of endotoxin concentration and duration of measurement.

Generally, for interpretation of our results, it must be taken into account the relatively small number of sampling sites. On the other hand, we had 3 measurements on different days with 2 samples each (1 day sample and one night sample) for each animal house, so that we had a representative overview over diurnal and seasonal patterns. Due to the fact that all 12 animal houses investigated were located in the southern region of Bavaria, the outside climatic conditions during the seasons were well comparable for all sites.

Numerous previous studies collected their samples in conventionally conducted animal house systems (e.g. confinement buildings), which are usually enclosed structures with a high density of livestock. Nevertheless, considerable endotoxin exposures were found for most types of livestock species in our study, which was conducted in modern and ecologically orientated stable systems specialising in animal health and welfare in an appropriate way for the species. As in previous studies, the poultry houses showed the highest airborne endotoxin concentrations compared to pig and cattle houses. This phenomenon could be explained by the higher activity of poultry [30]. This is also supported by the fact that day time and nighttime measurements differed largely for poultry houses, with considerably higher daytime values. In contrast, the day/nighttime variation was small for pig and cattle houses. Moreover, our results for pig farms were usually lower than in earlier studies [12, 30] mainly conducted in conventionally closed swine buildings. However, Chang *et al.* [5] investigated endotoxin concentrations in 30 open-style swine houses with natural ventilation and within a radius of 600 m. They found total endotoxin concentrations between 14.4-818 EU/m³ with an overall mean of 140 EU/m³ in 1.5 m above the floor in the central area of the stables (surrounding: range 3.2-32.9 EU/m³, mean 8.9 EU/m³). But it has to be borne in mind that these measurements took place under subtropical conditions using a different method. Another reason for the generally lower values in our study compared to other studies may be that the animal houses investigated in our study became operational only few months before measurements took place.

In most of the published studies [8, 10, 21, 26, 29], endotoxin samples were collected by personal sampling attached to the workers. In our study, stationary sampling (with personal samplers) took place in the middle of the animal houses to determine the exposure both for humans and for animals. For the sampling period during the time we chose 1 hour during feeding time, when high activity of the animals is expected to cause high dust and endotoxin exposure. However, since these data are from area samplers, they are most likely lower (even during feeding period) than they would be by personal sampling. Our data therefore tends to underestimate the personal endotoxin burden, which should be kept in mind when comparing with other studies. This fact is confirmed by another study [1] where personal dust sampling as well as stationary sampling took place in the same sheds, calculating a factor of 7.3 for the inhalable dust and a factor of 11.3 for the respirable dust fraction.

But even in studies using stationary sampling, the recorded results for total and/or respirable endotoxin exposure in dust from pig, cattle or poultry houses varied greatly, but were usually higher than our results, or approximately in the same range, independent if sampled during work rests [37], during work activities [23, 36], or over longer periods [30]. These and other findings were summarised in a review by Omland [22] who presented the results of some international studies from Northern Europe, USA and Canada, applying personal and/or stationary sampling in different animal houses (pigs, cows, poultry).

Diurnal differences. Only limited data on nighttime exposure levels for endotoxin are available. The reason is that most studies focussed on farmers' health. However, nighttime endotoxin levels might be crucial for animal welfare. We found much lower endotoxin levels in poultry houses during the night and high concentrations during the feeding period (1 h). In 1 poultry farm (laying hens) there was a 10 time higher endotoxin concentration (inhalable) in the day measurement, compared to the night measurement. This was probably due to the diurnal rhythm in animal activities with reduced animal and human activities in the night, and to the higher activities of both animal and livestock workers during the day and the load of endotoxin in grain dust from feed and manure (handling, clearing out, bedding). Because of the larger size of particles being released during those activities, and due to the fact that endotoxin is sticked to larger particles, endotoxin in inhalable dust fraction usually increased more than the respirable endotoxin concentrations. Likewise, endotoxin levels in the study by Seedorf *et al.* [30] differed between day and night time in poultry, as well as in pig and cattle houses in 4 European countries. The endotoxin levels in houses for dairy cattle were comparable to data we have found recently [1]. In that study, we reported median concentrations of 36 EU/m³ (range 4-561 EU/m³) for inhalable samples and 2 EU/m³ (range 0-18 EU/m³) for respirable samples. In the house for beef cattle, the concentrations in the present study were higher, particularly during the daytime, than in dairy cattle houses. In contrast, the higher median concentrations of inhalable endotoxin at night in some pig farms could only be assumed, and therefore possibly be caused by agitation or higher part of more active piglets/weaners.

