Environmental Research 243 (2024) 117821

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

# Short-term residential exposure to endotoxin emitted from livestock farms in relation to lung function in non-farming residents

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ARTICLE INFO

*Keywords:*
Air pollution
Emissions
Endotoxin
Livestock farming
Public health
Respiratory health

ABSTRACT

*Background:* Evidence on the public health relevance of exposure to livestock farm emissions is increasing. Research mostly focused on chemical air pollution, less on microbial exposure, while endotoxins are suggested relevant bacterial components in farm emissions. Acute respiratory health effects of short-term exposure to livestock-related air pollution has been shown for $NH_3$ and $PM_{10}$, but has not yet been studied for endotoxin. We aimed to assess associations between lung function and short-term exposure to livestock farming emitted endotoxin in co-pollutant models with $NH_3$ and $PM_{10}$.

*Methods:* In 2014/2015, spirometry was conducted in 2308 non-farming residents living in a rural area in the Netherlands. Residential exposure to livestock farming emitted endotoxin during the week prior to spirometry was estimated by dispersion modelling. The model was applied to geo-located individual barns within 10 km of each home address using provincial farm data and local hourly meteorological conditions. Regional week-average measured concentrations of $NH_3$ and $PM_{10}$ were obtained through monitoring stations. Lung function parameters ($FEV_1$, FVC, $FEV_1$/FVC, MMEF) were expressed in %-predicted value based on GLI-2012. Exposure-response analyses were performed by linear regression modelling.

*Results:* Week-average endotoxin exposure was negatively associated with FVC, independently from regional $NH_3$ and $PM_{10}$ exposure. A 1.1% decline in FVC was estimated for an increase of endotoxin exposure from 10th to 90th percentile. Stratified analyses showed a larger decline (3.2%) for participants with current asthma and/or COPD. $FEV_1$ was negatively associated with week-average endotoxin exposure, but less consistent after co-pollutant adjustment. $FEV_1$/FVC and MMEF were not associated with week-average endotoxin exposure.

*Conclusions:* Lower lung function in non-farming residents was observed in relation to short-term residential exposure to livestock farming emitted endotoxin. This study indicates the probable relevance of exposure to microbial emissions from livestock farms considering public health besides chemical air pollution, necessitating future research incorporating both.

## Introduction

Evidence is increasing on the health impact of livestock-related air pollution on people living in rural areas(Casey et al., 2015; Douglas et al., 2018; Smit and Heederik, 2017). Emissions of livestock farms contain several gases like ammonia, and particulate matter which predominantly consists of biological components including particles from manure, feed, hair, and feathers with typically high bacterial loads. (Cambra-López et al., 2011; Erisman et al., 2008). Endotoxins are important toxins of bacterial origin considering their potency in causing airway inflammation and their use as a generic marker for microbial air pollution. Livestock farms are major contributors to elevated endotoxin concentrations in ambient air in rural areas(De Rooij et al., 2018; Rolph et al., 2018), warranting comprehensive research on its public health relevance.

Endotoxin exposure resulting in adverse respiratory health effects is well described in experimental studies; and in multiple observational epidemiological studies mainly involving occupational populations like farmers and workers employed in farm related industries (Douwes et al., 2003; Heederik et al., 2007; Liebers et al., 2020). The most pronounced

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https://doi.org/10.1016/j.envres.2023.117821
Received 5 October 2023; Received in revised form 27 November 2023; Accepted 28 November 2023

effects include lung function changes expressed by several lung function parameters indicating airway obstruction and possibly also restriction (Bolund et al., 2017; Heederik et al., 2007). The evidence synthesis performed by Farokhi et al. (2018) on (relatively) low-level airborne endotoxin exposure (studies investigating concentrations >100 EU/$m^3$ were excluded) indicated a probable negative effect on lung function with concentrations in this (relatively) low range. Ambient endotoxin concentrations at residencies of inhabitants of livestock dense areas, including vulnerable subgroups, may reach these levels during short-term peak exposures. Previous work studying long-term (annual-average) exposure to airborne endotoxin from livestock farms in non-farming residents showed a negative trend for lung function(de Rooij et al., 2019). Dose-dependent stimulation of immunologic pathways is a potentially relevant biological mechanism underlying lung function changes in relation to endotoxin exposure (Mack et al., 2020; Mazgaeen and Gurung, 2020). Such effects may be more pronounced for elevations in exposure that might occur during shorter periods of time, resulting in stronger associations compared with annual-average exposure. Occurrence of such elevations in endotoxin concentrations during short periods has been demonstrated in various residential exposure assessment studies. Distinct variation in concentrations over time occurred, highest values in the range were 15 times higher than lowest values (De Rooij et al., 2017, 2018; Rolph et al., 2018).

Studying effects on lung function related to short-term residential exposure to endotoxin emitted from livestock farms is challenging. This is because intensive measurement campaigns are required and modelling efforts to estimate concentrations at residential addresses capturing short-term spatial-temporal variation. Because livestock farm emissions consist of several air pollutants, multi-pollutant modelling needs to be considered. For example, short-term exposure to ammonia measured on a regional scale was associated with acute effects on lung function (Borlée et al., 2017a; van Kersen et al., 2020). Ammonia was used as a proxy of temporal variation in air pollution from livestock farms. Ammonia is more specifically related to farm emissions than $PM_{10}$ which captures also other sources than livestock farms. Distinction variation in patterns of ambient concentrations of endotoxin versus ammonia and $PM_{10}$ is expected. Despite all being emitted by livestock farms, the sources and formation processes at the farm are very different as well as their characteristics as air pollutants (Cambra-López et al., 2010; Hendriks et al., 2013; Sutton et al., 2011). Ammonia is a gaseous air pollutant and is formed by bacteria mediated processes in the manure (Sutton et al., 2011). $PM_{10}$ in the farm is mainly characterized by small particles emanating from biological matter, but there are also non-biological sources like sand used for bedding and emissions from machinery(Cambra-López et al., 2010; Hendriks et al., 2013). Endotoxins are absorbed onto the surface of particles and mainly originate from decay of gram-negative bacteria(Bolund et al., 2017).

