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Endotoxin and particulate matter emitted by livestock farms and respiratory health effects in neighboring residents

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ABSTRACT

Background: Living in livestock-dense areas has been associated with health effects, suggesting airborne exposures to livestock farm emissions to be relevant for public health. Livestock farm emissions involve complex mixtures of various gases and particles. Endotoxin, a pro-inflammatory agent of microbial origin, is a constituent of livestock farm emitted particulate matter (PM) that is potentially related to the observed health effects. Quantification of livestock associated endotoxin exposure at residential addresses in relation to health outcomes has not been performed earlier.
Objectives: We aimed to assess exposure-response relations for a range of respiratory endpoints and atopic sensitization in relation to livestock farm associated PM_{10} and endotoxin levels.
Methods: Self-reported respiratory symptoms of 12,117 persons participating in a population-based cross-sectional study were analyzed. For 2494 persons, data on lung function (spirometry) and serologically assessed atopic sensitization was additionally available. Annual-average PM_{10} and endotoxin concentrations at home addresses were predicted by dispersion modelling and land-use regression (LUR) modelling. Exposure-response relations were analyzed with generalized additive models.
Results: Health outcomes were generally more strongly associated with exposure to livestock farm emitted endotoxin compared to PM_{10}. An inverse association was observed for dispersion modelled exposure with atopic sensitization (endotoxin: $p = .004$, PM_{10}: $p = .07$) and asthma (endotoxin: $p = .029$, PM_{10}: $p = .022$). Prevalence of respiratory symptoms decreased with increasing endotoxin concentration at the lower range, while at the higher range prevalence increased with increasing concentration ($p < .05$). Associations between lung function parameters with exposure to PM_{10} and endotoxin were not statistically significant ($p > .05$).
Conclusions: Exposure to livestock farm emitted particulate matter is associated with respiratory health effects and atopic sensitization in non-farming residents. Results indicate endotoxin to be a potentially plausible etiologic agent, suggesting non-infectious aspects of microbial emissions from livestock farms to be important with respect to public health.

1. Introduction

Epidemiological studies performed worldwide have shown associations between living in livestock dense areas and health effects, suggesting airborne exposures to livestock farms at residential level to be relevant for public health (Borlée et al., 2015, 2017a, 2017b, 2018; Douglas et al., 2018; Elliott et al., 2004; Mirabelli et al., 2006; Pavilonis et al., 2013; Radon et al., 2007; Rasmussen et al., 2017; Schinasi et al., 2011; Schulze et al., 2011; Sigurdarson and Kline, 2006; Smit et al., 2014). Adverse effects on health identified included increased respiratory symptoms (wheezing, cough) and decreased lung function (Borlée et al., 2017a; Radon et al., 2007; Schulze et al., 2011); protective effects included lower prevalence of asthma and atopy (Borlée et al., 2015; Elliott et al., 2004; Smit et al., 2014). Most studies have used exposure proxies such as distance to nearest farm and farm densities in the surroundings to represent exposure to livestock-related air pollution at residences. Underlying mechanisms and etiologic agents are difficult to establish as livestock farm emissions consist of complex mixtures of various gases and particles (Cambra-López et al., 2010; Hamon et al., 2012).

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Organic particulate matter (PM), also called biological particulate matter or bio-aerosols, are aerosolized solid or liquid particles of biological origin like bacteria, fungi, plant materials etc. Organic PM is an important air pollutant emitted by livestock farms. A constituent of organic PM is endotoxin, a potent pro-inflammatory component of the cell wall of gram-negative bacteria (Liu, 2002). Since PM concentrations in livestock farms are high and buildings are intensively ventilated (Winkel et al., 2015), endotoxins are emitted into the atmosphere in large quantities (Pillai and Ricke, 2002; Seedorf et al., 1998; Thorne et al., 2009). Endotoxins are dispersed in the near surroundings and beyond, resulting in exposure to livestock farm emitted endotoxin at residential sites (De Rooij et al., 2017; Hiranuma et al., 2011).

Endotoxin exposure has been linked to both adverse and protective effects on respiratory health (Farokhi et al., 2018; May et al., 2012). Adverse effects, such as upper respiratory symptoms and chronic bronchitis, are caused by endotoxin inducing production of cytokines and proteins that cause airway inflammation (Liu, 2002; Poole and Romberger, 2012). Understanding of protective effects, such as decreased atopy, allergic rhinitis, and atopic asthma, is incomplete, but endotoxin induced alterations in immune responses leading to suppression of allergy-promoting responses seems to play a role (Liu, 2002; Poole and Romberger, 2012). Health effects of endotoxin have been firmly established for exposure to high concentrations (predominantly short term) in the various experimental/occupational studies performed (Castellan et al., 1987; May et al., 2012; Michel et al., 2002; Smid et al., 1994). However, less is known on health effects of environmental exposure to considerably lower airborne endotoxin concentrations as measured at residential sites (Farokhi et al., 2018).

Quantification of exposure to livestock-emitted PM and endotoxin at residential addresses is needed to increase insight in the mechanisms behind livestock-related health effects observed in non-farming populations. State of the art modelling and monitoring methods aimed at endotoxin are needed to specifically predict ambient concentrations enabling exposure-response analyses. Land-use regression (LUR) modelling and dispersion modelling are extensively used modelling techniques in urban air pollution studies to predict exposures (De Hoogh et al., 2014). Implementation of these techniques to model exposure to livestock farm emitted endotoxin at residential addresses is novel. LUR modelling uses geospatial predictor variables to explain spatial contrasts in measured airborne concentrations. (De Rooij et al., 2018) Dispersion modelling uses mathematical equations to compute airborne distribution of pollutants resulting from emission from a source to the surroundings conditional on aerial and meteorological circumstances (De Hoogh et al., 2014). Methodological principles are distinctly different between both approaches, each faced with their own challenges and validation issues. Both approaches are expected to be of use to model exposure to livestock farm emitted endotoxin at residential addresses, but it cannot be foreseen if and how predictive ability would differ. We hypothesize that livestock farmemitted PM, and especially its endotoxin content, plays a role in observed health effects among residents living in livestock dense areas. We performed exposure-response analyses for a range of respiratory endpoints and atopic sensitization in relation to livestock farm emitted PM_{10} (particulate matter with a nominal aerodynamic diameter below 10 μm) and endotoxin among 12,117 participants of the VGO project (Dutch acronym for Livestock Farming and Neighboring Residents' Health Study). Both dispersion modelling and LUR modelling were applied to predict exposure at residential addresses.