Seasonal differences. Only a few publications have looked for the seasonal differences of airborne endotoxin concentration from stationary samples in livestock farms, but some of them applied personal sampling. Our results are supported by Preller *et al.* [23] who found higher endotoxin levels in winter (assuming a conversion factor of 10 EU/m³ for 1 ng/m³: geometric mean 1090 EU/m³) than in summer (assuming a conversion factor of 10 EU/m³ for 1 ng/m³: geometric mean 780 EU/m³) in pig houses using personal sampling for 8 hours on a single day in each of the 2 seasons. In contrast, Seedorf *et al.* [30] did not observe a significant seasonal variation in airborne endotoxin concentrations for cattle, pigs and poultry. Other studies measured seasonal variations of dust concentrations, which may be somewhat comparable because endotoxin is bound to dust particles. Takai *et al.* [32] measured dust concentrations in winter and summer in more than 300 livestock buildings in England, Germany and the Netherlands. They found only a weak seasonal effect for cattle, but a higher winter dust concentration for pig and poultry houses, similar to our seasonal distribution. Lee *et al.* [16] measured the airborne dust load as well as airborne microbial concentrations, such as bacteria and fungal spores, in different animal confinements (swine, poultry, dairy) under summer and winter conditions, and found on the swine farm higher concentrations of particles in winter than in summer, blaming the additional space enclosure in winter to protect the animals from the cold weather. The concentration of bacteria and fungal spores measured on the swine and dairy farms in summer was higher than in winter. The highest bacterial concentration was observed in the swine confinement during summer. Most likely, endotoxin levels in our study were also influenced by other sources that could not be included in our analyses, for example, weather conditions on the measurement days [4, 13], ventilation rate, amount of young active animals in the stables, variability in work practices (building size, density of animals).

Endotoxin in settled dust. Endotoxin concentrations in settled dust of dairy cattle were the lowest among the different animal types, and compare well with our previous results which showed a geometric mean of 258 EU/mg settled dust from about 300 animal houses in Austria, Germany and Switzerland [35], and a median endotoxin concentration of 202 EU/mg (range 22-832 EU/mg dust) in settled dust from 36 cow barns in Bavaria [1], respectively. Von Mutius *et al.* [34] measured a geometric mean of 649 EU/mg endotoxin in settled dust in livestock buildings which all housed cattle; some of them additionally housed pigs, sheep, goats or horses. Settled dust is a good marker for long term endotoxin exposure in stables. This dust can pass into the airborne dust fraction when activities inside the stables occur. Interestingly, the endotoxin concentrations in settled dust showed the same pattern as the inhalable endotoxin fraction in airborne dust for all animal species. Therefore, settled dust might be a useful marker of the endotoxin exposure in animal houses and has the additional advantage of easier sampling procedure.

CONCLUSION

In conclusion, the present study has indicated that even in modern, naturally ventilated and ecological housing systems, considerable exposure for airborne and settled endotoxin existed both during the day and night, and over all seasons. We have shown that the proposed Dutch endotoxin threshold of 50 EU/m³ could only be kept in modern dairy cattle houses. Endotoxin levels were influenced by sampling sites and animal species, but were generally lower than in conventionally conducted stables presented in earlier papers. In the inhalable dust fraction, endotoxin concentrations were higher than in the respirable fraction. Poultry houses generally showed the highest endotoxin levels compared to cattle and pigs. They also had higher endotoxin levels during the daytime than at night, while day and night concentrations in cattle stables were balanced and high median endotoxin concentrations at night were found in some pig farms. In winter, median concentrations for inhalable endotoxin for all animal types were usually higher than in spring or summer. The endotoxin levels in settled dust followed the same pattern as the airborne samples.

Our results therefore indicate that endotoxin in livestock houses, particularly in poultry houses, still give cause for concern both for the health of farmers, as well as for animal health and performance, therefore a further reduction of exposure is necessary. Further research is desirable to find practical methods to reduce endotoxin exposure in animal houses.

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REFERENCES

1. Berger I, Schierl R, Ochmann U, Egger U, Scharrer E, Nowak D: Concentrations of dust, allergens and endotoxin in stables, living rooms and mattresses from cattle farmers in southern Bavaria. *Ann Agric Environ Med* 2005, **12**, 101-107.