The aim of the present study was to assess associations between lung function in 2308 non-farming residents and short-term residential exposure to endotoxin emitted from livestock farms. We assessed the robustness of associations between endotoxin and lung function after adjustment for co-pollutants including ammonia and $PM_{10}$. This study was part of the VGO project (Dutch acronym for "Livestock Farming and Neighboring Residents' Health"), a long-running and comprehensive study on the health effects of livestock farming in the Netherlands, a densely populated country with a large livestock sector. Initial analyses within the VGO project involved associating exposure proxies (distance to farms, farm density) of livestock farm emissions and regional concentrations of air pollutants to lung function(Borlée et al., 2017a). In a later phase, after exposure models were developed, associations with annual average endotoxin concentrations were assessed(de Rooij et al., 2019).

## Methods

### Study design and study area

Lung function data was used from a population-based cross-sectional study, conducted as part of the VGO project. The study population consisted of adult non-farming residents living in a rural area and recruited via general practices. For a detailed description of the study population and the recruitment see Borlée et al.(Borlée et al., 2015, 2017b) In total 2494 study participants underwent a medical examination including spirometry in between March 2014 and February 2015. The study protocol (METC 13/533) was approved by the Medical Ethical Committee of the University Medical Centre Utrecht. All participants signed informed consent.

Study participants lived in a rural region (3000 $km^2$ in size) in the southeastern part of the Netherlands (provinces of Noord-Brabant and Limburg) comprising areas with high livestock densities. The number of farms in the proximity of the residential address ranged from 11 farms within 3000m circular buffers up to 171 farms, see Supplemental Figure S.1. for a map of the study region. In the Netherlands, livestock is typically kept in enclosed animal houses; besides some dairy cows, sheep and horses which are kept on pastures at periods during the year. Dutch farms are typically highly organized intensive operations that are specialized meaning that a single animal species is kept for a specific production target (e.g. laying hen farms, broiler farms). The number of animals kept on a farm varies but commonly is in the order of hundred(s) of cows at a cattle farm, thousands of pigs at a pig farm and ten-thousands of chickens at a poultry farm.

### Exposure assessment

#### Residential endotoxin exposure – week average

For each VGO health study participant, exposure to livestock farm emitted endotoxin in $PM_{10}$ fraction at the home address during the week prior to lung function testing was computed. This was done by means of dispersion modelling, taking into account spatial-temporal variation. In short, the dispersion model is based on the Gaussian plume model and implements the Netherlands New National Model (see De Rooij et al., 2019 for details). This model estimates dispersion of endotoxin emitted from livestock farms using geolocated data on source-level (e.g. farm-type, number of animals, barn characteristics, production cycle) and data relevant for dispersion in the surroundings of the source like local terrain roughness and meteorological conditions. For each participant during the week prior to lung function testing, the dispersion model was applied to geolocated individual barns within 10 km of their residential address. This was done using provincial livestock farm data of 2015 and using hourly meteorological conditions of a nearby monitoring station of the Dutch Royal Meteorological Institute (KNMI). To obtain week-average residential exposure to endotoxin in $PM_{10}$ size fraction, summation of hourly contributions of individual barns in that week was performed. This time resolution was chosen based on model performance as it was considered the shortest period for which exposure could be reliably estimated given the lack of real-time livestock data. Moreover, Gaussian plume models are not well suitable to predict hourly concentrations paired in both space and time, as is shown in many validation studies(Snoun et al., 2023). Model performance was evaluated by comparing modelled concentrations to concentrations repeatedly measured during 14-day periods for 3–5 times at 61 residential gardens in the same study area.

#### Short-term $NH_3$ and $PM_{10}$ and long-term endotoxin exposure

Week-average levels of $NH_3$ and $PM_{10}$ for each health study participant in the week prior to spirometry were taken into account analogously with previous work(Borlée et al., 2017a). Borlée et al. estimated exposure based on regional measured concentrations $NH_3$ and $PM_{10}$ as dispersion modelled concentrations were unavailable. They computed the temporal variation on regional scale by taking the average of concentrations measured at stations from the Dutch Air Quality Monitoring network situated within the study area, being two stations for $NH_3$ and four stations for $PM_{10}$. Annual-average residential exposure to endotoxin emitted from livestock farms was computed by dispersion modelling as described previously using meteorological data of 2014–2015 (De Rooij et al., 2019). Annual-average endotoxin concentrationswere included to adjust for long-term exposure, thus focusing on spatial variation.

