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AMBIENT ENDOTOXIN LEVEL IN AN AREA WITH INTENSIVE LIVESTOCK PRODUCTION

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Abstract: High levels of endotoxin are found inside and near to animal houses. However, there is a lack of data on environmental endotoxin in areas with intensive animal production facilities. We conducted a cross-sectional study of respiratory health in two villages of Lower Saxony with intensive livestock production. We assessed the level of endotoxin exposure in the backyards of 32 participants with two 24-hours measurements of inhalable fraction (one in winter and one in summer). The geometric mean (geometric standard deviation) of the levels of endotoxin varied between 2.0 (2.9) EU/m³ in winter and 2.9 (2.4) EU/m³ in summer. Potential predictors - season, sampling sites, and weather conditions - explained 24% of the variability in ambient endotoxin concentration in the study area. The results indicate that, compared with urban residents, exposure to endotoxin is greater among people living in rural areas with intensive animal production. This might affect their respiratory health. However, these exposures are characterized by a large spatial variability.

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INTRODUCTION

During recent decades, intensive livestock facilities became more common in many countries including Netherlands, Denmark, France, USA, Canada, China, and more recently Poland. In Germany, intensive production of pigs and poultry is widespread especially in parts of Lower Saxony [12] and North Rhine Westphalia. The increasing size, complexity, specialisation, and concentration of livestock and poultry farms have often resulted in a conflict with neighbours not involved in farming [3, 14]. Overall, neighbours are frequently annoyed by the odours from large-scale animal houses and from the fields [8, 12, 14]. In addition, they are often concerned about potential effects of gaseous and particulate emissions from animal houses on respiratory health and well-being [12, 14, 15].

Work in agriculture exposes the respiratory system to many different agents, such as inorganic and organic dust containing endotoxin, bacteria, allergens, and fungi, as well as gases (e.g., ammonia) and chemicals (e.g., disinfectants, pesticides) [13]. Endotoxin is a component of the outer membrane of gram-negative bacteria. Due to its

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pro-inflammatory capacity it is thought to cause adverse health effects among workers of animal houses: organic dust toxic syndrome (ODTS), chronic obstructive pulmonary diseases (COPD) and asthma-like syndrome [10-12, 17]. In contrast, endotoxin has recently been implicated in the protective effect of early-life contact to animal farms on respiratory allergies in children [4, 6, 18].

Several environmental studies have shown that the high concentrations of endotoxins inside animal houses are also found in close proximity to them [13, 16]. However, data on endotoxin exposure in ambient air are limited [2, 5, 7, 9] and to our knowledge, no study on ambient endotoxin levels in an area with intensive animal production has been carried out.

This study was part of the Lower Saxony Lung Study (NiLS) of respiratory health in adults living in a rural area with a high concentration of intensive animal production facilities [12]. The area has a high density of animal farming with many animal houses directly neighbouring residential areas.

We aimed to compare levels of endotoxin in rural area with those in urban areas. In addition, the spatial variation was assessed with respect to weather conditions and sampling site.

METHODS

Sampling locations. From the villages included in the NiLS-study, we selected two with the highest number of animal production facilities. We will refer to them as villages A and B for privacy purposes. The respective counts of animals in villages A and B were 270 and 690 cattle, 12,200 and 12,100 pigs, 702,000 and 680,000 hens, 42,800 and 45,000 ducks, 6,000 and 155,500 turkeys.

Samples of endotoxin were taken in the backyards of 32 participants of the NiLS-study. Participants were selected in order to represent a wide spatial distribution of sampling sites within the villages. Oral informed consent was obtained from participants for conducting measurements in their backyards. Thus, 24 sampling sites were in village A, and eight were in village B (Fig. 1). In order to account for seasonal variation, we took one measurement in winter and one in summer of 2004 [2] resulting in 64 measurements. To cover the day-night-variation [16] measurements were conducted over 24 hours.