2. Methods

2.1. Study design and population

Exposure-response analyses were performed in a population based cross-sectional study which was part of the “Livestock Farming and Neighboring Residents' Health” (VGO) project. The VGO study population is a general, non-farming population sample enrolled in 2012 through a questionnaire survey among randomly selected patients from 21 general practices (aged 18–70 years) living in a rural area in the southeast of the Netherlands (the VGO study area; 3000km^2 in size) (Borlée et al., 2015). Analyses were conducted on 12,117 responders who lived at their home address for at least 1 year. Responders who were willing to participate in a follow-up study (asked in questionnaire), and who were living within 10 km of one of twelve temporary research centres were invited to the nearest centre for a medical examination (n = 7180). From March 2014 to February 2015, 2494 subjects participated in the medical examination that included spirometry and peripheral blood collection, see Borlée et al. for a flow chart of the data collection(Borlée et al., 2017b). Detailed non-response analyses indicated no signs that selection bias influenced the observed associations (Borlée et al., 2015, 2017a, 2017b, 2018). The study protocol (13/533) was approved by the Medical Ethical Committee of the University Medical Centre Utrecht. All 2494 subjects signed informed consent. Initial analyses within the VGO project involved associating exposure proxies (distance to farms, farm density) of livestock farm emissions to health outcomes (Borlée et al., 2015, 2017a, 2017b, 2018). In the current study, analyses on health outcomes in relation to predicted residential exposures to livestock farm emitted PM_{10} and endotoxin are included.

2.2. Description of study area

The study area (3000 km^2 in size) contained regions of the Netherlands with high livestock densities. The study area was situated in the provinces of Noord-Brabant and Limburg, these provinces have a combined surface area of 7290km^2 on which in total 17,250 farms are present (provincial databases on farm licenses of 2015, http://bvb.brabant.nl and http://limburg.vaa.com/webbvb). Farms are not geographically evenly spread; instead farms are mainly concentrated in the region where the two provinces border. In the Netherlands, livestock is commonly kept in enclosed animal houses, apart from some dairy cows, sheep, and horses that also are kept on pastures during parts of the year (Central Bureau of Statistics, 2015). Most farms are highly specialized intensive operations meaning that only one animal species is kept aimed at a specific production type (e.g., broiler farms, laying hen farms). The number of animals kept on a farm vary highly; on average, the number of animals kept in the study area on pig farms, chicken farms, cattle farms were 2659; 76,718; 205, respectively (Borlée et al., 2017a).

2.3. Exposure assessment

2.3.1. Dispersion modelling

Dispersion modelling was applied to estimate livestock farm emitted annual average PM_{10} mass concentration and annual average endotoxin concentrations in PM_{10} fraction at residential addresses. The dispersion model used is based on the Gaussian plume model and implements the Netherlands New National Model (see Supplementary Methods tables M1–3 for details). The model was applied to estimate dispersion of livestock farm emitted PM and its endotoxin content. PM dispersion was modelled using farm-type specific PM_{10} emission factors, farm-type specific distribution of PM size-fractions, barn characteristics and emission rate (both mass and heat), meteorological conditions, and terrain roughness. To obtain endotoxin dispersion, additional model input included farm-type specific endotoxin content per PM size-fraction (see Supplementary methods Tables M1–3).

To estimate endotoxin concentrations at residential addresses of VGO study participants, we applied the dispersion model to individual barns within 10 km of each address using provincial livestock farm data including the exact location, farm-type and licensed animal numbers. Per residential address' geographic coordinate (based on centroid of the home), annual averages of PM_{10} and endotoxin concentrations were obtained by summation of contributions of individual barns. Meteorological data used was matched to the period of the study, being 2012 for the questionnaire study and 2014–2015 for the medical examination study. Data on licensed animal numbers were available for the years 2012 and 2015, as provided by the provinces of Noord-Brabant and Limburg.

2.3.2. LUR modelling

Annual average endotoxin exposure at residential addresses were estimated through application of a previously described endotoxin LUR model developed for the VGO study area (De Rooij et al., 2018). In brief, the LUR model was developed based on repeatedly measured endotoxin concentrations in PM_{10} fraction at 61 residential sites geographically distributed in the VGO study area. The measurement campaign started in May 2014 and ended in December 2015, 2-week average air samples were collected. The 61 sites were sampled three to five times spread over the four seasons and in addition a background location was included which was sampled consecutively (see De Rooij et al., 2018 for details). GIS predictors used were based on provincial livestock data of 2015. A forward supervised stepwise selection procedure was performed to select GIS predictors. The resulting LUR model contained the following variables: 'number of sows weighted to distance in a 1000 m buffer', 'number of laying hens weighted to distance in a 3000 m buffer', 'number of poultry animals in a 500 m buffer', and 'number of horse farms in a 3000 m buffer'. Together these variables explained 64% of spatial variation in endotoxin concentrations. In the current study, geocoded residential addresses of VGO study participants were combined with geocoded livestock data to compute livestock characteristics of the residential address' surroundings enabling application of the endotoxin LUR model function. As described in De Rooij et al., 2018, LUR modelling on measured PM10 concentrations using livestock-related GIS predictors was evaluated. As the resulting LUR model explained the spatial variation in PM10 concentrations only modestly (19%), model performance was regarded too limited to be applied for predicting exposure.

2.3.3. Validation and comparative analyses

Dispersion modelling was also applied to estimate endotoxin concentrations at the 61 VGO sites where endotoxin concentrations had been measured. This enabled validation of dispersion modelled endotoxin concentrations against measured endotoxin concentrations. Furthermore, dispersion modelled endotoxin concentrations were compared against LUR modelled endotoxin concentrations for both the 61 VGO measurement sites as well as residential addresses of the health study participants.