### *Lung function assessment*

Lung function was assessed by spirometry performed according to European Respiratory Society (ERS) guidelines and the European Community Respiratory Health Survey III (ECRHS-III) as described before by Borlée et al., (2017). In short, EasyOne Spirometers (NDD Medical Technologies Inc.) measuring flow and volume by ultra-sound transit time were used by trained field workers. Besides initial quality checks in the field, a lung function specialist reviewed the quality of all lung function curves in NDD software. Out of the multiple spirograms per participant, the three curves with the highest quality according to ATS/ERS criteria were selected. In total, 2308 participants had a quality of C or higher (meaning at least two reproducible curves or reproducibility within 200 ml) for their pre-bronchodilator spirometry. We analyzed the following lung function parameters: Forced Expiratory Volume in 1 s ($FEV_1$), Forced Vital Capacity (FVC), the $FEV_1$/FVC ratio and Maximum Mid-Expiratory Flow (MMEF). Lung function parameters were expressed in age-, sex- and height-adjusted percentage predicted values based on the Global Lung Function Initiative (GLI) 2012 reference equations(Quanjer et al., 2012).

### *Data analysis*

The distribution of week-average endotoxin concentrations in $PM_{10}$ fraction at the residential addresses in the week prior to spirometry was evaluated. To avoid outlying unrealistically high values, winsorizing to the 99.5 percentile was performed. To enable direct comparisons of effect sizes between the exposure parameters, each exposure parameter was scaled to the 10th-90th percentile range. Correlations between exposure parameters were computed.

Associations between week-average residential endotoxin exposure and lung function parameters were analyzed by means of linear regression modelling. This was chosen as no deviations from linearity were indicated by means of likelihood ratio testing of linear models versus penalized regression splines (p-value >0.20). Associations were adjusted for smoking habits, living on a farm during childhood and being born in the study area(Borlée et al., 2017b). Several sensitivity analyses were performed: 1) annual-average endotoxin exposure was included besides week-average endotoxin exposure to explore differences in short-term versus long-term exposure; 2) co-pollutant models including regional week-average $NH_3$ and $PM_{10}$ to explore robustness for other livestock-related exposures; 3) stratified analyses were performed to gain insight into possible differences in associations for potential vulnerable subgroups. Analyses were performed on the subgroup of participants without asthma/COPD versus the subgroup of participants with either current asthma (defined as an asthma attack, woken by an attack of shortness of breath, and/or currently taking any medicine for asthma in the last 12 months) and/or COPD (defined as self-reported doctor's diagnosis and/or based on spirometry either GOLD/LLN criteria, see Borlée et al. (2017b)).

## Results

### *Characteristics study population and exposures*

The study population had an average age of 56 years, over half (55%) were females and most (76%) of the participants were born in the study area (see Borlée et al. (2017b) and Supplemental Table S1 for descriptive characteristics). Considering respiratory status, 12.9% of the participants reported current asthma and 12.7% of the participants had COPD.

Week-average concentrations of endotoxin in $PM_{10}$ fraction at the home address prior to spirometry ranged from 0.01 to 1.65 EU/$m^3$ overall (see Supplemental Table S2 for descriptive characteristics of exposure). Fig. 1 shows the considerable contrast per week in endotoxin exposure at the study participants' home addresses. Week-average residential endotoxin exposure was strongly correlated with annual-average residential endotoxin exposure (Pearson's r = 0.82, $p < 0.001$; see Fig. 2), suggesting the importance of the spatial component of the modelled weekly average endotoxin exposure. The variation in week-average residential endotoxin concentrations was more profound than for the annual average concentration because spatial-temporal variation was captured resulting in a wider range (coefficient of variation of 84% and 66% respectively). Week-average residential endotoxin exposure was weakly correlated with regional week-average $NH_3$ concentrations measured at routine monitoring stations (Pearson's r = 0.25, $p < 0.001$) and even weaker with measured regional week-average $PM_{10}$ (Pearson's r = 0.093, $p < 0.001$). Evaluation of predictions on short-term exposure by the endotoxin model indicated moderate correlation between modelled and measured concentrations (Pearson's r = 0.35, $p < 0.001$) and thus a limited degree of overall variation explained by the model. Model performance was more robust at some locations than others as indicated by multiple comparisons (measured versus predicted) per site; of all Pearson r's computed per site (N = 61 sites) the overall median Pearson's r was 0.46; while median Pearson's r was 0.32 for sites within 250m distance from the nearest farm (N = 24), 0.57 for sites between 250 and 500m distance from the nearest farm (N = 21), 0.40 for sites between 500 and 1000m distance from the nearest farm (N = 11), and 0.67 for sites further than 1000m distance from the nearest farm (N = 5).

### *Main analyses exposure-response*

The overall outcomes of the exposure-response analyses are visually represented in Fig. 3 and the accompanying modelling results are reported in more detail in Table 1 for percentage predicted values of $FEV_1$ and FVC and in Supplemental Table S.3 for $FEV_1$/FVC and MMEF. All reported associations were adjusted for the confounders: smoking habits, living on a farm during childhood and born in study area. In brief, for $FEV_1$ a statistically significant negative association with week-average residential endotoxin exposure was observed. After co-pollutant adjustment (model 2) for regional week-average $NH_3$ concentrations, this negative association did not remain statistically significant but a trend remained ($p = 0.112$, $\beta = -1.02$). The association observed for regional week-average $NH_3$ concentrations and $FEV_1$ in this co-pollutant model was slightly affected compared to previous $NH_3$ single-pollutant modelling ($\beta = -1.9$ vs. $-2.2$, $p = 0.014$ vs. 0.003 (Borlée et al., 2017a)). For FVC a statistically significant negative association with residential week-average endotoxin exposure was observed. This association remained after adjustment for regional week-average $NH_3$. The estimate of the effect size of 1.1% decrease in FVC with a 10th-90th percentile increase in week-average endotoxin exposure was not affected by adjustment for long-term endotoxin exposure: the confidence interval widened but this was not unexpected considering the high correlation (see Supplemental Table S4., VIF = 3.1). For $FEV_1$/FVC no significant association with residential week-average endotoxin exposure was observed. No clear association was observed for MMEF with week-average endotoxin exposure. Sensitivity analyses including $PM_{10}$ adjustment did not affect the findings.

