



# Spatial and temporal variation in endotoxin and PM10 concentrations in ambient air in a livestock dense area

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## ABSTRACT

Several studies have reported associations between farming and respiratory health in neighboring residents. Health effects are possibly linked to fine dust and endotoxin emissions from livestock farms. Little is known about levels of these air pollutants in ambient air in livestock dense areas. We aimed to explore temporal and spatial variation of PM10 and endotoxin concentrations, and the association with livestock-related spatial and meteorological temporal determinants.

From March till September 2011, one week average PM10 samples were collected using Harvard Impactors at eight sites (residential gardens) representing a variety of nearby livestock-related characteristics. A background site was included in the study area, situated at least 500 m away from the nearest farm. PM10 mass was determined by gravimetric analysis and endotoxin level by means of Limulus-Amebocyte-Lysate assay. Data were analyzed using mixed models.

The range between sites of geometric mean concentrations was for PM10 19.8–22.3  $\mu\text{g}/\text{m}^3$  and for endotoxin 0.46–0.66 EU/ $\text{m}^3$ . PM10 concentrations and spatial variation were very similar for all sites, while endotoxin concentrations displayed a more variable pattern over time with larger differences between sites. Nonetheless, the temporal pattern at the background location was highly comparable to the sites mean temporal pattern both for PM10 and endotoxin (Pearson correlation: 0.92, 0.62). Spatial variation was larger for endotoxin than for PM10 (within/between site variance ratio: 0.63, 2.03). Spatial livestock-related characteristics of the surroundings were more strongly related to endotoxin concentrations, while temporal determinants were more strongly related to PM10 concentrations.

The effect of local livestock-related sources on PM10 concentration was limited in this study carried out in a livestock dense area. The effect on endotoxin concentrations was more profound. To gain more insight in the effect of livestock-related sources on ambient levels of PM10 and endotoxin, measurements should be based on a broader set of locations.

## 1. Introduction

Several studies have reported associations between livestock farming and respiratory health in neighboring residents (Elliott et al., 2004; Pavilonis et al., 2013; Radon et al., 2007; Schiffman et al., 2005; Schinasi et al., 2011; Smit et al., 2012, 2014; Wing and Wolf, 2000; Mirabelli et al., 2006). Associations were explored using exposure proxies such as distance to farm and farm density. Health effects are possibly linked to fine dust and endotoxin exposure, which are known to be emitted from farms (Radon et al., 2007; Schinasi et al., 2011; Smit et al., 2014). Endotoxins are components of the cell-walls of gram-negative bacteria. Aerosolized endotoxins can be inhaled and are capable of causing inflammation (Douwes et al., 2003). Exposure to endotoxins is known to induce a variety of acute and chronic clinical

signs, predominantly adverse respiratory health (Liebers et al., 2008). The agricultural sector is suggested as a major source of environmental endotoxins (Mueller-Anneling et al., 2004; Thorne et al., 2009). High endotoxin concentrations have been measured in and directly around farms (Thorne et al., 2009; Jonges et al., 2015). Endotoxin-contaminated organic dust is regarded as the most important respiratory hazard within livestock related occupational settings (Basinas et al., 2015).

Endotoxins are absorbed onto the surface of particles, predominantly coarse particulate matter (Soukup and Becker, 2001; Schins et al., 2004; Morgenstern et al., 2005; Chen and Hildemann, 2009). Particles with an aerodynamic diameter of 10  $\mu\text{m}$  or less (Particulate Matter 10/PM10) are able to enter the tracheobronchial and alveolar regions of the respiratory tract and are associated with adverse health

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effects (Lippmann et al., 1980). Elevated PM10 exposure leads to increased morbidity and mortality from respiratory and cardiovascular diseases (Adar et al., 2014; Pope and Dockery, 2006). PM10 concentrations can be high inside livestock operations (Heber et al., 2006; Winkel et al., 2015) and when emitted to the environment may lead to an increase in PM10 concentrations in ambient air around farms.

Only a few studies have been performed measuring endotoxin concentrations in ambient air showing endotoxin concentrations to vary notably spatially as well as temporarily (Mueller-Anneling et al., 2004; Morgenstern et al., 2005; Carty et al., 2003; Bari et al., 2014; Tager et al., 2010; Kallawicha et al., 2015). Higher temperatures were found to be consistently positively associated with endotoxin concentrations in these studies (Mueller-Anneling et al., 2004; Carty et al., 2003; Bari et al., 2014; Tager et al., 2010; Kallawicha et al., 2015). The role of relative humidity, wind speed and precipitation as well as other meteorological factors remains unclear. Kallawicha et al. (2015) looked into spatial variation of endotoxin in the Greater Taipei Area with varying degrees of urbanization and did not find a relationship between endotoxin and land-use type. Tager et al. reported an ecological association between elevated endotoxin concentrations and proximity of pasture-land, cropland and Confined Animal Feeding operations in California (Tager et al., 2010). An earlier study in California (USA) reported highest levels of endotoxins in a community in close proximity to an area with high dairy farm density (Mueller-Anneling et al., 2004). Yet, these studies lack detailed livestock information.

Overall, well-designed exposure studies for the assessment of PM10 and endotoxin concentrations in ambient air related to agriculture, especially livestock farming, are virtually lacking. We thus aimed to explore temporal and spatial variation in PM10 concentrations and endotoxin concentrations in a livestock dense area. Secondary aim was to identify spatial determinants using detailed livestock-related characteristics of the surroundings and temporal determinants using local meteorological conditions.

## 2. Material and methods

### 2.1. Study design

The study took place in an area in the Netherlands with the highest-livestock density of the country (see below for a description of the Dutch farming system). We performed secondary analyses on an air measurement network in The Netherlands originally set up to detect airborne *Coxiella burnetii* (causative agent of Q fever) related to ruminant farming. The design of the measurement network including sampling site selection has been described previously (de Rooij et al., 2016). Briefly, one-week averaged PM10 measurements were performed concurrently at eight measurement locations in the east of the Province Noord-Brabant. All sites were located near a farm (within 300 m) and at least 500 m away from a highway and/or railway. Measurements were collected from March till September 2011.