2.4. Health outcomes

2.4.1. Questionnaire study

In 2012, a questionnaire survey was performed including questions on respiratory health adapted from the European Community Respiratory Health Survey (ECRHS)-III postal questionnaire (Jarvis et al., 2018). The following outcomes were included in the current analysis: current asthma, doctor diagnosed COPD, wheeze (with or without shortness of breath/having a cold), and daily cough (See Supplementary Methods Table M.4. for the definition of the self-reported respiratory conditions and symptoms).

2.4.2. Medical examination study

The medical examination included spirometry and peripheral blood collection (Borlée et al., 2017a, 2017b, 2018). Pre- and post-bronchodilator spirometry was conducted as described before (Borlée et al., 2017a) according to European Respiratory Society (ERS) guidelines. The current study analyzes the pre-bronchodilator lung function parameters forced expiratory volume in 1 s (FEV_1), Forced Vital Capacity (FVC), FEV_1/FVC and Maximum Mid-Expiratory Flow (MMEF). Lung function parameters were expressed as percentage predicted based on the GLI-2012 reference equations; thus adjusting for age, sex and height (Quanjer et al., 2012). Atopy was defined as elevated levels of specific serum IgE antibodies ($>$ 0.35 U/ml) to one or more common allergens (cat, dog, grass and house dust mite) and/or a total IgE higher than 100 IU/ml, assessed by ELISA as previously described (Borlée et al., 2018; Doekes et al., 1996).

2.5. Exposure-response analyses

Analyses were performed using R studio (version 3.0.2) (R. Core-Team, 2017). Predicted concentrations at residential addresses were truncated to 99.5 percentile for both LUR modelled endotoxin and dispersion modelled endotoxin and PM_{10} to avoid outlying unrealistically high values (see Table 1 for values of 99.5 percentile). Associations between exposures and health outcomes were analyzed by means of regression splines as previously performed analyses with exposure proxies showed associations to be non-linear (Borlée et al., 2015, 2017a, 2018). Penalized regression splines using the (default) "thin plate" basis as implemented in the mgcv R-package were used. To enable comparisons between predicted concentrations at residential addresses and earlier reported exposure proxies, analyses on health outcomes in relation to 'number of farms within 1000 m buffer' and 'distance to nearest farm' were included. Shape of the associations, smooth term p-values, Akaike Information Criterion (AIC) values were compared between the livestock exposure parameters.

Previously identified confounders were included. Associations with respiratory symptoms were adjusted for age, sex and smoking habits (never smoker, ex-smoker, current smoker) (Borlée et al., 2015). Associations with lung function parameters were adjusted for living on a farm during childhood, born in study area and smoking habits (Borlée et al., 2017a). The week average ambient ammonia concentration prior to lung function testing was taken into account in addition (visualized by means of dashed lines) as this was found to be inversely associated with lung function (Borlée et al., 2017a). Associations with atopic sensitization were adjusted for sex, age, smoking habits (ever smoking and pack years), education (high versus middle/low education), born in study area, and history of living on a farm during childhood (Borlée et al., 2018).

3. Results

3.1. Study population characteristics

Of the total study population, participants had on average 8 farms in a buffer of 1000 m around their house and on average the closest farm was at 490 m distance (see Table 1). Modelled annual average concentrations of livestock farm emitted PM_{10} at residential addresses ranged from the 10th percentile to the 90th percentile from 0.07 to 0.40 $\mu g/m^3$. The mean value of modelled annual average concentrations of endotoxin at residential addresses was for LUR modelling, dispersion modelling, 0.25, 0.18 endotoxin units (EU) per m^3, respectively (see Supplementary Table S.1. for other study population characteristics). At relatively close distances, distinctly different endotoxin concentrations were predicted, resulting in a substantial contrast in predicted residential exposures across the study area (see Supplementary Fig. S.1. for a geographical overview).

3.2. Comparative analyses: LUR versus dispersion

Comparisons between dispersion modelled endotoxin concentrations and measured endotoxin concentrations at the VGO measurement sites (n = 61) showed reasonable agreement overall and absolute concentrations to match despite clear differences at certain sites (Pearson correlation 0.51, see Supplementary Fig. S.2.). The level of agreement between LUR modelled endotoxin concentration and dispersion

Table 1
Residential characteristics of study population.

		Mean	SD	10%ile	50%ile	90%ile	99.5%ile
Total study population (*n* = 12,117)	Distance to nearest farm in meters	488	275	170	440	870	1374
	Number of farms within 1000 m buffer	8	6	1	7	15	26
	Predicted annual average endotoxin concentration (EU/m^3) by LUR model	0.25	0.09	0.16	0.24	0.35	0.70
	Predicted annual average endotoxin concentration (EU/m^3) by dispersion model	0.18	0.13	0.07	0.15	0.33	0.98
	Predicted annual average PM$_{10}$ concentration (μg/m^3) by dispersion model	0.20	0.15	0.07	0.18	0.40	0.94
Subset of study population (*n* = 2494, medical examination participants)	Distance to nearest farm in meters	439	266	135	401	794	1360
	Number of farms within 1000 m buffer	9	6	2	9	17	27
	Predicted annual average endotoxin concentration (EU/m^3) by LUR model	0.27	0.10	0.17	0.25	0.38	0.72
	Predicted annual average endotoxin concentration (EU/m^3) by dispersion model	0.25	0.16	0.09	0.22	0.42	1.26
	Predicted annual average PM$_{10}$ concentration (μg/m^3) by dispersion model	0.31	0.18	0.12	0.29	0.53	1.22

modelled endotoxin concentration at residential addresses was substantial (Pearson correlation for the full study population was 0.64, for the subset with medical examination 0.68; see Fig. 1). Dispersion modelled endotoxin concentrations showed a broader range, a lower minimum and higher maximum, than LUR modelled concentrations.

3.3. *Livestock exposure parameters: proxies, PM$_{10}$ and endotoxin*

Pearson correlations between modelled livestock farm emitted PM$_{10}$ concentrations and modelled endotoxin concentrations were 0.84 for dispersion modelled endotoxin, and 0.74 for LUR modelled endotoxin in the total study population (see Supplementary Fig. S.3). Correlation between modelled concentrations (PM$_{10}$ and endotoxin) with the exposure proxy 'number of farms within 1000 m buffer' was moderate (Pearson correlation: 0.48–0.58). The correlation between modelled concentrations and the exposure proxy 'distance to nearest farm' was weaker (Pearson correlation: 0.37–0.41).