### *Stratified analyses exposure-response*

Analyses on FVC in the subset of participants with current asthma

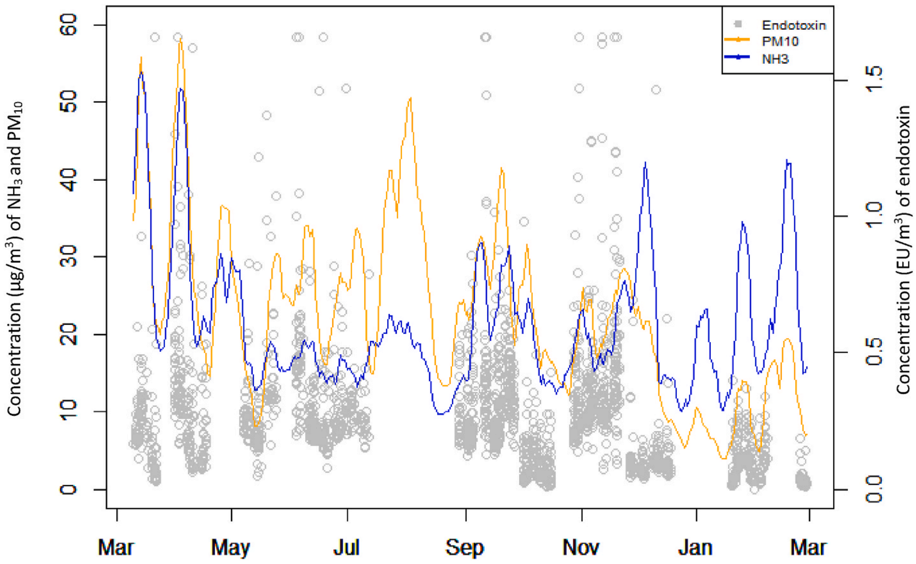

**Fig. 1.** Overview over time of exposure parameters during the study period (March 2014–March 2015); week-average of measured concentrations of $NH_3$ and $PM_{10}$ (µg/$m^3$) in study region* and modelled week-average endotoxin exposure (EU/$m^3$) at home addresses of study participants
Note. * Week-average of concentrations measured at stations from the Dutch Air Quality Monitoring network being two stations for $NH_3$ and four stations for $PM_{10}$. In August 2014 and in the end of December 2014 until beginning of January 2015 no lung function measurements were performed.

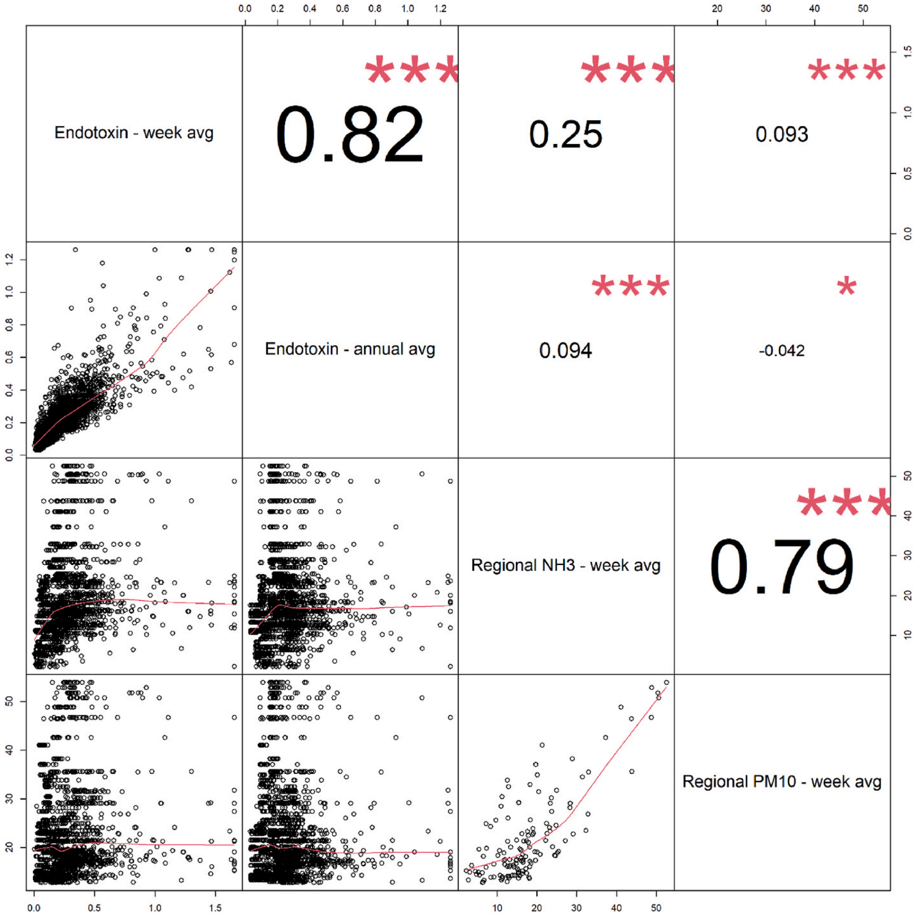

**Fig. 2.** Correlation plot showing scatterplots and accompanying Pearson's correlations between modelled week average endotoxin exposure (EU/$m^3$), modelled annual average endotoxin exposure (EU/$m^3$), regional measured weekly $NH_3$ concentration (µg/$m^3$) and regional measured weekly $PM_{10}$ concentration (µg/$m^3$)
Note. Line is based on LOESS smoothed fit.
*** = P-value <0.001
* = P-value <0.05.