Sampling sites covered a range of number of farms present in the vicinity and different animal species were represented including poultry, pigs and cattle. As the measurement network was primarily set up to detect *C. burnetii*, all sites were situated nearby goat farms. This permitted studying associations between PM10 and endotoxin concentrations and type of farm (for example poultry farm, cattle farm, goat farm) in a secondary analysis.

To explore the use of a background location to characterize area-wide temporal variation in analyses of PM10 and endotoxin concentrations, an additional measurement location was included from May 2011 onwards, ten weeks after the start of the campaign. This location was situated in the same rural area, however at larger distances from livestock farms (> 500 m) than the eight sites. Inclusion of a background site is especially important in designs including a high number of sites where it is impossible to measure all sites simultaneously. The background site is then used to account for temporal variation,

provided that the site reflects temporal patterns representative for the covered area. In the current study, because of the limited number of sites, all sites could be measured simultaneously. Thus the background site was only used to investigate applicability of adjusting for temporal variation in PM10 and endotoxin (see statistical analyses).

### 2.2. Description of livestock farming practices in the Netherlands

In the Netherlands, livestock is generally kept year-round in enclosed animal houses; except for some dairy cows and sheep which, during part of the year, also stay at pasture land. Dairy farms are commonly naturally ventilated whereas pig and poultry farms are mechanically ventilated. Various mechanical and natural ventilation systems are used in these animal houses. Manure is stored at the farm until application or treatment. Land application of manure is only allowed during specific periods defined by type of manure, soil type and land use. As a rule of thumb application of manure is allowed between February and September.

### 2.3. Sampling method and processing

Harvard Impactors (Air Diagnostics and Engineering Inc., Naples, ME, USA) at 2.5 m height were used to sample PM10 dust on Teflon filters (Teflo W/ring 37 mm with 2 µm pore size; Pall Corporation, Michigan, Ann Arbor, USA). The flow was maintained at 10 L/min by means of Derenda pumps (LVS 3.1; Comde-Derenda GmbH, Stahnsdorf, Germany). Pumps were programmed to sample air for 30 min of each hour to avoid filter overloading. Thirty-eight duplicate, side-by-side, measurements were included, these were performed at different locations. A field blank control was collected every sampling week, and each week this was performed at a different site. All samples were stored within 24 h after collection at −20 °C. Strict procedures were followed to avoid endotoxin contamination of materials. Filters were handled with cleaned and disinfected (flamed) tweezers and stored until use in sterile petridishes in ziplock bags. All other sampling materials were cleaned with soap and disinfected with 70% ethanol before use.

Particle mass was determined by gravimetric analysis using a microbalance (1 µg precision) as described previously (Eeftens et al., 2012). Filters were conditioned for 24 h prior to pre- and post-weighing in a climate controlled room: temperature (21 ± 0.5 °C) and relative humidity (35 ± 5%) (Eeftens et al., 2012). Subsequently, samples underwent endotoxin extraction and analysis as described previously (de Rooij et al., 2016). Filters, including support ring, were transferred to 50-ml tubes (Greiner Bio-one) by using two disinfected tweezers on opposite sides bending the filter slightly. Next, 5 ml of pyrogen-free water (Aqua B. Braun) supplemented with 0.05% Tween 20 (Calbiochem, United States) was added. After shaking for 1 h in an end-over-end roller, tubes were centrifuged at 1000×g for 15 min and 1 ml of supernatant was stored at −20 °C. Endotoxin was analyzed by means of a Limulus Amebocyte Lysate (LAL) assay as previously described (Spaan et al., 2007, 2008). In short, 100 µl of a 1 in 25 diluted sample was used in the quantitative kinetic chromogenic LAL assay (Lonza, Walkersville, MD, USA; LAL-lysate lot number KL089V). Results are expressed as endotoxin units (EU) per cubic meter of sampled air.

### 2.4. Temporal determinants

Meteorological data was obtained from the weather station Volkel from the Royal Netherlands Meteorological Institute, which is located in the study area. From the hourly data, one-week averages were computed for temperature, atmospheric pressure, global radiation, wind speed, relative humidity, amount and duration of precipitation and duration of sunshine. Wind direction was not included because of variable wind direction in a one-week period and because sites were

surrounded by farms in all directions.

## 2.5. Spatial determinants

Livestock related spatial determinants were analyzed by means of geographical information system software (ArcGIS, version 10.2.2). Geographic coordinates of all livestock farms and the number of licensed animals per farm in the year 2012 were obtained as these were considered most representative for actual numbers of 2011 due to an administrative time lag. The data were provided by the province of Noord-Brabant and were extracted from the provincial database of mandatory environmental licenses for livestock-keeping.

The number of animals (pigs, poultry, cattle, goats, sheep, and other animals) in buffer zones of respectively 500 and 1000 m around the measurement sites were computed. Distance to the nearest farm per farm type (cattle farm, poultry farm, pig farm etc.) was also taken into account to assess local effects (included next to distance absolute also inverse distance ( $1/m^2$ ) to reflect the typical non-linear, exponential decline of concentration with distance to source). To account for both source strength and air pollution dilution with increasing distance from the source, squared distance weighted variables were computed per farm type being: distance weighted number of animals on the nearest farm.

## 2.6. Statistical analyses

Statistical analyses were carried out using R studio (version 3.0.2) (R Foundation for Statistical Computing, 2015) and SPSS (version 22; IBM Corp. Armonk, NY, USA). PM10 and endotoxin concentrations were natural log-transformed prior to analysis because of the skewed distribution. Between locations Pearson correlations of exposure concentrations were calculated to gain more insight in concordant temporal trends.