3.4. *Exposure-response analyses*

For the majority of health outcomes, strength of associations of exposure-response analyses followed a similar pattern: strongest associations (lowest *p*-values, lowest AIC values) were found with endotoxin concentrations, weakest associations with livestock exposure proxies, and intermediate with livestock farm emitted PM$_{10}$ concentrations (see Figs. 2–5).

3.4.1. *Atopic sensitization*

A significant inverse association between dispersion modelled endotoxin concentration and atopic sensitization (based on serological data, available for subset of total study population) was observed (see Fig. 2). Predicted prevalence decreased from 0.32 to 0.24 when comparing the 5th percentile of dispersion modelled endotoxin concentration to the 95th percentile, thus a prevalence ratio of 0.75. The protective association was also observed with the proxy 'distance to nearest farm' however the association was weaker. Associations with LUR modelled endotoxin concentrations and dispersion modelled PM$_{10}$ concentrations showed a similar trend (non-significant) of decreased prevalence with increased exposure.

3.4.2. *Respiratory symptoms*

Modelled endotoxin concentrations (both LUR modelled and dispersion modelled) were significantly associated with prevalence of wheeze with shortness of breath and daily cough (based on questionnaire data collected from total study population), see Fig. 3. At the lower range of endotoxin concentrations, predicted prevalence of wheeze with shortness of breath decreased with increasing endotoxin concentration from 0.11 for the lowest exposed (5th percentile) to 0.08 for dispersion modelled endotoxin exposure of 0.32 EU/m^3 (prevalence ratio of 0.80), for daily cough the predicted prevalence decreased from 0.14 for the lowest exposed (5th percentile) to 0.12 for dispersion modelled endotoxin exposure of 0.32 EU/m^3 (prevalence ratio of 0.82). At the higher range of endotoxin concentrations, predicted prevalence of wheeze with shortness of breath/daily cough increased with increasing endotoxin concentration but confidence intervals were wide due to a lower number of observations. The pattern with livestock farm emitted PM$_{10}$ concentration was comparable, but non-significant. The shape of the association between 'number of farms within 1000 m buffer' and prevalence of wheeze with shortness of breath was distinctly different, no change in prevalence was observed up to around 24 farms followed by a sharp increase in prevalence with increasing number of farms.

3.4.3. *Asthma and COPD*

Fig. 4 shows the associations between increased livestock exposure with decreased prevalence of both asthma and COPD (based on questionnaire data collected from total study population). The shape of the

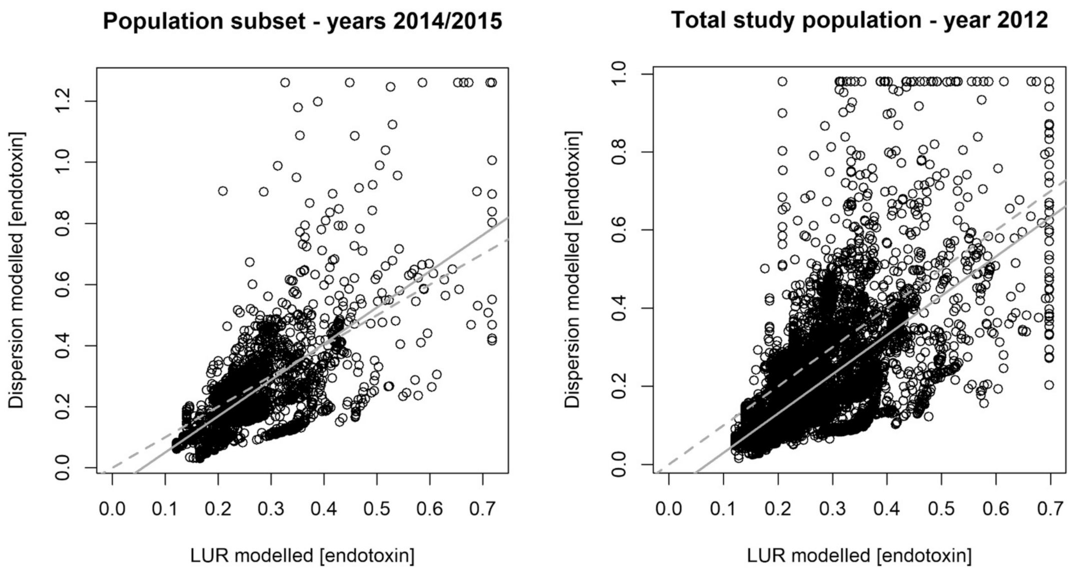

Fig. 1. Comparison between dispersion modelled versus LUR modelled annual average endotoxin concentrations (in EU/m^3); left panel for years 2014–2015, in line with medical examination period; right panel for year 2012, in line with period when questionnaires were collected.
Note. Modelled concentrations for years 2014-2015: Pearson correlation is 0.68, Spearman correlation is 0.65.
Modelled concentrations for year 2012: Pearson correlation is 0.64, Spearman correlation is 0.66.
Solid grey line is linear regression fit.
Dashed grey line is line of unity.

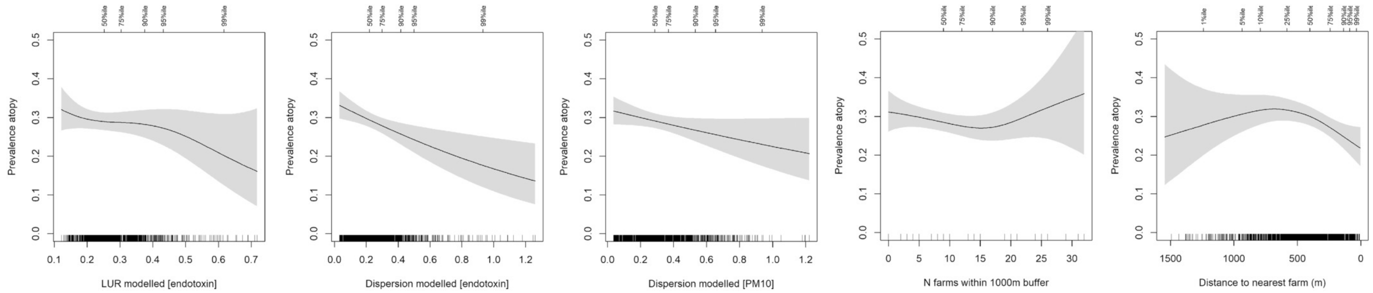

Fig. 2. Splines of associations between different livestock exposure parameters with atopic sensitization.