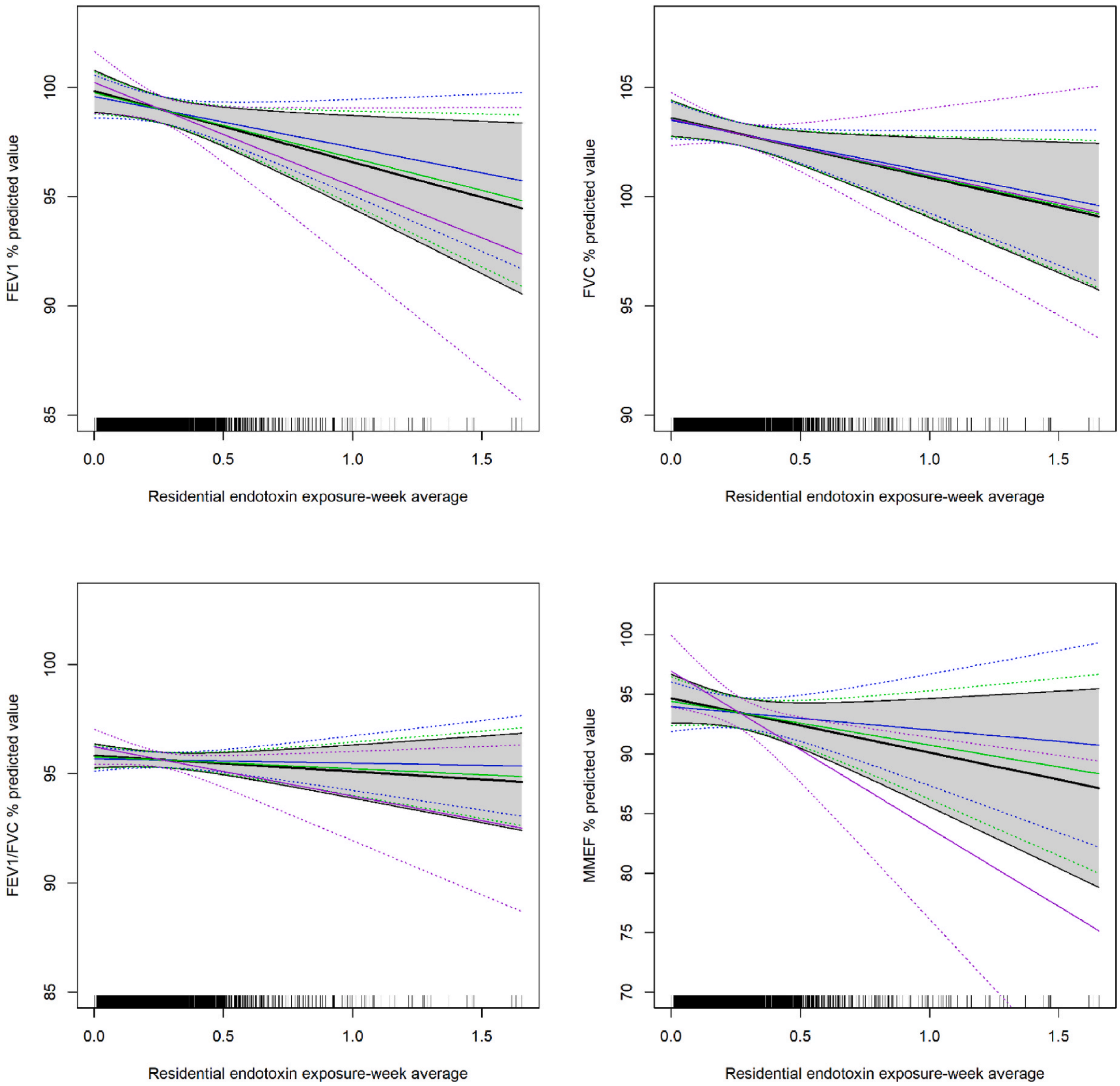

**Fig. 3.** Associations for the different lung function parameters (expressed in percentage predicted value) with modelled week-average residential endotoxin exposure (EU/$m^3$) at the home address in single-pollutant and co-pollutant linear regression models.
Note. Black lines: model with week-average endotoxin exposure, adjusted for smoking habits (never smoker, ex-smoker, current smoker), living on a farm during childhood, born in study area; the basic confounders set.
Blue lines: model with week-average endotoxin exposure and basic confounders set plus further adjustment for week-average regional $NH_3$ levels prior to the lung function test.
Green lines: model with week-average endotoxin exposure and basic confounders set plus further adjustment for week-average regional $PM_{10}$ levels prior to the lung function test.
Purple lines: model with week-average endotoxin exposure and basic confounders set plus further adjustment for residential annual-average endotoxin exposure.
Dashed lines of corresponding colors depict confidence intervals.

and/or COPD showed a significant negative association with residential week-average endotoxin exposure of considerable effect size. A 10th-90th percentile increase in week-average residential exposure was associated with 3.2% decrease in FVC predicted value (see Table 1 and Supplemental Figure S.2.). Albeit this effect size being larger compared to the estimate in non-respiratory patients, there was no statistically significant difference ($p = 0.11$ for interaction term). The effect size and p-value remained stable after adjustment for regional week-average co-pollutants ($NH_3$, $PM_{10}$). The estimate was not affected by adjustment for long-term endotoxin exposure. Analyses on FEV1 in the subsets showed no remaining association after adjustment for regional-week average NH3 (see Table 1). For $FEV_1$/FVC no clear association with residential week-average endotoxin exposure in the subsets was observed, likewise for MMEF (see Supplemental Table S.3.).