2. Bottcher MF, Bjorksten B, Gustafson S, Voor T, Jenmalm MC: Endotoxin levels in Estonian and Swedish house dust and atopy in infancy. *Clin Exp Allergy* 2003, **33**, 295-300.

3. Braun-Fahrlander C, Riedler J, Herz U, Eder W, Waser M, Grize L, Maisch S, Carr D, Gerlach F, Bufe A, Lauener RP, Schierl R, Renz H, Nowak D, von Mutius E: Environmental exposure to endotoxin and its relation to asthma in school-age children. *N Engl J Med* 2002, **347**, 869-877.

4. Carty CL, Gehring U, Cyrys J, Bischof W, Heinrich J: Seasonal variability of endotoxin in ambient fine particulate matter. *J Environ Monit* 2003, **5**, 953-958.

5. Chang CW, Chung H, Huang CF, Su HJJ: Exposure assessment to airborne endotoxin, dust, ammonia, hydrogen sulfide and carbon dioxide in open style swine houses. *Ann Occup Hyg* 2001, **45**, 457-465.

6. Chun DTW, Bartlett K, Gordon T, Jacobs RR, Larsson BM, Larsson L, Lewis DM, Liesivuori J, Michel O, Milton DK, Rylander R, Thorne PS, White EM, Brown ME, Gunn VS, Wurtz H: History and results of the two inter-laboratory round robin endotoxin assay studies on cotton dust. *Am J Ind Med* 2006, **49**, 301-306.

7. Danuser B, Weber C, Kunzli N, Schindler C, Nowak D: Respiratory symptoms in Swiss farmers: An epidemiological study of risk factors. *Am J Ind Med* 2001, **39**, 410-418.

8. Donham KJ, Cumro D, Reynolds SJ, Merchant JA: Dose-response relationships between occupational aerosol exposures and cross-shift declines of lung function in poultry workers: Recommendations for exposure limits. *J Occup Environ Med* 2000, **42**, 260-269.

9. Dutch Expert Committee on Occupational Standards: *Endotoxins: health based recommended exposure limit*. A report of the Health Council of the Netherlands, publication no. 1998/03WGD. Health Council of the Netherlands, Rijkswijk 1998.

10. Eduard W, Douwes J, Omenaas E, Heederik D: Do farming exposures cause or prevent asthma? Results from a study of adult Norwegian farmers. *Thorax* 2004, **59**, 381-386.

11. Gehring U, Bischof W, Fahlbusch B, Wichmann HE, Heinrich J: House dust endotoxin and allergic sensitization in children. *Am J Respir Crit Care Med* 2002, **166**, 939-944.

12. Heederik D, Brouwer R, Biersteker K, Boleij JS: Relationship of airborne endotoxin and bacteria levels in pig farms with the lung function and respiratory symptoms of farmers. *Int Arch Occup Environ Health* 1991, **62**, 595-601.

13. Heinrich J, Pitz M, Bischof W, Krug N, Borm PJA: Endotoxin in fine (PM2.5) and coarse (PM2.5-10) particle mass of ambient aerosols. A temporo-spatial analysis. *Atmos Environ* 2003, **37**, 3659-3667.

14. Kabesch M, Lauener RP: Why Old McDonald had a farm but no allergies: genes, environments, and the hygiene hypothesis. *J Leukoc Biol* 2004, **75**, 383-387.

15. Lane SR, Nicholls PJ, Sewell RDE: The measurement and health impact of endotoxin contamination in organic dusts from multiple sources: Focus on the cotton industry. *Inhal Toxicol* 2004, **16**, 217-229.

16. Lee SA, Adhikari A, Grinshpun SA, McKay R, Shukla R, Reponen T: Personal exposure to airborne dust and microorganisms in agricultural environments. *J Occup Environ Hyg* 2006, **3**, 118-130.

17. Leynaert B, Guilloud-Bataille M, Soussan D, Benessiano J, Guenegou A, Pin I, Neukirch F: Association between farm exposure and atopy, according to the CD14 C-159T polymorphism. *J Allergy Clin Immunol* 2006, **118**, 658-665.