Model	LUR modelled [endotoxin] (EU/m^3)	Dispersion modelled [endotoxin] (EU/m^3)	Dispersion modelled [PM_{10}] (μg/m^3)	N farms within 1000 m buffer	Distance to nearest farm (m)
AIC	2813.11	2806.69	2812.25	2814.17	2806.89
P-value smooth term	0.278	0.00391*	0.0695	0.414	0.0245*

Note. Associations were adjusted for sex, age, smoking habits (ever smoking and pack years), education (high versus middle/low education), born in study area, and history of living on a farm during childhood. Rug plot shown on lower x-axis, percentiles shown on upper x-axis. Predicted concentrations at residential addresses were truncated to 99.5 percentile for both LUR modelled endotoxin and dispersion modelled endotoxin and PM_{10}. Panel 'number of farms within 1000 m buffer' and 'distance to nearest farm' reprinted in adapted format with permission of the BMJ Publishing group Ltd. Copyright © 2019.Borlée F., Yzermans C.J., Krop E.J.M., Maassen C.B.M., Schellevis F.G., Heederik D.J.J., Smit L.A.M.; Residential proximity to livestock farms is associated with a lower prevalence of atopy, *Occup Env. Med* **75**, 2018, 453–460, https://doi.org/10.1136/oemed-2017-104769.

association with COPD prevalence was comparable between all livestock exposure parameters ('distance to nearest farm' lowest *P*-value: 0.015). For asthma prevalence, the protective effect with modelled concentrations (PM_{10} and endotoxin) was observed in the lower concentration range; predicted prevalence decreased from 0.13 for the lowest exposed (5th percentile) to 0.10 (prevalence ratio of 0.80).

3.4.4. Lung function

Analyses with FEV_1 and FEV_1/FVC (based on spirometry data, available for subset of total study population) showed no significant associations with livestock exposure parameters (see Supplementary Fig. S.4). Dispersion modelled endotoxin concentration was borderline significantly ($p = .06$) associated with FVC (see Fig. 5). This downward trend was not observed with the other parameters of livestock exposure. The significant association between MMEF and 'number of farms within 1000 m buffer', was not found for the other livestock exposure parameters (see Fig. 5).

4. Discussion

This study is the first to use both dispersion modelling and LUR modelling to study health effects in relation to livestock farm emissions. Results indicate livestock farm emitted PM_{10}, and especially its endotoxin content, play a role in the earlier identified associations

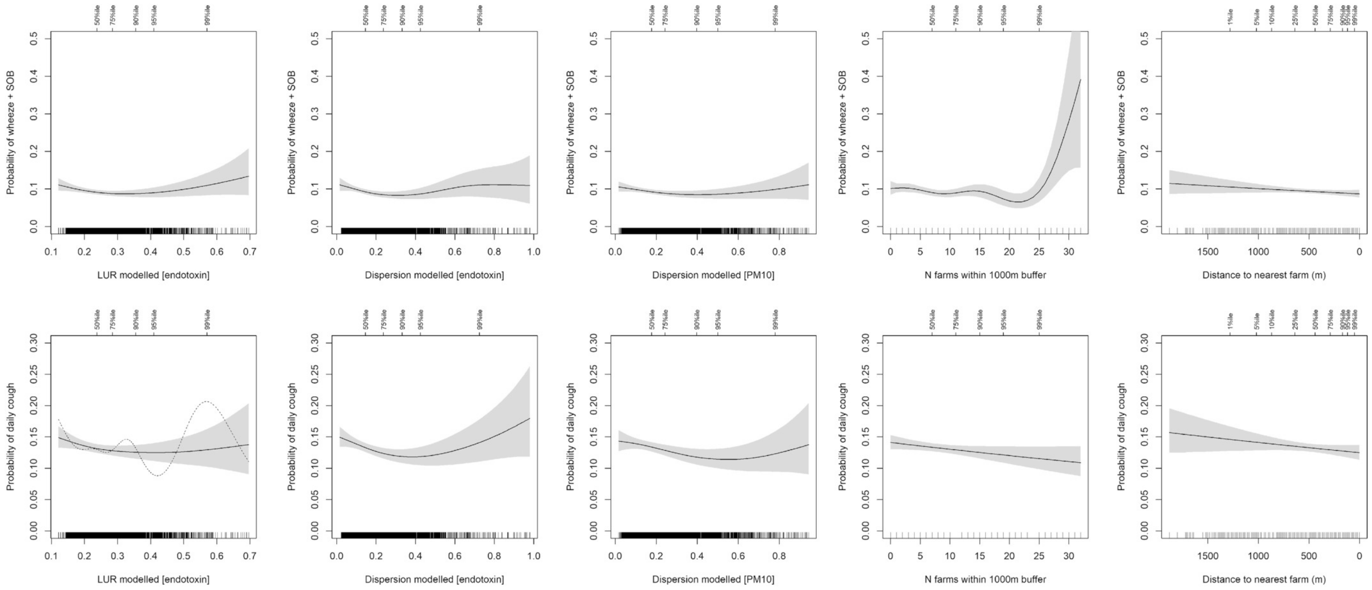

Fig. 3. Splines of associations between different livestock exposure parameters with respiratory symptoms; upper row – wheeze and shortness of breath, lower row - daily cough.