## Discussion

Main findings of the current study indicate a reduction in lung function in inhabitants of a rural area related to short-term exposure to endotoxin emitted from livestock farms. Associations were robust to adjustment for co-pollutants $NH_3$ and $PM_{10}$.

This study shows that associations of short-term residential endotoxin exposure were stronger compared to previous findings assessing long-term endotoxin exposure(de Rooij et al., 2019). As foreseen, correlation between short and long-term exposure was high thus complicating interpretation of modelling results with both short and long-term

**Table 1**

Results of linear regression analyses on associations between residential exposure to week-average endotoxin and lung function parameters FEV1 and FVC obtained by single-pollutant modelling (Model 1) and co-pollutant modelling (Models 2,3,4) on the overall dataset and stratified for asthma/COPD.

| | | Model 1 | | | | | | Model 2 | | | | | | Model 3 | | | | | | Model 4 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Data | Variable | β | p | 2.5% | 97.5% | | Variable | β | p | 2.5% | 97.5% | | Variable | β | p | 2.5% | 97.5% | | Variable | β | p | 2.5% | 97.5% | |
| FEV1 % predicted value | All participants | Residential EU- week avg | −1.41 | 0.022* | −2.63 | −0.2 | | Residential EU-week avg | −1.02 | 0.112 | −2.27 | 0.24 | | Residential EU-week avg | −1.3 | 0.037* | −2.52 | −0.08 | | Residential EU-week avg | −2.07 | 0.053∼ | −4.17 | 0.03 | |
| | | | | | | | | Regional $NH_3$-week avg | −1.91 | 0.014* | −3.43 | −0.38 | | Regional $PM_{10}$-week avg | −1.37 | 0.058∼ | −2.78 | 0.04 | | Residential EU-annual avg | 0.83 | 0.453 | −1.33 | 2.98 | |
| | Subset | Residential EU- week avg | −1.23 | 0.046* | −2.43 | −0.02 | | Residential EU-week avg | −0.76 | 0.233 | −2.01 | 0.49 | | Residential EU-week avg | −1.1 | 0.075∼ | −2.31 | 0.11 | | Residential EU-week avg | −1.91 | 0.068∼ | −3.96 | 0.14 | |
| | No asthma/ COPD | | | | | | | Regional $NH_3$–week avg | −2.02 | 0.009* | −3.52 | −0.51 | | Regional $PM_{10}$-week avg | −1.33 | 0.061∼ | −2.71 | 0.06 | | Residential EU-annual avg | 0.88 | 0.421 | −1.26 | 3.01 | |
| | Subset | Residential EU- week avg | −2.36 | 0.210 | −6.04 | 1.32 | | Residential EU-week avg | −2.28 | 0.235 | −6.04 | 1.47 | | Residential EU-week avg | −2.35 | 0.212 | −6.04 | 1.34 | | Residential EU-week avg | −4.17 | 0.251 | −11.28 | 2.94 | |
| | With asthma/ COPD | | | | | | | Regional $NH_3$-week avg | −0.5 | 0.839 | −5.35 | 4.35 | | Regional $PM_{10}$-week avg | −0.18 | 0.936 | −4.72 | 4.35 | | Residential EU-annual avg | 2.1 | 0.56 | −4.95 | 9.15 | |
| FVC% predicted value | All participants | Residential EU- week avg | −1.19 | 0.026* | −2.23 | −0.15 | | Residential EU-week avg | −1.03 | 0.061∼ | −2.11 | 0.05 | | Residential EU-week avg | −1.15 | 0.031* | −2.2 | −0.11 | | Residential EU-week avg | −1.12 | 0.222 | −2.92 | 0.68 | |
| | | | | | | | | Regional $NH_3$-week avg | −0.75 | 0.262 | −2.06 | 0.56 | | Regional $PM_{10}$-week avg | −0.42 | 0.495 | −1.63 | 0.79 | | Residential EU-annual avg | −0.08 | 0.932 | −1.93 | 1.77 | |
| | Subset | Residential EU- week avg | −0.86 | 0.139 | −2 | 0.28 | | Residential EU-week avg | −0.68 | 0.262 | −1.87 | 0.51 | | Residential EU-week avg | −0.83 | 0.156 | −1.98 | 0.32 | | Residential EU-week avg | −0.78 | 0.429 | −2.73 | 1.16 | |
| | No asthma/ COPD | | | | | | | Regional $NH_3$-week avg | −0.79 | 0.276 | −2.22 | 0.63 | | Regional $PM_{10}$-week avg | −0.33 | 0.623 | −1.64 | 0.98 | | Residential EU-annual avg | −0.1 | 0.923 | −2.12 | 1.92 | |
| | Subset | Residential EU- week avg | −3.19 | 0.051∼ | −6.37 | 0 | | Residential EU-week avg | −3.34 | 0.045* | −6.59 | −0.09 | | Residential EU-week avg | −3.2 | 0.05* | −6.4 | −0.01 | | Residential EU-week avg | −4.46 | 0.157 | −10.62 | 1.7 | |
| | With asthma/ COPD | | | | | | | Regional $NH_3$-week avg | 1.03 | 0.632 | −3.17 | 5.22 | | Regional $PM_{10}$-week avg | 0.45 | 0.821 | −3.47 | 4.38 | | Residential EU-annual avg | 1.48 | 0.636 | −4.63 | 7.59 | |

Note. associations adjusted for smoking habits (never smoker, ex-smoker, current smoker), living on a farm during childhood, born in study area; the basic confounders set.

