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Air pollution from livestock farms is associated with airway obstruction in neighboring residents

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ABSTRACT

Rationale: Livestock farm emissions may not only affect respiratory health of farmers but also of neighboring residents.

Objectives: To explore associations between both spatial and temporal variation in pollutant emissions from livestock farms and lung function in a general, non-farming, rural population in the Netherlands.

Methods: We conducted a cross-sectional study in 2,308 adults (age 20-72 years). A pulmonary function test was performed measuring pre- and post-bronchodilator FEV_1, FVC, FEV_1/FVC and MMEF. Spatial exposure was assessed as 1) number of farms within 500 m and 1,000 m of the home, 2) distance to the nearest farm, and 3) modelled annual average fine dust emissions from farms within 500 m and 1,000 m of the home address. Temporal exposure was assessed as week-average ambient PM_{10} and ammonia (NH_3) concentrations prior to lung function measurements. Data were analyzed with generalized additive models (smoothing).

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Results: A negative association was found between the number of livestock farms within a 1,000 m buffer from the home address and MMEF, which was more pronounced in non- atopic participants. No associations were found with other spatial exposure variables. Week- average PM_{10} and NH_3 levels were negatively associated with FEV_1, FEV_1/FVC and MMEF.

In a two-pollutant model, only NH_3 remained associated. A 25 µg/m^3 increase in NH_3 was associated with a 2.22% lower FEV_1 (95%CI -3.69 to -0.74), FEV_1 /FVC: -1.12% (-1.96 to - 0.28) and MMEF: -5.67% (-8.80 to -2.55).

Conclusion: Both spatial and temporal variation in livestock air pollution emissions are associated with lung function deficits in non-farming residents.

INTRODUCTION

Recent studies have highlighted the large contribution of agriculture to fine particulate matter (PM) air pollution, and the public health impact that may result from agricultural emissions (1–4). In the Netherlands – a small country with one of the highest population densities in the world and one of the highest livestock farm densities - neighboring residents are concerned about potential health risks of farm emissions (5).The air inside livestock farms contains high levels of (organic) dust which is known to lead to adverse respiratory health effects in those occupationally exposed (6, 7). Livestock farms may also emit air pollutants into the atmosphere, consisting of a mixture of gases such as ammonia (NH_3) and hydrogen sulfide (H_2S), and PM contaminated with micro-organisms and toxins such as endotoxins: cell-wall components of Gram-negative bacteria (8, 9). Raised endotoxin levels were measured up to 200 meters downwind from farms (10, 11). NH_3 is an irritant gas that is formed by enzymes in animal waste, and used as a marker for livestock farm emissions (12). NH_3 is also an important precursor of secondary inorganic aerosols (SIA) and highly contributes to atmospheric PM concentrations of amongst others PM_{10} and $PM_{2.5}$, of which especially $PM_{2.5}$ can be transported over great distances (4).

Despite the substantial contribution of livestock farming to ambient air pollution, potential respiratory health risks have been poorly studied. Three longitudinal panel studies have been conducted in the United States. Two were conducted among 51 children with asthma and reported a temporal effect of NH_3 and $PM_{2.5}$ exposure on forced expiratory volume in 1s (FEV_1) (12, 13). The other panel study also found a temporal effect of farm-related pollutants on self-reported respiratory symptoms and FEV_1 in 101 adults (14). Two other American studies described spatial associations between residential proximity to livestock farms and self-reported respiratory effects (15, 16).

Studies from Europe have been conducted in Germany and the Netherlands. A cross-sectional German study found a decrease in FEV_1 in adults with more than 12 stables within 500 m of the home address (17), and among subjects exposed to higher annual

NH_3 levels (18). In the same area a cross-sectional study among 3,867 children found an association between asthmatic symptoms and modeled endotoxin exposure among children with atopic parents (19). Conversely, two Dutch studies found inverse associations between livestock farm proximity and asthma and COPD prevalence using medical records and self-reported data (20, 21). However, COPD patients reported more symptoms in areas with high farm density (20, 22).