Health outcome	Model	LUR modelled [endotoxin] (EU/m^3)	Dispersion modelled [endotoxin] (EU/m^3)	Dispersion modelled [PM_{10}] (μg/m^3)	N farms within 1000m buffer	Distance to nearest farm (m)
Wheeze + SOB	**AIC**	7483.02	7482.71	7485.25	7478.73	7488.56
	P-value smooth term	0.0379*	0.0361*	0.0855	0.007*	0.142
Daily cough	**AIC**	9391.19	9395.54	9400.58	9403.11	9404.50
	P-value smooth term	0.0026*	0.0089*	0.0603	0.062	0.142

Note. Associations were adjusted for age, sex and smoking habits (never smoker, ex-smoker, current smoker). Smoothed plot for probability of daily cough in relation to LUR modelled endotoxin concentrations based on the "thin plate" basis showed an overfitted pattern (see dashed line), the non-overfitted pattern depicted (solid line) was based on number of knots set at 5. Rug plot shown on lower x-axis, percentiles shown on upper x-axis. Predicted concentrations at residential addresses were truncated to 99.5 percentile for both LUR modelled endotoxin and dispersion modelled endotoxin and PM_{10}.

between livestock farming exposure proxies and respiratory health of persons living in livestock dense areas. Associations with modelled endotoxin concentrations were stronger than with modelled PM_{10} concentrations for atopic sensitization, asthma and respiratory symptoms; supporting our hypothesis of the relevance of endotoxin with respect to observed health effects. A clear decrease in prevalence of atopic sensitization was found in relation to increased endotoxin concentration at the residential address, indicative of a protective effect. Also for prevalence of asthma, indications for a protective effect of endotoxin exposure were observed. Analyses of respiratory symptoms suggested a protective effect at the lower range of endotoxin concentrations and an possibly adverse effect at the higher range of concentrations given the power limitations at the higher range of exposure (minority of participants highly exposed). Spirometry results showed some suggestions for a small decrease in lung function in relation to livestock exposure, but associations were weak with all livestock exposure parameters.

4.1. Concentrations: PM_{10} and endotoxin

Both dispersion modelling and LUR modelling were successful in quantifying endotoxin concentrations in ambient air at residential addresses. Model performance (expressed as measured spatial variation explained) was comparable between both approaches (dispersion R^2: 0.26, LUR hold-out validation R^2: 0.32). A good level of agreement was observed between dispersion modelled concentrations and measured concentrations. The overall good level of agreement between dispersion modelled concentrations and LUR modelled concentrations at the majority of residential addresses indicates robustness of exposure predictions between two distinctly different modelling approaches. At certain individual addresses, however, modelled concentrations were distinctly different. In a LUR model, only a restricted number of variables are selected that describe the general pattern in spatial variation of residential concentrations well. The endotoxin LUR model contains four variables (De Rooij et al., 2018), farm types not included are represented indirectly by means of overall spatial correlation in the study area. Dispersion modelling uses emission from a single farm as the starting point of modelling of exposure and thus includes all farm types in a direct manner, however emission characterization is generalized per farm type.

Dispersion modelled PM_{10} concentrations and dispersion modelled endotoxin concentrations showed some divergence. This can be explained by variability in endotoxin content per size fraction depending on the farm type. For example, at addresses with many poultry farms in the vicinity, endotoxin concentrations are relatively low compared to PM_{10} concentrations, whereas the opposite holds at addresses with many pig farms in the vicinity.

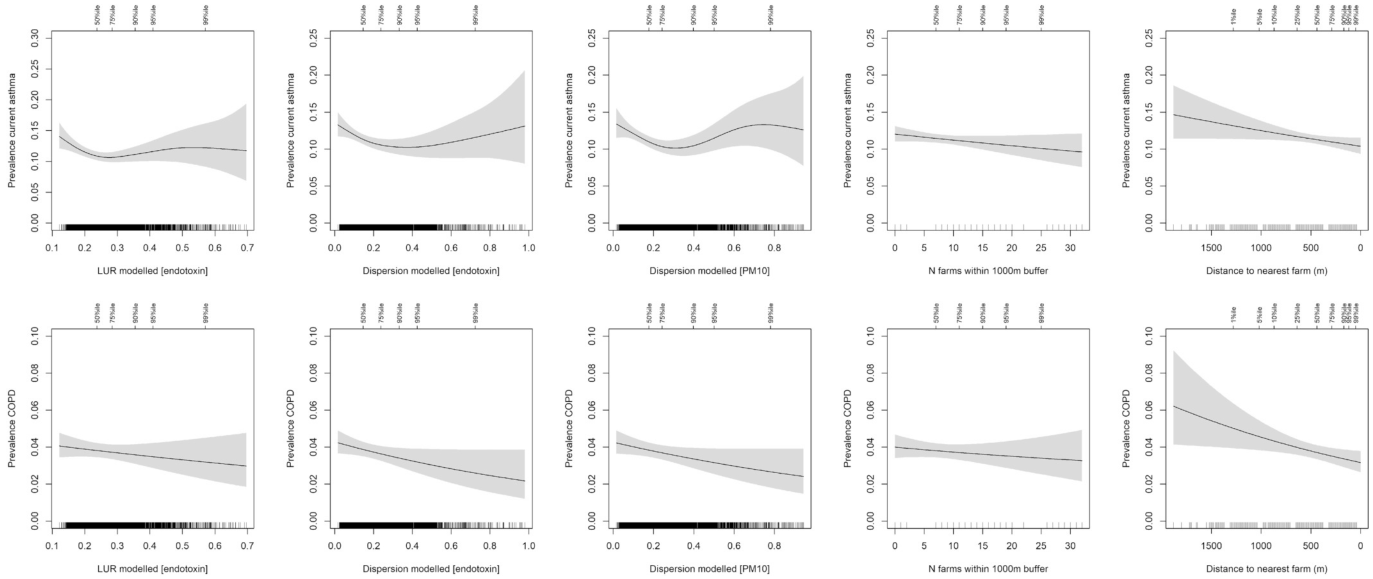

Fig. 4. Splines of associations between different livestock exposure parameters with asthma (upper row) and COPD (lower row).