β = estimate of association for increase in exposure from 10th-90th percentile.

p = p-value of association.

2.5% = lower bound of 95% confidence interval.

97.5% = upper bound of 95% confidence interval.

* = p-value is $<$ 0.05.

∼ = p-value is $>$ 0.05 and $<$ 0.10.

exposure included. However, the modest widening of the confidence intervals and associated moderate variance inflation factors (slightly higher than 3) suggest model results can be interpreted. The effect of short-term exposure to air pollution from livestock farms on lung function has previously been demonstrated by Borlée et al. (2017a), who included regional concentrations of $NH_3$ and $PM_{10}$. The current co-pollutant modelling indicates that endotoxin is also likely to be a relevant livestock-related air pollutant for acute changes in lung function, meaning that alterations in lung function may change over time related to the temporal variation in residential exposure. A panel study performed in the same study region among COPD patients explored the effects of short-term $NH_3$ exposure on daily repeated lung function measurements (van Kersen et al., 2020). They showed decreased lung function after days with higher livestock-related air pollution indicating temporal changes in lung function.

People residing in areas with more farms in the surroundings have a higher exposure to livestock farm emissions overall, but there can be considerable variation in levels of exposure week by week. This was clearly demonstrated by the modelled week-average endotoxin exposures in this study and was also shown earlier in measurement studies (De Rooij et al., 2017; Rolph et al., 2018). It can be interpreted as a logical consequence of time-varying aspects like variation in emission due to e.g. animal activity and production cycles; and meteorological conditions affecting dispersion from the farm to the residential address and hence exposure (Cambra-López et al., 2010; de Rooij et al., 2019; Winkel et al., 2015). However as these processes take place on a very local scale and require detailed data which are not (yet) existing, modelling short-term exposure is prone to misclassification as shown by the evaluation of model performance. As measured versus modelled annual averages have a satisfactory correlation, the dispersion model can be considered adequate in predicting the overall distribution of concentrations at the receptor point (e.g. measurement site), so including peak exposures. However zooming in on concentrations during shorter time periods, some periods are being overestimated by the model and others underestimated resulting in misclassification which is deemed non-differential (or random). Non-differential misclassification in exposure, usually leads to attenuation of exposure-response relations (Armstrong, 1998). While we incorporated both spatial and temporal variation in our modelling approach for estimating endotoxin exposure, we were not able to consider this for $NH_3$ and $PM_{10}$.Concentrations of these pollutants were based on regional monitoring data which solely covered temporal variation on a regional scale. Consequently misclassification of exposure to $NH_3$ and $PM_{10}$ at home addresses is inherent but the extent of it is not known. Regional measured concentrations of $NH_3$ and $PM_{10}$ had a weak correlation with modelled week-average residential endotoxin exposure. This can be explained by the local versus regional scale on the one hand and differences between air pollutants on the other hand. $PM_{10}$ is not specific for livestock farming, there are many other sources like traffic and industries. For ambient $NH_3$ concentrations, livestock farming is the main source but because of the gaseous form, contributions have a regional scale (and beyond) due to its dispersion over long distances (Hendriks et al., 2013; Sutton et al., 2011). In comparison, for endotoxins, long-distance transport is assumed to be less far (typically several kilometers from the source) because of its association with particulate matter mostly its coarse fraction and thus more a concern of (relatively) local scale(Godoy et al., 2009; Rolph et al., 2018). The weak correlation between concentrations of $NH_3$ and $PM_{10}$ with endotoxin exposure allowed for the independent assessment of associations between the air pollutants and lung function.

The co-pollutant models indicated FVC to be more strongly associated with short-term exposure to endotoxin than $NH_3$ (and $PM_{10}$) and annual-average endotoxin exposure. Interestingly, the decrease in FVC associated with week-average residential endotoxin exposure was more pronounced in the asthma and COPD patients indicating that these might be especially vulnerable. An 10th-90th percentile increase in week-average endotoxin exposure resulting in an average reduction of 3.3% in FVC. This is not a large effect size, and might be an underestimation of the actual effect size considering attenuation due to misclassification. Still even small population-wide lung function differences are known predictors of increased morbidity in the general population (Knuiman et al., 1999; Singh-Manoux et al., 2011). One should realize that endotoxin exposure may act as a proxy for microbial and/or organic exposures because livestock farm emissions are complex mixtures (Cambra-López et al., 2011). On the other hand, a causal role for endotoxin is biologically plausible and probable as (hourly) peak exposures in the order of tens of EU/$m^3$ seem possible in our study area. We base this on modelled and measured levels in this specific region and given observations from short-term measurement series performed in comparable farm-dense areas(Heederik et al., 2019; Rolph et al., 2018; Wouters et al., 2019). The incidental peak exposures are levelled out when assessing annual average exposures. For residential addresses in our study area, modelled annual-average concentrations ranged between 0.1 and 1.3 EU/$m^3$ in the $PM_{10}$ fraction. So relatively elevated long-term average concentrations can be considered indicators of more frequent or higher short-term exposures occurring during the course of a year and thereby linked to health effects.