18. Liu AH, Redmon AH: Endotoxin: Friend or foe? *Allergy Asthma Proc* 2001, **22**, 337-340.

19. Michel O: Systemic and local airways inflammatory response to endotoxin. *Toxicology* 2000, **152**, 25-30.

20. Monso E, Magarolas R, Radon K, Danuser B, Iversen M, Weber C, Opravil U, Donham KJ, Nowak D: Respiratory symptoms of obstructive lung disease in European crop farmers. *Am J Respir Crit Care Med* 2000, **162**, 1246-1250.

21. Nieuwenhuijsen MJ, Noderer KS, Schenker MB, Vallyathan V, Olenchock S: Personal exposure to dust, endotoxin and crystalline silica in California agriculture. *Ann Occup Hyg* 1999, **43**, 35-42.

22. Omland O: Exposure and respiratory health in farming in temperate zones - A review of the literature. *Ann Agric Environ Med* 2002, **9**, 119-136.

23. Preller L, Heederik D, Kromhout H, Boleij JS, Tielen MJ: Determinants of dust and endotoxin exposure of pig farmers: development of a control strategy using empirical modelling. *Ann Occup Hyg* 1995, **39**, 545-557.

24. Radon K: The two sides of the "endotoxin coin". *Occup Environ Med* 2006, **63**, 73-78.

25. Radon K, Danuser B, Iversen M, Jorres R, Monso E, Opravil U, Weber C, Donham KJ, Nowak D: Respiratory symptoms in European animal farmers. *Eur Respir J* 2001, **17**, 747-754.

26. Radon K, Danuser B, Iversen M, Monso E, Weber C, Hartung J, Donham KJ, Palmgren U, Nowak D: Air contaminants in different European farming environments. *Ann Agric Environ Med* 2002, **9**, 41-48.

27. Radon K, Peters A, Praml G, Ehrenstein V, Schulze A, Hehl O, Nowak D: Livestock odours and quality of life of neighbouring residents. *Ann Agric Environ Med* 2004, **11**, 59-62.

28. Radon K, Schulze A, Nowak D: Inverse association between farm animal contact and respiratory allergies in adulthood: protection, underreporting or selection? *Allergy* 2006, **61**, 443-446.

29. Radon K, Weber C, Iversen M, Danuser B, Pedersen S, Nowak D: Exposure assessment and lung function in pig and poultry farmers. *Occup Environ Med* 2001, **58**, 405-410.

30. Seedorf J, Hartung J, Schroder M, Linkert KH, Phillips VR, Holden MR, Sneath RW, Short JL, White RP, Pedersen S, Takai H, Johnsen JO, Metz JHM, Koerkamp P, Uenk GH, Wathes CM: Concentrations and emissions of airborne endotoxins and microorganisms in livestock buildings in Northern Europe. *J Agric Engng Re* 1998, **70**, 97-109.

31. Singh J, Schwartz DA: Endotoxin and the lung: Insight into the host-environment interaction. *J Allergy Clin Immunol* 2005, **115**, 330-333.

32. Takai H, Pedersen S, Johnsen JO, Metz JHM, Koerkamp P, Uenk GH, Phillips VR, Holden MR, Sneath RW, Short JL, White RP, Hartung J, Seedorf J, Schroder M, Linkert KH, Wathes CM: Concentrations and emissions of airborne dust in livestock buildings in Northern Europe. *J Agric Engng Res* 1998, **70**, 59-77.

33. Von Essen S: The role of endotoxin in grain dust exposure and airway obstruction. *Curr Opin Pulm Med* 1997, **3**, 198-202.

34. von Mutius E, Braun-Fahrlander C, Schierl R, Riedler J, Ehlermann S, Maisch S, Waser M, Nowak D: Exposure to endotoxin or other bacterial components might protect against the development of atopy. *Clin Exp Allergy* 2000, **30**, 1230-1234.

35. Waser M, Schierl R, von Mutius E, Maisch S, Carr D, Riedler J, Eder W, Schreuer M, Nowak D, Braun-Fahrlander C: Determinants of endotoxin levels in living environments of farmers‘ children and their peers from rural areas. *Clin Exp Allergy* 2004, **34**, 389-397.

36. Zucker BA, Draz AM, Muller W: Comparison of filtration and impingement for sampling airborne endotoxin. *J Aerosol Sci* 2000, **31**, 751-755.

37. Zucker BA, Muller W: Concentrations of airborne endotoxin in cow and calf stables. *J Aerosol Sci* 1998, **29**, 217-221.