Health outcome	Model	LUR modelled [endotoxin] (EU/m^3)	Dispersion modelled [endotoxin] (EU/m^3)	Dispersion modelled [PM_{10}] (μg/m^3)	N farms within 1000m buffer	Distance to nearest farm (m)
Current asthma	**AIC**	8442.66	8442.67	8441.51	8448.52	8446.65
	P-value smooth term	0.0348*	0.0289*	0.0222*	0.14	0.0428*
COPD	**AIC**	4274.19	4271.22	4271.52	4274.56	4269.41
	P-value smooth term	0.307	0.0523	0.0607	0.427	0.0147*

Note. Associations were adjusted for age, sex and smoking habits (never smoker, ex-smoker, current smoker). Rug plot shown on lower x-axis, percentiles shown on upper x-axis. Predicted concentrations at residential addresses were truncated to 99.5 percentile for both LUR modelled endotoxin and dispersion modelled endotoxin and PM_{10}.

4.2. Exposure-response relations

For atopic sensitization, asthma and respiratory symptoms, weakest exposure-response associations were found with livestock exposure proxies, strongest with endotoxin and intermediate with PM_{10}. Considering attenuation of associations in epidemiological studies due to misclassification, this pattern supports the hypothesis of the etiological relevance of livestock farm emitted PM_{10} and in particular its endotoxin content. Earlier work showed livestock exposure proxies to only moderately explain spatial variation in endotoxin concentrations measured at residential sites (r-squared: 15.5%, 12.5% for 'number of farms within 1000 m buffer', 'distance to nearest farm'; respectively) (De Rooij et al., 2018). Modelled concentrations are naturally an approximation of true levels, hence some misclassification will always be present. Exposure-response relations for endotoxin concentration modelled independently by both approaches corresponded well, this substantiates identified associations.

The earlier identified significant negative association between the lung function parameter MMEF and 'number of farms within 1000 m buffer' (Borlée et al., 2017a) was not observed with modelled concentrations. On the contrary, FVC appeared (borderline) significantly negatively associated with dispersion modelled endotoxin concentration while livestock exposure proxies did not suggest a trend for FVC.

4.3. Endotoxin and health

Significant associations between endotoxin exposure and health outcomes were identified, even though predicted annual average levels of endotoxin exposure (on average 0.25 EU/m^3) were markedly below proposed health based exposure limits (Dutch Health Council: occupational limit of 90 EU/m^3, tentative limit of 30 EU/m^3 for the general population) (Health Council of the Netherlands, 2010, 2012). However, these cannot be directly compared as exposure limits specifically aim at short-term exposures (6-hour exposure) in inhalable dust. Occurrence of short-term peak concentrations at residential sites are likely, especially at sites with elevated long-term average concentrations, since farm emissions are not constant over time (Winkel et al., 2015) and dispersion depends on ever-changing atmospheric conditions.

Our results showed an inverse association between endotoxin exposure and atopic sensitization and asthma prevalence. For children, an inverse association between livestock exposure and allergic diseases has been firmly established, this protective association is believed to be mediated by exposure to microbes (Campbell et al., 2015). Recent studies also identified this inverse association in adult populations (adjusted for childhood farm exposure) suggesting continuous exposure to be the most effective to prevent development of allergic diseases (Elholm et al., 2013, 2018; Smit et al., 2010; Spierenburg et al., 2017). For both atopic sensitization as asthma, the prevalence ratio observed in our study was substantial (0.75, 0.80; respectively) suggesting the size of the effect of livestock-related environmental exposure in a non-farming population to be considerable. The majority of studies looking into respiratory symptoms in relation to low levels of airborne endotoxin exposure (defined as < 100 EU/m^3) found an increase in symptoms with increased exposure but most did not reach statistical significance (Farokhi et al., 2018). Most of these studies focused on short-term exposure, research regarding long-term exposure and

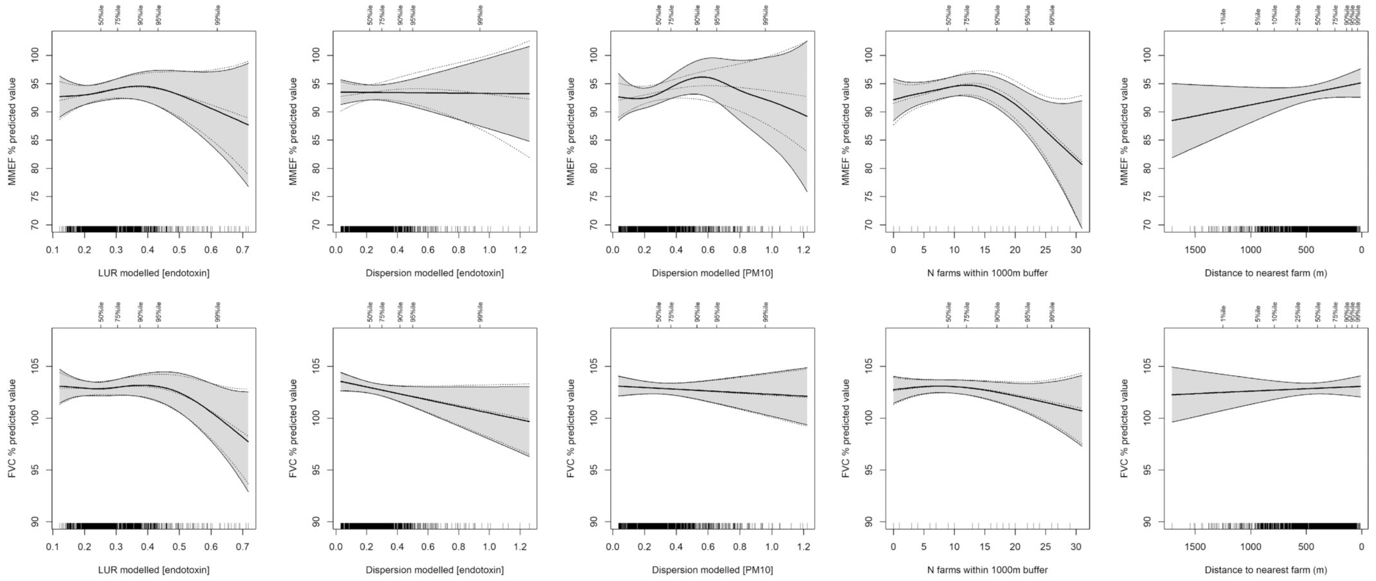

Fig. 5. Splines of associations between different livestock exposure parameters with lung function parameters; upper row – MMEF, lower row - FVC.