Data were analyzed using mixed models to take into account repeated measurements at the same locations. Inclusion of a random

intercept per location fitted the data best on basis of Akaike Information Criterion (AIC). Spatial and temporal variation were quantified by determining between and within location variance in endotoxin and PM10 concentrations, respectively. All predictors were scaled to their Inter Quartile Range (IQR). Meteorological (temporal) and agricultural (spatial) determinants were first analyzed by univariable generalized linear mixed regression to explore possible relations. Multivariable generalized linear mixed modelling was performed following a supervised forward stepwise procedure based on improvement of AIC, taking into account a predetermined parameters direction of effect when possible. Such a strategy is common in Land Use Regression modelling as a precautionary measure to limit the chance of selecting models that are not plausible and/or stable (Hoek et al., 2008). Based on theoretical knowledge the parameters direction of effect were: number of animal in buffers: positive, distance to nearest farm: negative, distance weighted variables: positive, and for all other variables no predefined direction of effect could be established due to lack of prior knowledge. Variables were excluded if  $p > 0.10$ , and models checked for influential observations (Cook's distance  $< 0.25$ ), multi-collinearity ( $VIF < 3$ ) and the general assumptions for mixed models. Models were made with 3 subsets of data namely: (A) model based on all measurement weeks, (B) model based on weeks in which the background location was measured, (C) model based on weeks in which the background location was measured and level at background location was taken into account as input parameter.

## 3. Results

### 3.1. PM10 and endotoxin concentrations

Figure 1 shows the geographic distribution of the sampling sites over the study area along with the locations of livestock farms. Average concentration over the whole sampling period taken all eight locations together for PM10 was  $23.3 \mu\text{g}/\text{m}^3$  ( $\text{SD}=10.4$ ,  $N=211$ ) and for endotoxin  $0.657 \text{ EU per m}^3$  ( $\text{SD}=0.397$ ,  $N=223$ ). All field blank samples

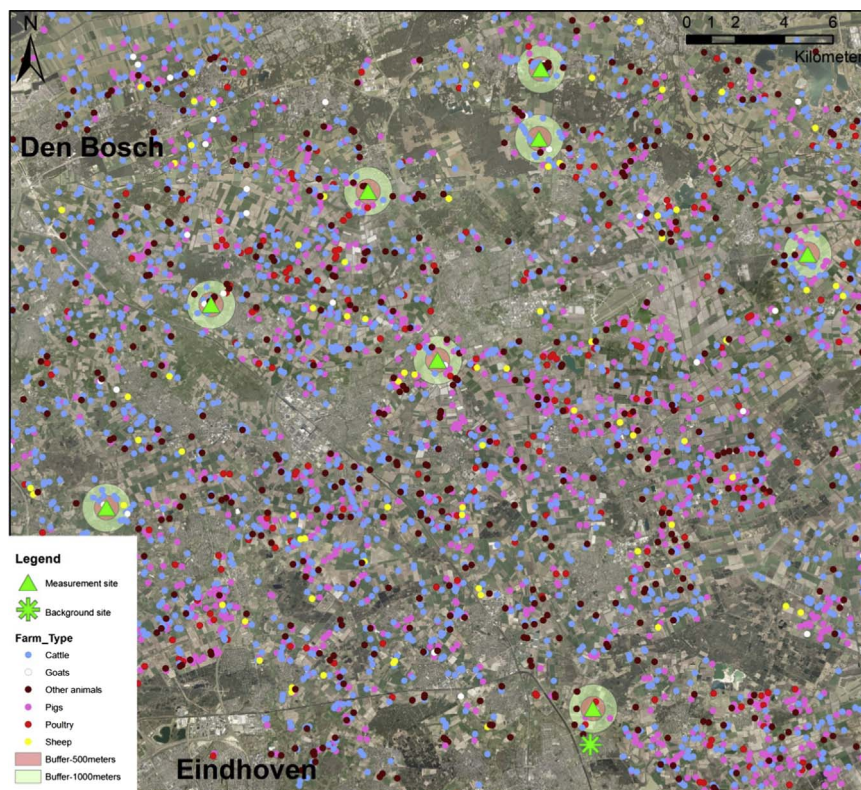


Fig. 1. Overview of the geographical positions of measurement sites and farms in the research area situated in the province Noord-Brabant in the Netherlands.



**Table 1**  
Descriptives of PM10 and endotoxin concentrations and livestock characteristics per sampling location.

		Measurement Site							
		1	2	3	4	5	6	7	8
PM10 ( $\mu\text{g}/\text{m}^3$ )	AM	24.2	22.8	24.1	21.6	23.9	21.1	23.3	25.0
	SD	10.5	9.8	11.9	10.3	10.2	9.0	10.2	11.6
	GM	22.3	21.1	22.1	19.8	22.2	19.8	21.5	22.3
	GSD	1.52	1.49	1.50	1.50	1.46	1.42	1.47	1.52
Endotoxin (units/ $\text{m}^3$ )	AM	0.709	0.712	0.754	0.541	0.766	0.604	0.600	0.553
	SD	0.360	0.463	0.508	0.314	0.428	0.334	0.341	0.343
	GM	0.626	0.578	0.622	0.463	0.656	0.526	0.524	0.626
	GSD	1.67	1.97	1.87	1.78	1.78	1.72	1.69	1.67
Distance site to nearest farm (m)	Pig farm	282	519	1119	1682	478	207	318	444
	Poultry farm	687	839	117	1574	286	1528	469	259
	Cattle farm	717	282	67	426	901	242	261	33
	Goat farm	253	349	271	144	91	249	256	591
Number of animals within 1000 m buffer	Pigs	21,900	17,674	432	0	6800	26,482	7083	7380
	Chicken	38,673	150,127	18,080	0	114,811	0	108,050	17,100
	Cows	341	649	1333	3544	241	1717	1454	4220
	Goats	300	1325	4139	3095	688	1400	1500	9561
	Sheep	0	30	25	607	25	82	48	802
	Other animals	756	67	676	4	1758	57	156	49
Number of farms within 1000 m buffer	Farms	15	20	15	13	8	22	18	16

Note. AM, arithmetic mean; SD, standard deviation; GM, geometric mean; GSD, geometric standard deviation.

except one, contained endotoxin and PM10 concentrations at or below the analytical limit of detection (LOD). Since all sampled filters contained concentrations of PM10 and endotoxin above the LOD, no adjustment for blanks were performed.