Thus, only a limited number of studies measured pulmonary function in neighboring residents, and most studies focused on spatial variation, e.g. distance to the nearest farm. However, we expect that time-varying exposure to air pollutants resulting from agricultural activities also plays a role. The current study aims to explore associations between proxies of both temporal and spatial variation in air pollution from livestock farms and lung function in a general, non-farming population of 2,308 adults in the Netherlands. Results presented in this manuscript are part of the VGO-study (Dutch acronym for Livestock Farming and Neighboring Residents' Health) and have been previously presented as abstracts (23, 24).

METHODS

Study population and design

A questionnaire survey was conducted among patients of 21 general practitioner practices resulting in 14,875 participating adults (53.4% response) from the general population as described earlier (20). Subjects who were working or living on a farm (n=712; 4.8%) were excluded. Questionnaire respondents who were willing to participate in a follow-up study were eligible for a medical examination (n=8,714). Based on their home addresses, twelve temporary research centers were established (Figure 1). Between March 2014 and February 2015, all participants living within a distance of approximately 10 km of a temporary research center (n=7,180) were invited to the nearest research center for medical examination which resulted in 2,494 participants (response 34.7%) (25). Non-response was analyzed by comparing characteristics and association between respiratory health indicators and livestock exposure in different population subsets. The study protocol (13/533) was approved by the Medical Ethical Committee of the University Medical Centre Utrecht. All 2,494 participants signed informed consent.

Medical examination

Population characteristics were collected with an extended questionnaire. Atopy was defined as the presence of specific serum IgE antibodies to one or more common allergens (house dust mite, grass, cat and dog) and/or a total IgE higher than 100 IU/ml (26). Pre- and post- bronchodilator (BD) spirometry was conducted. FEV_1, Forced Vital Capacity (FVC), FEV_1/FVC and Maximum Mid-Expiratory Flow (MMEF) were expressed as percentage predicted based on the GLI-2012 reference equations (27). COPD was defined as a post-BD measurement of FEV_1/FVC below

the lower limit of normal AND/OR a post-BD measurement of FEV_1/FVC <0.70 (GOLD) (25).

Spatial and temporal livestock farm exposure proxies

One of the aims of the current study is to replicate the association between farm density around the home and lung function in German adults (17). In addition, other spatial and temporal exposure proxies were considered. The following spatial livestock farm exposure proxies were studied for each subject: 1) total number of farms within 500 and 1,000 m; 2) distance (m) to the nearest farm (general and specific animal farms: pigs, poultry, cattle, goats and mink); 3) inverse-distance weighted fine dust emissions from all farms within 500 m and 1,000 m as described previously (21). Ambient NH_3 and PM_{10} levels prior to the spirometry measurement were studied as temporal exposure proxies. Average daily ambient NH_3 and PM_{10} levels were obtained from air monitoring stations located in the study area, which are part of the Dutch Air Quality Monitoring Network(28).

Statistical analysis

Relationships between spirometry variables and livestock farm exposure variables (both spatial and temporal) were studied using a penalized regression spline using the (default) “thin plate” basis as implemented in the mgcv (mixed generalized additive model computation vehicle) R package. Based on the results of the spline analyses, exposure cut-off values were chosen for further analyses. Multiple linear regression analyses were used to study associations between these dichotomized exposure variables and spirometry values. For all these analyses pre-BD spirometry values were used. These models were adjusted for smoking habits, living on a farm during childhood, and growing up in the study area. Multicollinearity between confounders was checked with Spearman’s rank correlation and variance inflation factors.

Sensitivity analyses were conducted to investigate the effect of spirometry technician, influenza-season, humidity, temperature and traffic related air pollution at the home address of NO_2, $PM_{2.5}$ and $PM_{2.5}$ absorbance (soot) using land use regression models from the ESCAPE project (29, 30). In addition, analyses were stratified for atopy, COPD (based on spirometry (GOLD and/or LLN), asthma, and smoking habits and interaction between these groups and exposure variables was tested.
More details on the study methodology are provided in the online supplement.