To the best of our knowledge, no other study assessed the association between short-term exposure to endotoxin in ambient air and lung function of inhabitants living in rural areas. Long-term effects of (indicators of) exposure to livestock farm related air pollution and lung function has been studied in the Dutch VGO project, in Germany(Radon et al., 2007) and recently in adolescents from a Dutch birth cohort(Kiss et al., 2023). These studies indicated an association between lower $FEV_1$ and higher residential exposure to livestock farming (different exposure indicators used), but not with FVC. Results from panel studies on short-term lung function changes over time related to livestock farming exposure showed $FEV_1$ decrease with increasing ammonia exposures for school-attending children in the US(Loftus et al., 2015) as well as for Dutch COPD patients(van Kersen et al., 2020); FVC was not measured. Most studies on lung function in relation to occupational endotoxin exposure that assessed both $FEV_1$ and FVC, found a decrease in both parameters(Farokhi et al., 2018). In our study, we found FVC to be clearly associated with short-term residential exposure to livestock farming, specifically endotoxin. This association between ambient short-term endotoxin exposure and airway restriction is a relevant finding which warrants further research including panel studies to gain deeper insights into individuals' lung function patterns over time.

The main strength of this study is exposure estimation for endotoxin at individual home addresses combined with high quality lung function data collected from a large population-based cohort. Spirometry testing on so many participants requires huge effort but ensures objective and standardized data on airway performance. Moreover, it also provided the opportunity to assess the relatively subtle changes in lung function that were expected (and indeed observed) that can occur sub-clinically even in respiratory patients. To allow the spatial-temporal modelling of endotoxin exposure at individual home addresses, extensive work on endotoxin measurements and model development has been done since the beginning of the VGO project. Studying week-average residential endotoxin exposure instead of regional concentrations of $PM_{10}$ and $NH_3$ used as proxies of temporal variation in livestock-related air pollution, enabled individual exposure assessment and deeper insights into relevance of endotoxin as a livestock-related microbial air pollutant.

This study should be viewed as a starting point to further investigate the effects of short-term exposure to livestock farm emissions on the respiratory health of the general population living in rural areas. The study design was limited due to its cross-sectional set-up. Another limitation is solely inclusion of adults, studying children would also be interesting as they might be more susceptible as their airways and immune system are still developing. A study limitation related to exposure assessment is modelling of endotoxin emission based on number of animals present and stable type, and a lack of real-time data on emission-strength for each farm. In the Netherlands, the agricultural data is already quite detailed in comparison to other countries, animal numbers are included and even information on the exact type of housing system. Future advancements would be to have a data platform in which farmers upload real-time data from their climate/ventilation computers (if present) and animal status/occupancies (especially relevant for broiler farms). Using more refined data in the modelling, would then lead to less exposure misclassification so further enhancing insights into exposure-response relations in future epidemiological studies.

The impact of livestock farm emissions on public health has long been neglected(Smit and Heederik, 2017); as is the relevance of biological exposures in environmental epidemiology(Nazaroff, 2019). This study adds to the growing body of evidence on effects of livestock farming on the airways of people living in rural areas. Specifically it warrants for more attention to microbial air pollution given its probable public health relevance, besides chemical air pollution. The Netherlands is exceptionally densely populated and contains multitudes of livestock. However there are more areas in the world with these characteristics, and given foreseeable increasing demands in food consumption and thus food production, likely more to come. Sustainable animal production protecting the environment and ensuring health and welfare of humans and animals in and around the farms would be the way forward.

To conclude, a lower lung function in non-farming residents was observed related to short-term exposure at the home address to endotoxin emitted from livestock farms. This finding emphasizes the public health relevance of livestock-related microbial air pollution. Future research should not only be focused on livestock-related chemical air pollution but also involve microbial air pollution.

## Funding

The study was funded by the Ministry of Infrastructure and Water Management; the Province of Noord-Brabant; the Ministry of Health, Welfare and Sports and the Ministry of Economic Affairs of the Netherlands; and supported by a grant from the Lung Foundation Netherlands (3.2.11.022).The funders were not involved in designing the study, data collection, analysis and interpretation, writing of the article, nor the decision to publish.

## Ethics approval statement

The study protocol (METC 13/533) was approved by the Medical Ethical Committee of the University Medical Centre Utrecht. All participants signed informed consent prior to conducting the study.

## CRediT authorship contribution statement

**Myrna M.T. de Rooij:** Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Software, Validation, Visualization, Writing - original draft, Writing - review & editing. **Hans J. Erbrink:** Conceptualization, Data curation, Formal analysis, Methodology, Software, Writing - review & editing. **Lidwien A.M. Smit:** Conceptualization, Data curation, Funding acquisition, Methodology, Writing - review & editing. **Inge M. Wouters:** Conceptualization, Funding acquisition, Methodology, Project administration, Writing - review & editing. **Gerard Hoek:** Methodology, Writing - review & editing. **Dick J.J. Heederik:** Conceptualization, Funding acquisition, Methodology, Project administration, Writing - original draft, Writing - review & editing.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

All authors reports financial support was provided by Netherlands Ministry of Infrastructure and Water Management. All authors reports financial support was provided by Province of Noord-Brabant. All authors reports financial support was provided by Netherlands Ministry of Health Welfare and Sport. All authors reports financial support was provided by Netherlands Ministry of Economic Affairs. All authors reports financial support was provided by Lung Foundation Netherlands. Co-author Gerard Hoek is associate editor at Environmental Research.

## Data availability

Data will be made available on request.

## Acknowledgements

We gratefully acknowledge Thomas Hagenaars, Nico Ogink, Albert Winkel (Wageningen University & Research) for providing information on farm emissions and Floor Borlée (Institute for Risk Assessment Sciences, Utrecht University) for contributing to collection of health data. We gratefully thank all study participants.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2023.117821.

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