Mean PM10 concentrations varied only modestly across locations, whereas endotoxin concentrations showed a higher degree of variability (Table 1). Geometric mean site PM10 concentrations at sites ranged from 19.8 to 22.3  $\mu\text{g}/\text{m}^3$  whereas endotoxin concentrations ranged from 0.46 to 0.66 EU/ $\text{m}^3$ , thus a difference between the lowest and highest concentration of 11% for PM10 and 30% for endotoxin. Distances to (small) ruminant farms were on average closer compared to distances to pig and poultry farms. The number of farms within 1000 m buffer was quite variable between sites and ranged from 8 to 22 farms. Only three farms were situated within 1000 m from the background location. One farm was situated at a 510 m distance (60 sheep, 4 horses), one farm at a distance of 775 m (150 cows) and one farm at a

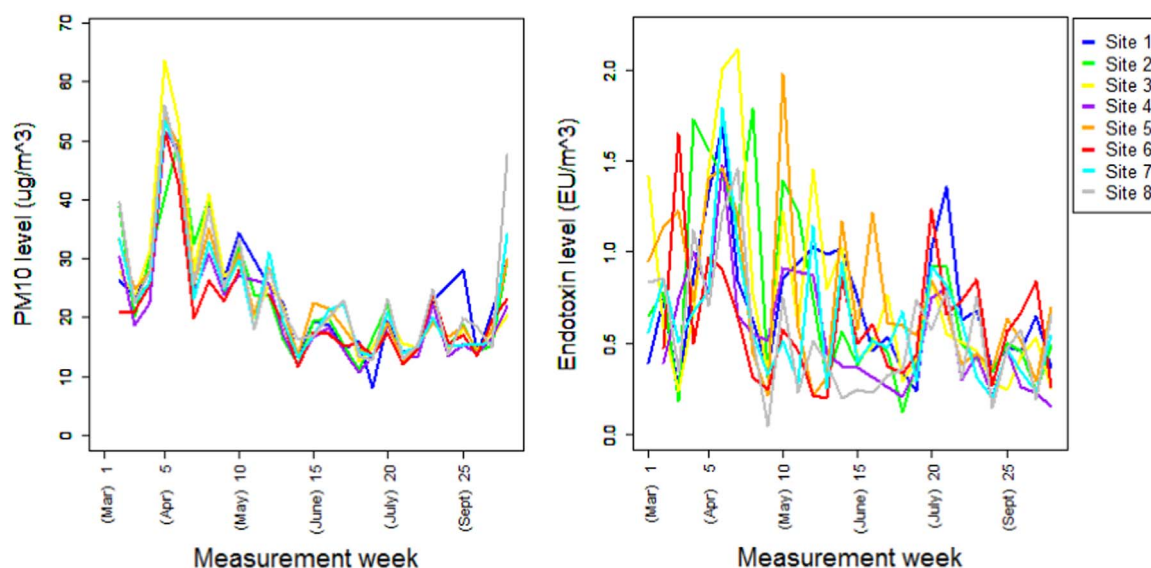
distance of 845 m (99,000 chickens).

### 3.2. Correlations

Endotoxin concentrations were moderately correlated to PM10 concentrations (Pearson correlation 0.44). Side-by-side collected parallel (duplicate) samples showed also lower Pearson correlation for endotoxin (0.59,  $p < 0.001$ ) than for PM10 (0.94,  $p < 0.001$ ), suggesting occurrence of inherent variability for endotoxin due to influence of sampling and analytical variability.).

### 3.3. Temporal and spatial variability in concentrations

An overview of the variability in PM10 and endotoxin concentrations over time is provided in Fig. 2. A clear temporal pattern is shown for PM10 with similar PM10 concentrations per week for the different



**Fig. 2.** Concentrations of PM10 and endotoxin over time per sampling week over the course of the whole sampling campaign displayed per measurement site.

**Table 2**

Between sites Pearson correlation coefficients for PM10 concentrations (left side of the table, in light grey) and endotoxin concentrations (right side of the table, in dark grey).

Endotoxin \ PM10	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	BG site
Site 1	1	0.52	0.65	0.72	0.35	0.2	0.72	0.44	0.58
Site 2	0.86	1	0.53	0.68	0.38	-0.04	0.42	0.59	0.41
Site 3	0.91	0.91	1	0.67	0.5	0.12	0.71	0.63	0.26
Site 4	0.91	0.95	0.97	1	0.48	0.31	0.71	0.59	0.66
Site 5	0.94	0.94	0.95	0.97	1	0.46	0.46	0.45	0.00
Site 6	0.93	0.86	0.95	0.95	0.95	1	0.25	0.12	0.36
Site 7	0.89	0.91	0.93	0.96	0.98	0.92	1	0.57	0.52
Site 8	0.86	0.88	0.85	0.90	0.92	0.85	0.96	1	0.48
BG site	0.73	0.87	0.90	0.95	0.83	0.88	0.85	0.87	1

Note. Pearson correlations computed on PM10 concentrations (in  $\mu\text{g}/\text{m}^3$ ); and on endotoxin concentrations (in units/ $\text{m}^3$ ).

sampling sites, while endotoxin concentrations display more discordant variation patterns over time for the different measurement locations.

The variation in endotoxin concentrations between sites was larger than the variation within sites, whereas the opposite holds for PM10 concentrations (Ln(endotoxin): between sites variance 0.58, within sites variance 0.37; within sites/between sites variance ratio=0.63; Ln(PM10): between sites variance 0.077, within sites variance 0.156; within sites/between sites variance ratio=2.03).