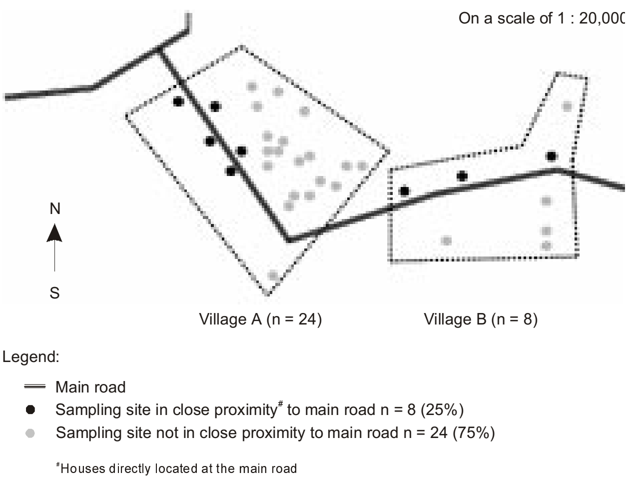

Figure 1. Classification of sampling sites.

In our analyses we accounted for temperature, wind direction, wind force, and precipitation. The weather data were obtained from the German National Meteorological Service (Deutscher Wetterdienst [DWD]). Data from two monitoring stations 10 and 44 km away from the village were used. The monitoring station located closer to the study area provided the data of precipitation. The other station provided the temperature, wind direction, and air velocity.

Field work and endotoxin analyses. The field worker arranged appointments with the participants by telephone and helped the research staff to find a suitable measurement site. A suitable site was a place where the pump could be placed outdoors in the vicinity of a power supply. The pumps were placed into wooden cases in order to protect them against extreme weather conditions. The protection cases were installed at the height of 1.5 m away from garbage cans or compost heaps. Inhalable dust was collected on 37 mm diameter glass fibre filters (Whatman, Glass Micro-fibre Filters) fixed in threaded holders. Power-operated pumps (BUCK-VSS) provided a constant airflow of 3.5 l/min. The flow was calibrated using a rotameter at the beginning and at the end of each 24-hour measurement. Six duplicate measurements were conducted with two pumps at the same location. The results of these duplicates were used to calculate the relative measurement error (38%). In addition, 16 field blanks yielded a detection limit (mean of blanks plus three times standard deviation) of 1.3 endotoxin units per m³ (EU/m^3).

The levels of endotoxin in all samples were determined in the laboratory of the Institute for Occupational and Environmental Medicine of the University of Munich by a kinetic Limulus assay (kinetic-QCL, BioWhittaker Inc., Walkersville, MD, USA) [19]; standard guidelines were followed [11]. The standard endotoxin had a potency of *E. coli* 055: B5 of 11 EU/ng. The laboratory background endotoxin level over 24 hours with a flow of 3.5 l/min amounted to 0.1 EU/m³.

Statistical analysis. Dividing the amount of measured endotoxin by the sampled air volume sucked through the pumps the endotoxin concentration (in EU/m³) was obtained. Endotoxin concentration was best described by lognormal distribution. All results below the detection limits were set to 50% of the detection limit.

The sampling sites were *a priori* grouped based on proximity to the main road (Fig. 1). This was done as in the study area large animal production facilities are not located in direct proximity to the main road. Sampling sites classified as “in close proximity to main road” were those where the houses were located directly at the main road.