Health outcome	Model	LUR modelled [endotoxin] (EU/m^3)	Dispersion modelled [endotoxin] (EU/m^3)	Dispersion modelled [PM_{10}] (μg/m^3)	N farms within 1000m buffer	Distance to nearest farm (m)
MMEF % predicted value	**AIC**	22009.60	22010.77	22008.92	22003.61	22008.46
	P-value smooth term	0.506	0.955	0.337	0.0456*	0.129
FVC % predicted value	**AIC**	17914.32	17913.96	17917.18	17915.49	17917.27
	P-value smooth term	0.216	0.0613	0.588	0.317	0.655

Note. Adjustment for sex, age and height was made by calculating percentage predicted spirometry variables based on GLI-reference values. Associations are also adjusted for farm childhood, smoking habits and born in study area. The dotted lines show the models for further adjustment for week-average ambient NH3 levels (ug/m3) prior to the lung function test. Rug plot shown on lower x-axis, percentiles shown on upper x-axis. Predicted concentrations at residential addresses were truncated to 99.5 percentile for both LUR modelled endotoxin and dispersion modelled endotoxin and PM_{10}. Panel 'number of farms within 1000m buffer' reprinted in adapted format with permission of the American Thoracic Society. Copyright © 2019 American Thoracic Society. Cite: Borlée F., Yzermans C.J., Aalders B., Rooijackers J., Krop E.J.M., Maassen C.B.M., Schellevis F.G., Brunekreef B., Heederik D.J.J., and Smit L.A.M.; Air pollution from livestock farms is associated with airway obstruction in neighboring residents, *Am. J. Respir. Crit. Care Med.* **196**, 2017a, 1152–1161, https://doi.org/10.1164/rccm.201701-0021OC. The *American Journal of Respiratory and Critical Care Medicine* is an official journal of the American Thoracic Society.

potential health effects is lacking. Splines displaying the relation between endotoxin exposure and respiratory symptoms in our study population had a remarkable shape, displaying inverse associations at the lower range and adverse associations at the higher range. It might be that this pattern represents protective and adverse effects of endotoxin and that exposure characteristics determine the effect. This would be in line with suggestions made that (besides individual susceptibility) dose, duration and frequency of exposure largely determines the response (Gautam et al., 2018; Liu, 2002; Wunschel and Poole, 2016). Besides a plausible causative agent, endotoxin is also a marker for livestock-related microbial exposures in general. Other microbial components like glucans and peptidoglycans could also play a role in observed health effects among residents (Poole and Romberger, 2012).

An inverse association was observed in our study for self-reported COPD prevalence with livestock exposure parameters. This inverse association has been observed before in this rural area in the Netherlands by a study assessing associations with livestock exposure proxies in relation to health using electronic medical records of over 90,000 patients (Smit et al., 2014). Our study findings showed the association with COPD prevalence to be strongest with 'distance to nearest farm', dispersion modelled concentrations were borderline significant. It might be that associations are influenced by self-reported COPD overlapping with asthma (Borlée et al., 2015). Biologically, it does not seem plausible that endotoxin exposure protects against COPD. Taking in mind misclassification induced attenuation, this also argues against livestock emitted PM underlying the protective association between 'distance to nearest farm' and COPD.

A clear decline in lung function after short term exposure to organic dust, specifically endotoxin, has been firmly established in experimental and occupational studies (Castellan et al., 1987; Michel et al., 2002; Möller et al., 2012; Smid et al., 1994). Evidence with respect to long-term exposure and accelerated lung function decline is limited (Bolund et al., 2017). In our study population, we did not observe a clear decrease in lung function in relation to long-term endotoxin exposure. Findings reported by Borlée et al. (2017a) indicated short-term elevated levels of exposure to livestock farm emissions, assessed by variations in ammonia concentrations over time, being potentially relevant with respect to lung function in this study population.

4.4. Strengths and limitations

Exposures were estimated based on two distinct state-of-the-art modelling approaches, both being validated against measured concentrations. Comparability of exposure-response relations for endotoxin concentration modelled independently by both approaches, substantiates identified associations. Health outcomes used were obtained from a large population based study including validated questionnaires, high quality lung function testing and objectively assessed atopic sensitization.

The main limitation of this study is its cross-sectional design hampering interpretation of results with respect to causality and timing of effects. No insight was gained in short-term variation of exposures to livestock farm emissions and health effects, which remains a major knowledge gap. Longitudinal health studies in combination with time-resolved concentrations at residential addresses are warranted. Other limitations of this study include the lack of information on home-related exposures and the use of self-reported data to determine current asthma/COPD status which might not reflect the actual status in all cases.

4.5. Implications

This study supports the hypothesis that emissions from livestock farms are of public health relevance. Results indicated microbial emissions from farms to have sizable effects on the respiratory system of people living in livestock dense areas. Besides relevance of microbial emissions with respect to transmission of infectious disease, this also draws attention to non-infectious health effects (Leibler et al., 2017). Microbial air pollution is, compared to other types of air pollution, understudied (Nazaroff, 2019). Additional research is required to substantiate findings of this study, most importantly shape of the associations and effect sizes given the confidence intervals observed at the higher range of exposure. As agricultural practices and rural populations vary, performing similar research in various countries/regions enabling comparisons would be valuable. Besides studying effects in the general population, there is a need to focus on specific groups potentially more vulnerable to livestock-related air pollution (e.g. respiratory patients, elderly and children).

4.6. Conclusion

Exposure to livestock farm emitted particulate matter seems to affect respiratory health and atopic sensitization of non-farming residents. Results indicate endotoxin as the particulate matter constituent potentially underlying observed health effects. Exposure-response analyses suggested both protective and adverse health effects to be related to exposure to livestock farm emitted endotoxin, indications of a protective effect were particularly strong for atopic sensitization. In view of residential health effects of microbial exposures related to livestock farming, focus thus far lies on zoonotic infections. Our findings draw attention to non-infectious aspects of microbial emissions with respect to public health. It is essential to gain further insights in this, especially since many people worldwide live in rural areas of which some are potentially more vulnerable to livestock farm emissions.

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Declaration of Competing Interest

No conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.105009.

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