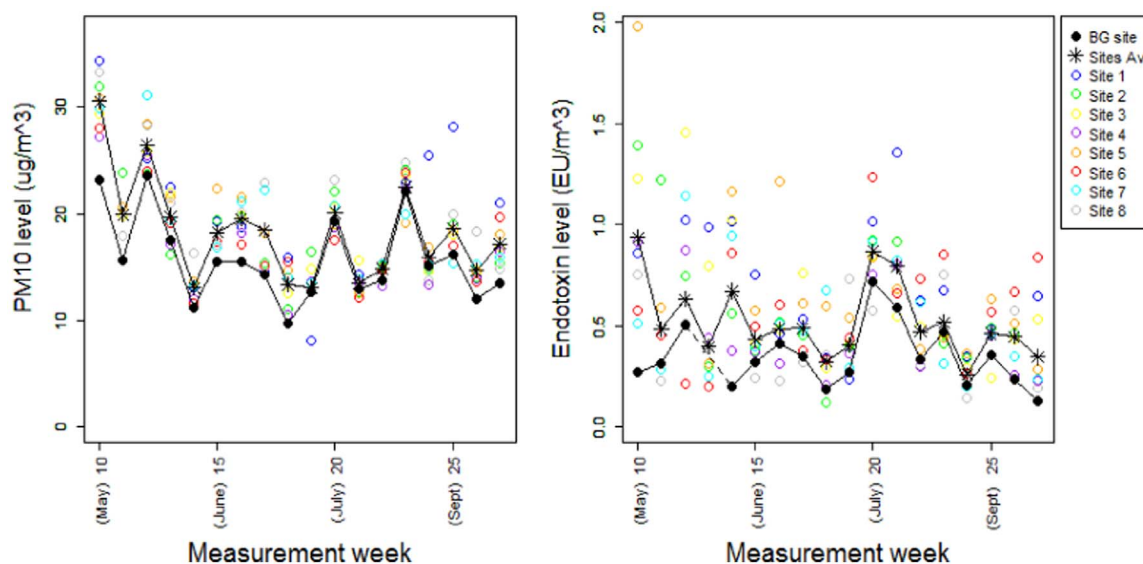
### 3.4. Between sites correlations

Between sites correlation coefficients of PM10 concentrations were constantly high, while for endotoxin more variation in correlation between sites was observed (Table 2). Several endotoxin correlation coefficients especially for site 6 were lower than the side-by-side

duplicate correlation, suggesting the influence of local sources.

### 3.5. Concentrations at background site

Fig. 3 shows the variability of sites-average levels of PM10 and endotoxin over time alongside with levels measured at the background location (a site situated in the same rural area but at greater distances from livestock farms). Concentrations of PM10 and endotoxin are on average 14% and 34% respectively, lower for the background site compared to the sites-average levels. The pattern over time observed at the background site is highly comparable to the pattern of the averaged level of the sites for PM10 and somewhat less strong endotoxin, suggesting that the background site exposure level may be used to adjust for regional temporal variation for endotoxin as well as PM10. The correlation between the sites-average concentrations (Ln) and background concentration (Ln) was 0.92 for PM10 and 0.62 for



**Fig. 3.** Concentrations of PM10 and endotoxin measured over time at the background site alongside with the sites-averaged level. Only measurements weeks 10–27 are depicted as only in these weeks the background site was included. The line through the asterisks represent the averaged level of the measurement sites. The open circles represent the concentrations measured at the different sites. The line through the points represent the level measured at the background location. The endotoxin outcome of measurement week 13 is missing, hence the dashed line.

**Table 3**  
Pearson correlation coefficients between meteorological parameters.

	Wind speed						
Temperature	0.20	Temperature					
Sunshine duration	–0.24	–0.21	Sunshine duration				
Precipitation amount	0.14	0.41	–0.45	Precipitation amount			
Precipitation duration	0.28	0.25	–0.44	<b>0.79</b>	Precipitation duration		
Relative humidity	–0.03	0.04	<b>–0.72</b>	0.37		Relative humidity	
Global radiation	–0.09	0.15	<b>0.82</b>	–0.23	–0.24	<b>–0.80</b>	Global radiation
Atmospheric pressure	<b>–0.62</b>	–0.45	0.52	–0.48	–0.50	–0.11	0.19

Note. Pearson correlation more extreme than 0.6/–0.6 marked in bold.

**Table 4**  
Multiple generalized linear mixed regression models of spatial and temporal variables associated with PM10 concentrations.

Variable (scaled to IQR)	M1. All weeks measured Marginal R squared 0.68		M2. Subset weeks Marginal R squared 0.59		M3. Subset weeks incl input background Marginal R squared 0.77	
	Estimate	CI (90%)	Estimate	CI (90%)	Estimate	CI (90%)
Hourly duration of sunshine (0.1 h)	0.184	0.109; 0.259	0.291	0.235; 0.348	0.067	0.002; 0.132
Wind speed (m/s)	–0.219	–0.265; –0.174	–0.089	–0.139; –0.039	.	.
Hourly duration of precipitation (0.1 h)	–0.187	–0.232; –0.142	–0.124	–0.165; –0.084	–0.039	–0.070; –0.007
Relative humidity (%)	–0.145	–0.194; –0.096	.	.	–0.077	–0.115; –0.039
Temperature (°C)	.	.	0.100	0.042; 0.158	.	.
Distance site to nearest poultry farm (meters*–1)	0.053	0.025; 0.080	0.042	0.015; 0.068	0.042	0.022; 0.061
PM10 level (Ln) at background location	NA	NA	NA	NA	0.751	0.649; 0.852

Note. Outcome is Ln(PM10) in  $\mu\text{g}/\text{m}^3$ .

Marginal R squared=variance explained by fixed factors.

. =empty cell, thus not included in the model.

NA=Not applicable.

Distance site to nearest poultry farm taken into account as meters\*–1 to alter direction of estimate.

M1: Model based on all measurement weeks.

M2: Model based on subset measurement weeks (two-third of all weeks), namely only those weeks in which background location was measured.

M2: On basis of VIF > 3; global radiation and atmospheric pressure excluded from model.

M3: Model based on subset measurement weeks (two-third of all weeks). level at background location taken into account as input parameter.

endotoxin.