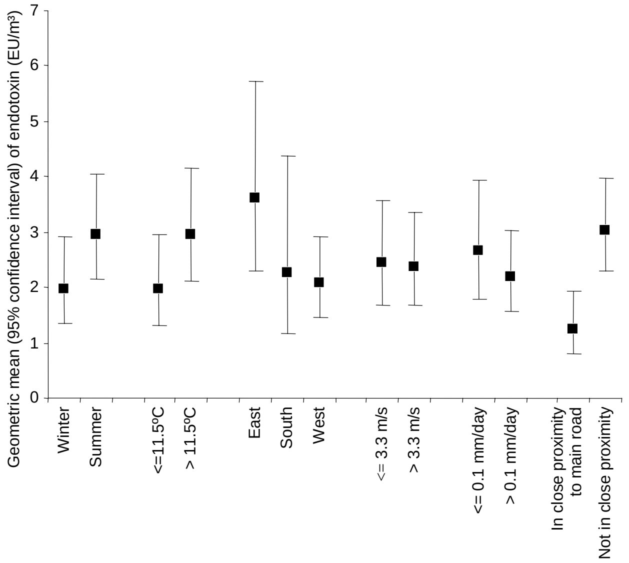

Figure 2. Association between season, weather conditions, sampling sites and ambient endotoxin concentrations.

Table 2. Analysis of variance between environmental parameters and ambient endotoxin levels (R^2=0.24; $p<0.05$).

	Log transformed endotoxin level [ln(EU/m³)]		
	d.f.[a]	Value of Fisher's F distribution	p-value
Season (winter or summer)	1	1.71	0.2
Weather:			
Temperature [ºC]	1	0.18	0.7
Wind direction (south, east or west)	2	1.54	0.2
Air velocity [m/s]	1	0.17	0.7
Precipitation [mm]	1	1.26	0.3
Proximity to main road	1	7.39	<0.01

[a]d.f. degree of freedom

We performed bivariate and multiple regression analyses (analysis of variance of repeated measurements) using SAS software (Version 9.1; SAS Institute Inc.) to assess the relationship of ambient endotoxin concentration in the rural study area with weather conditions and location of the sampling site.

RESULTS

To compare the levels of endotoxin between rural and urban areas, the background endotoxin levels were also determined at a sampling site in the city closest to the study area (Oldenburg, 44 km north). The endotoxin level (0.6 EU/m³) was found to be below the detection limit of the measurement devices (1.3 EU/m³).

Within the rural study area, endotoxin concentrations varied from below the detection limit to 20.0 EU/m^3 in winter and from below the detection limit to 23.2 EU/m^3 in summer (Tab. 1). Overall, 17 (26%) of the samples were below the detection limit. Median air temperature during sampling was 11.5ºC (52.6ºF), the main wind direction was west (56% of the measurements) with a median air velocity of 3.3 m/s. The precipitation ranged from 0–22.8 mm per day.

Higher temperatures were borderline significantly associated with higher endotoxin concentrations ($p_{Wilcoxon}$=0.10, Fig. 2). There were no significant differences in the endotoxin concentrations by wind direction or according to the air velocity. The ambient endotoxin concentration measured in close proximity to the main road was significantly lower than endotoxin concentration at other sampling sites ($p_{Wilcoxon}<0.01$).

The multiple regression analysis confirmed the results of the bivariate analysis (Tab. 2). The parameters included in the analysis of variance explained 24% of the variability of the ambient endotoxin concentration ($p<0.05$). In

Table 1. Ambient endotoxin concentrations and weather conditions during the measurements.

		Mean (SD[a])	GM[b] (GSD[c])	Quartile (1st; 2nd; 3rd)	Range
Endotoxin concentrations [EU/m^3]					
Winter (n=32)		3.55 (4.36)	1.98 (2.98)	0.66; 2.17; 5.06	0.66–19.98
Summer (n=32)		4.35 (4.65)	2.95 (2.41)	1.82; 2.71; 5.28	0.66–23.22
Air temperature [°C]		10.15 (6.72)	-	3.65; 11.45; 15.88	0.17–21.02
Air velocity [m/s]		3.21 (0.87)	-	2.50; 3.26; 3.59	1.85–5.19
Precipitation [mm per day]		2.14 (5.06)	1.76 (3.77)	0.00; 0.10; 2.00	0.00–22.80
Wind direction		(% measurements)			
	North	0.00			
	East	25.00			
	South	18.75			
	West	56.25			

[a]SD Standard deviation; [b]GM Geometric mean; [c]GSD Geometric standard deviation

this model the proximity to the main road was the main predictor of the ambient endotoxin concentration (F=7.4, $p<0.01$). Season, wind direction, and precipitation were not significantly associated with levels of endotoxin.