### 3.6. Spatio-temporal models

Many temporal variables as well as some spatial characteristics were identified to be related to PM10 and endotoxin concentrations in univariable analyses (Appendix Table A1). Correlation between some meteorological variables was strong (Table 3), precluding simultaneous application in multivariable models and complicating interpretation. Temporal variables were most strongly associated to PM10 concentrations in multivariable analyses (Table 4). Addition of spatial variables did not improve the PM10 models except for distance to the nearest poultry farm (difference in  $R^2$  with and without the spatial predictor

2%). The effect size of this spatial characteristic was consistent for the subset analyses, whereas estimates of temporal variables were affected by inclusion of background PM10 (Table 4). Compared to the other determinants, background location level was strongest associated with the concentrations at the measurement sites ( $R^2$  of model only background location included: 0.67). Overall explained variance was best ( $R^2$  0.77) for the model containing concentration of the background location along with temporal and spatial variables.

The multivariable models for endotoxin showed less overall explained variance than PM10 models (Table 5). However, the variance explained by spatial livestock characteristics was higher compared to PM10 (difference in  $R^2$  with and without 7%). Background location level was, compared to the other determinants, strongest associated

**Table 5**  
Multiple generalized linear mixed regression models of spatial and temporal variables associated with endotoxin concentrations.

Variables (scaled to IQR)	M1. All weeks measured Marginal R squared 0.22		M2. Subset weeks Marginal R squared 0.18		M3. Subset weeks incl input background Marginal R squared 0.26	
	Estimate	CI (90%)	Estimate	CI (90%)	Estimate	CI (90%)
Global Radiation ( $\text{J}/\text{cm}^2$ )	.	.	0.285	0.175; 0.395	0.191	0.080; 0.302
Hourly duration of sunshine (0.1 h)	0.448	0.348; 0.548	.	.	.	.
Number of other animals in 1000 m buffer	0.127	0.055; 0.199	0.145	0.060; 0.230	0.145	0.064; 0.225
Number of pigs in 1000 m buffer	.	.	0.108	0.025; 0.191	0.108	0.029; 0.186
Input endotoxin level (Ln) at background location	NA	NA	NA	NA	0.386	0.224; 0.547

Note. Outcome is Ln(EU) in units/ $\text{m}^3$ .

Marginal R squared=variance explained by fixed factors.

. =empty cell, thus not included in the model.

NA=Not applicable.

M1: Model based on all measurement weeks.

M2: Model based on subset measurement weeks (two-third of all weeks), namely only those weeks in which background location was measured.

M3: Model based on subset measurement weeks (two-third of all weeks), level at background location taken into account as input parameter.

with the concentrations at the measurement sites ( $R^2$  of model only background location included: 0.15). The endotoxin model including background endotoxin amidst other spatial and temporal characteristics had a considerably higher  $R^2$  (0.26) compared to the model based on the same set of measurement weeks. A meteorological determinant was included in each of the models, being either global radiation or sunshine duration. Livestock related variables explaining spatial variation were number of animals (pigs and other animals) in 1000 m buffer for the endotoxin models based on the subset of weeks, while for the model based on all weeks only the number of other animals was included.

## 4. Discussion

This study showed that ambient air concentrations of endotoxin and PM10 in a livestock dense area varied distinctly over time. For PM10 the temporal pattern was similar for the different measurement sites. For endotoxin, a more variable temporal pattern was observed for the different sites. Both for PM10 and endotoxin, we were able to identify associated temporal, meteorological, and spatial livestock-related, determinants.

### 4.1. Spatial-temporal patterns

Spatial-temporal patterns of endotoxin concentrations differed from PM10 concentration patterns. Analysis of variance showed that for endotoxin the variation between sites was larger than the within site temporal variation, whereas for PM10 the opposite was found. Endotoxin concentrations of the 8 sites were on average 34% higher than measured at a background site, while PM10 concentration was on average only 14% higher than at the background site. All of our 8 sites were located near a farm, except the background site. The correlation of concentrations measured simultaneously at the 8 sites was substantially lower for endotoxin than for PM10. These measured patterns imply that emissions from local livestock farming contribute substantially more to endotoxin concentrations than to PM10 concentrations. The implication is that measurements of PM10 do not sufficiently capture spatial patterns of livestock related emissions, as also indicated by the moderate correlation between PM10 and endotoxin measurements (Pearson correlation 0.44).

### 4.2. Comparison with previously reported concentrations

PM10 concentrations measured were comparable to the levels measured earlier in an explorative study performed in a nearby region in the Netherlands in 2010 using comparable measurement methods and assays. Endotoxin concentrations measured in the current study were however considerably higher compared to the explorative study (explorative study: GM per site ranged from 0.21 to 0.31 EU/m<sup>3</sup>, in total 5 sites measured) (Heederik and IJzermans, 2011). However as measurements were performed at different sites in different years (2010 and 2011) detailed comparisons are hampered. Potentially the level differences could be due to higher livestock density in the direct surroundings in the current study. Studies performed in California, USA, also measured highest endotoxin concentrations in farm dense areas (Mueller-Anneling et al., 2004; Tager et al., 2010). A direct comparison of endotoxin levels in the current study to those measured in California or previous studies in urban areas is difficult due to application of different sampling methods and of various endotoxin assay methodologies, which are known to affect outcomes. However, the explorative study by Heederik et al. showed increased endotoxin concentrations for sites within the livestock dense area compared to the background location of that study which was situated in an urban area (Heederik and IJzermans, 2011).

### 4.3. Correlation between side-by-side measurements

Observed lower correlations in parallel measured (duplicate samples, collected at a one meter distance) endotoxin concentrations compared to PM10 concentrations suggests occurrence of inherent variability for endotoxin due to influence of sampling and analytical variability. Accuracy of the endotoxin assay, which is known to be 20% expressed as coefficient of variation, likely partly explains disagreement between parallel collected samples. Other factors, e.g. variability as endotoxins originate from bacteria, which grow and amplify, and high very local scale (within meters) variation may also be important. The effect of high very local scale variation has been observed earlier for traffic-related air pollutants (Klompmaaker et al., 2015). This suggests that dispersion processes from local sources are expected to have a profound influence on endotoxin concentrations measured at a specific site. This may explain the observed differences in temporal patterns of endotoxin concentrations for the different measurement sites.

### 4.4. Temporal determinants

Several temporal, meteorological, variables were associated with PM10 and endotoxin concentrations. Specific meteorological parameters included in the final model differed depending on the subset included in analysis. Interpretation of these results is complicated because meteorological parameters are highly correlated. In addition emissions from sources are likely to vary over time, adding to the complexity of interpretation of temporal associations. For PM10, many different emitting sectors are present and local levels are predominated by the regional background. For endotoxin, local sources had a more profound influence on absolute levels. If endotoxin sources have seasonal varying emissions, which for the agricultural sector is plausible, this may affect the meteorological parameters identified. Besides effect of meteorology on dispersion, it may also influence endotoxin emission itself. Meteorological factors may affect growth and decay of bacteria which translates into even larger temporal variation.

Previous studies found temperature to be positively associated with endotoxin level (Mueller-Anneling et al., 2004; Carty et al., 2003; Bari et al., 2014; Tager et al., 2010; Kallawicha et al., 2015). Temperature was, however, not identified as a determinant in the current endotoxin multivariable model, yet global radiation was included. Previous studies reported also various effects of other meteorological conditions. Also, within our study different temporal associations can be found depending on the subset included in the analysis. Likely temporal relations are not, or only partially causal as several interlinked processes may distort associations. For future studies, it would be interesting to take variation over time of source emissions into account.

### 4.5. Spatial determinants

This study is the first implementing local livestock characteristics of the surroundings in spatiotemporal models for assessment of endotoxin and PM10. Previous studies did either not take into account livestock related sources or only generic indicators (like the number of farms) at regional level. Measured variation of PM10 between sites was low and the explained spatial variance was limited. We only focused on local livestock-related sources, thus no other local sources (like traffic) were taken into account, what could have contributed to the low explained spatial variance. However, previous traffic related measurement studies also showed that contribution of local sources are of minor importance in addition to regional background concentrations (Eeftens et al., 2012). Besides, measurements were performed in a region which has, compared to other regions in the Netherlands, a constant relatively high PM10 level. Potentially, when regional PM10 levels are already high, identification of local sources is compromised. Nonetheless, this study identified a relation for increasing concentrations of PM10 with closer distance to a poultry farm. The model



predicted an increase of about 5% in PM10 concentration for an IQR decrease (580 m) in distance. This finding is supported by emission studies performed at farm level, poultry farms were suggested to contribute significantly to PM10 levels in the direct surroundings of the farm (Winkel et al., 2015).

Livestock related characteristics considerably explained spatial variance in endotoxin concentrations. The number of other animals present in the direct surroundings was found to be positively associated in all models. In the model based on the subset of weeks, the number of pigs in the surroundings were identified as well. Since other measurement studies did not take into account local livestock characteristics no comparisons can be made. Also, as yet, little is known on endotoxin emission levels from livestock farms (Seedorf et al., 1998).

#### 4.6. Background location

PM10 and endotoxin concentrations measured at the background location were at the lower range of the concentrations measured at the sites. Total variation explained in PM10 and endotoxin concentrations substantially increased when background site concentrations were taken into account next to temporal and spatial variables. Thus, temporal variation was better explained by the background location than a combination of meteorological parameters alone. Inclusion of background concentrations substantially reduced the effect estimates for temporal but not spatial predictors. Because of the small size of the study area, meteorological impacts are well reflected in the background concentration. The use of a background location to adjust temporal variation of PM10 is standard in land use regression of PM and traffic-related air pollution in urban areas (Eeftens et al., 2012; Hoek et al., 2002). In this study we showed the applicability of this strategy for endotoxin as well for PM10 in a rural setting. This is important knowledge for future studies which include high numbers of measurement sites where it is impossible to measure all sites simultaneously.

## Appendix A

See Table A1 here.

**Table A1**

Univariable generalized linear mixed regression models for PM10 concentrations and endotoxin concentrations of spatial and temporal variables.

Variable type	Variables (scaled to IQR)	IQR	PM10			Endotoxin		
			Estimate	CI 90% LB	CI 90% UB	Estimate	CI 90% LB	CI 90% UB
Spatial: Nearest farm related	Number of chickens on nearest poultry farm	9.24E+04	-0.052	-1.23E-01	1.93E-02	-0.035	-1.70E-01	1.02E-01
	Number of pigs on nearest pig farm	3.45E+03	0.037	-4.04E-02	1.14E-01	-0.107	-2.41E-01	2.58E-02
	Number of cows on nearest cattle farm	1.06E+02	-0.026	-1.12E-01	6.11E-02	-0.091	-2.56E-01	7.26E-02
	Number of goats on nearest goat farm	1.56E+03	0.03	-6.72E-02	1.26E-01	-0.087	-2.67E-01	9.38E-02
	Distance site to nearest poultry farm (meters*-1)	5.80E+02	<b>0.052</b>	3.31E-03	1.01E-01	0.057	-3.16E-02	1.45E-01
	Distance site to nearest pig farm (meters*-1)	2.01E+02	0.007	-1.23E-02	2.57E-02	0.013	-2.30E-02	4.90E-02
	Distance to nearest cattle farm (meters*-1)	5.04E+02	-0.005	-8.32E-02	7.32E-02	<b>-0.173</b>	-3.22E-01	-1.37E-02
	Distance to nearest goat farm (meters*-1)	6.10E+01	-0.009	-2.83E-02	9.96E-03	<b>0.031</b>	2.00E-03	6.00E-02
	Weighted distance site to nearest poultry farm (1/squared meters)	1.35E-01	0.012	-1.41E-02	3.71E-02	0.025	-2.21E-02	7.18E-02
	Weighted distance site to nearest pig farm (1/squared meters)	6.16E-02	-0.015	-5.31E-02	2.41E-02	-0.001	-7.54E-02	7.38E-02
	Weighted distance site to nearest cattle farm (1/squared meters)	2.18E+00	0.019	-1.21E-02	5.01E-02	<b>-0.053</b>	-1.03E-01	-1.90E-03
	Weighted distance site to nearest goat farm (1/squared meters)	5.22E-02	0	-6.18E-03	6.46E-03	0.008	-3.49E-03	1.87E-02
	Number of chickens weighted to distance to nearest poultry farm (distance in hm)	4.18E+03	0.027	-3.36E-02	8.73E-02	0.046	-6.69E-02	1.60E-01
	Number of pigs weighted to distance to nearest pig farm (distance in hm)	1.56E+02	0.053	-1.37E-02	1.20E-01	-0.053	-1.78E-01	7.33E-02
	Number of cows weighted to distance to nearest cattle farm (distance in hm)	5.13E+01	0.004	-3.23E-03	1.14E-02	<b>-0.014</b>	-2.49E-02	-2.72E-03
	Number of goats weighted to distance to nearest goat farm (distance in hm)	2.51E+02	0	-4.73E-02	4.67E-02	0.061	-2.10E-02	1.42E-01