DISCUSSION

Inhalable samples from the backyards of 32 citizens in rural Lower Saxony were analysed for endotoxin content. We assessed potential predictors associated with ambient endotoxin concentration like sampling sites and weather conditions. Overall, our measurements in this rural area with intensive animal production facilities showed a higher level of endotoxin than control measurements in urban areas. In addition, the endotoxin concentrations varied widely by area. Weather and spatial distribution explained nearly one quarter of the variability of the ambient endotoxin level.

The measurements were conducted with standard methods at the laboratory of the Institute for Occupational and Environmental Medicine in Munich. All co-workers adhered to standard procedures. In addition, field visits for quality control were carried out. Different sampling sites could affect the results; therefore, the staff members paid special attention to use similar sampling locations in winter and summer. Field blanks and duplicates were performed to control for the different pumps and the background concentration.

The mean endotoxin level found in the urban area was significantly lower than those measured in the rural area. In addition, these background levels were comparable to measurements of Mueller-Anneling *et al.* in different communities in Southern California [7] (geometric mean: 0.3 EU/m³ *vs.* 0.4 EU/m³). In the rural study area, the endotoxin concentrations were higher than in the Californian study, most likely due to the influence of intensive livestock production in the area. Likewise, Heinrich *et al.* measured endotoxin levels in two cities in the east of Germany [5] and found endotoxin levels in the particulate matter (PM) less than 10 µm (PM_{10}) fraction, well below our measurements (GM, range; 0.07 EU/m³, 0.02–0.17 EU/m³). However, it has to be taken into consideration that endotoxin measurements performed in different laboratories and in different particle fractions are not directly comparable [10].

While Heinrich *et al.* used $PM_{2.5\text{-}10}$ and Mueller-Anneling *et al.* PM_{10}, we used inhalable samples. The PM_{10} fraction used by Mueller-Anneling is comparable to the inhalable fraction used in our study. Intensive farming might therefore be one reason for the higher endotoxin levels found in our study. In order to assess the impact of farming on the endotoxin concentrations we took into consideration sampling sites, weather conditions, and season. Large numbers of animal houses and animals were found in the study area. To assess their influence we took the proximity to the main road into account and found significant spatial differences in ambient endotoxin concentrations. Owing to privacy consideration we were unable to use data on number, type, and housing characteristics of the animal houses in close proximity to residents. If data on the number of animals inside the animal houses as well as the ventilation characteristics of these houses at the time of the measurements were available, it is likely that even more of the variation in endotoxin levels in the study area could be explained by intensive animal production. Most likely, endotoxin levels in our study were also influenced by other sources that we could not include in our analyses, for example, spraying liquid manure on the fields, or presence of slaughter-houses near to the sampling sites.

There are some studies showing some influence of weather conditions on levels of endotoxin (e.g. [2] and [5]). For our study, the association between weather conditions and levels of endotoxin was not significant. As no other weather data were available for the study area, we had to use weather monitoring stations that were 10 and 44 km away from the study area. However, because of the topography (no mountains, few forests) the distance is thought to only weakly influence weather conditions.

Carty *et al.* studied the seasonal variation and influence of temperature on the endotoxin level [2]. In their study, the influence of weather conditions was weak. This might be due to the different particular matter in aerodynamic diameter (PM2.5) used in their study and different sampling period (14 days, during which air was sampled for 15 minutes every two hours for a total of 42 hours each sampling period). However, levels of endotoxin are much better detectable in inhalable fraction [1]. In contrast, a seasonal influence was found in a study by Heinrich *et al.* [5] in two towns in Eastern Germany.

CONCLUSION

Our results confirm that people living in rural areas might be exposed to a higher level of endotoxin than urban residents. Our study has shown that ambient endotoxin concentrations in a rural area with intensive animal production facilities are strongly affected by sampling sites. This spatial distribution could be influenced by intensive animal production and weather conditions.

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