(continued on next page)

## 5. Conclusion

To conclude, the effect of local livestock-related sources on absolute PM10 mass concentration seems limited in this study carried out in a livestock dense area. For endotoxin we showed that the more sizable spatial variation could be explained by livestock related characteristics in the surroundings. This suggests livestock farms to be a major source of endotoxins in this study area. Although measuring and assessing levels of endotoxin are more challenging than for PM10, it is important to determine endotoxin levels as inferences from PM10 levels cannot be made. Including a background location as part of the measurement network, showed to be useful to account for temporal variation also for endotoxin. To gain more insight in the effect of livestock-related sources on ambient concentrations of PM10 and endotoxin, measurements should be based on a broader set of locations representing a variety of livestock related characteristics. A larger measurement network would enable more extensive modelling, providing opportunities for predictions of exposure levels based on spatial characteristics.

## Conflict of interest statement

No conflicts of interest to declare.

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Table A1 (continued)

Variable type	Variables (scaled to IQR)	IQR	PM10			Endotoxin		
			Estimate	CI 90% LB	CI 90% UB	Estimate	CI 90% LB	CI 90% UB
Spatial: Buffer related	Number of chicken within 500 m buffer	1.71E+04	0.005	−1.74E−02	2.65E−02	−0.009	−5.05E−02	3.36E−02
	Number of pigs within 500 m buffer	3.76E+03	0.055	−2.44E−02	1.34E−01	−0.07	−2.16E−01	7.80E−02
	Number of cows within 500 m buffer	8.86E+02	−0.012	−8.90E−02	6.46E−02	−0.213	−3.43E−01	−8.25E−02
	Number of goats within 500 m buffer	1.01E+03	−0.034	−8.49E−02	1.62E−02	−0.005	−1.04E−01	9.19E−02
	Number of sheep within 500 m buffer	2.50E+01	0.011	−5.46E−02	7.63E−02	0.006	−1.20E−01	1.33E−01
	Number of other animals within 500 m buffer	1.23E+02	0.039	−5.32E−02	1.31E−01	0.097	−6.84E−02	2.66E−01
	Number of livestock farms within 500 m buffer	6.00E+00	0.011	−6.00E−02	8.26E−02	−0.02	−1.56E−01	1.18E−01
	Number of chicken within 1000 m buffer	9.43E+04	0.019	−5.72E−02	9.43E−02	0.102	−3.50E−02	2.40E−01
	Number of pigs within 1000 m buffer	1.09E+04	−0.014	−6.66E−02	3.87E−02	0.028	−7.19E−02	1.28E−01
	Number of cows within 1000 m buffer	1.22E+03	−0.006	−4.52E−02	3.41E−02	<b>−0.127</b>	−1.94E−01	−6.06E−02
	Number of goats within 1000 m buffer	3.13E+03	0.021	−2.79E−02	6.94E−02	<b>−0.107</b>	−1.88E−01	−2.66E−02
	Number of sheep within 1000 m buffer	5.70E+01	0	−8.42E−03	8.75E−03	<b>−0.023</b>	−3.55E−02	−9.69E−03
	Number of other animals within 1000 m buffer	6.59E+02	0.027	−2.41E−02	7.77E−02	<b>0.126</b>	4.60E−02	2.07E−01
	Number of livestock farms within 1000 m buffer	3.00E+00	−0.015	−4.79E−02	1.77E−02	<b>−0.032</b>	−9.15E−02	2.80E−02
Temporal	Temperature (°C)	3.16E+00	<b>−0.139</b>	−1.89E−01	−8.78E−02	<b>−0.111</b>	−2.00E−01	−2.26E−02
	Relative Humidity (%)	1.22E+01	<b>−0.277</b>	−3.26E−01	−2.29E−01	<b>−0.286</b>	−3.63E−01	−2.10E−01
	Wind speed (m/s)	1.58E+00	<b>−0.313</b>	−3.79E−01	−2.46E−01	−0.091	−2.00E−01	1.71E−02
	Atmospheric pressure (in 1 hPa)	6.91E+00	<b>0.413</b>	3.56E−01	4.71E−01	<b>0.133</b>	4.15E−02	2.25E−01
	Hourly duration of sunshine (0.1 h)	1.89E+00	<b>0.485</b>	4.24E−01	5.45E−01	<b>0.449</b>	3.49E−01	5.49E−01
	Hourly global radiation (in J/cm <sup>2</sup> )	2.68E+01	<b>0.284</b>	2.19E−01	3.50E−01	<b>0.339</b>	2.40E−01	4.38E−01
	Hourly precipitation amount (in 0.1 mm)	1.28E+00	<b>−0.323</b>	−3.81E−01	−2.65E−01	<b>−0.184</b>	−2.80E−01	−8.80E−02
	Hourly duration of precipitation (0.1 h)	7.04E−01	<b>−0.367</b>	−4.21E−01	−3.13E−01	<b>−0.168</b>	−2.65E−01	−7.14E−02

Note. Estimates with p-value below 0.1 marked in bold. Distance site to nearest farm taken into account as meters\*−1 to alter direction of estimate. PM10 outcome is Ln(PM10) in µg/m<sup>3</sup>; endotoxin outcome is Ln(EU) in units/m<sup>3</sup>.

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