RESULTS

Study population characteristics

The medical examination was conducted in 2,494 persons aged 20-72 years, including more females (54.6%) than males (see Table 1). In total, 837 individuals

(33.6%) reported a farm childhood and 1,871 (75.0%) were born in the study area. Only subjects with lung function measurements with sufficient quality were included in the analyses which resulted in 2,308 pre-BD measurements and 2,169 post-BD measurements. Based on spirometry results we found indication of reversibility in 4.1% and COPD in 10.9% of all participants.

Non-response analysis

Differences in personal characteristics of different population subsets were found (e.g. age, gender, self-reported morbidity, distance to farms) indicating that selection-bias could have played a role (Table 2 and more details in online supplement). However, associations between farm exposure estimates and respiratory morbidity based on self-reported data in different population subsets - the source population, the population which participated in the questionnaire survey, the population invited for the medical examination and the population which participated in the medical examination - showed similar associations, in terms of direction and magnitude, with overlapping confidence intervals (online supplementary Table E3 online supplement).

Association between spatial variation in livestock exposure and lung function

Farms located in the study area are a mix of small farms with relatively few animals to large farms with thousands of animals (see online supplement Table E1). Smoothed plots suggested a non-linear negative association between the number of farms in a 1,000 m radius and FEV_1, FEV_1/FVC and MMEF (see Figure 2). The association was statistically significant for MMEF. No clear association was found for FVC, indicating that in particular obstructive lung function changes occurred. No associations were found with farm density for specific animal species or with the other spatial exposure proxies including distance to the nearest farm (general and specific animal farms) and modelled annual average fine dust emissions from farms within 500 m and 1,000 m. Spirometry results expressed as z-scores showed minimal differences compared to results presented as % predicted values (see online supplement Figure E1). Adjustment for week-average NH_3 and PM_{10} levels at the time of the medical evaluation resulted in only minor changes of the association between farm density and lung function (see striped and dotted lines in Figure 2). Based on the shape of the splines, a cut-off value of 17 farms or more was chosen to compare so-called ‘hotspot’ areas with a high farm density, with lower farm density areas. This cut-off value was based on a visual inspection of the smoothed plots; around 17 farms within 1,000 m, there is a turning point where the % predicted value is lower than the % predicted value at 0 farms. In total 11.3% participants lived in a ‘hotspot’ and they had a 4.5% lower MMEF (95%CI -8.64 to -0.36), and a 1.86% lower FEV_1 (95%CI -3.80 to 0.09) than participants from lower farm density areas.

Adjustment for spirometry technician, influenza-season, humidity, temperature and traffic- related air pollution (NO_2, $PM_{2.5}$ and soot) had minor effects on the association between lung function and ‘hotspot’(see sensitivity analyses Figure E2 online supplement). No association

between living in a 'hotspot' and lung function was observed when COPD patients were removed from the analyses. Associations were also stronger when analyses were restricted to subgroups of non-atopics and ever smokers. A significant interaction was observed between atopy and 'hotspot' in models with lung function variables FEV_1/FVC and MMEF ($p<0.05$),
indicating significantly different associations between living in a 'hotspot' and lung function among non-atopic and atopic subjects. Minor changes were observed when atopy was defined as specific serum IgE to one or more common allergens (instead of the combination specific serum IgE and/or total IgE > 100 IU/ml): associations were still stronger when analyses were restricted to non-atopics and (borderline) significant interaction was observed between atopy and 'hotspot' in FEV_1/FVC and MMEF models (results not shown).

Association between temporal variation in livestock exposure and lung function

During the period the medical survey took place, ambient NH_3 levels ranged from 1.6 to 52.5 µg/m^3 (week-average values; see Figure 3) with a median NH_3 level of 16.3 µg/m^3. Higher NH_3 peaks were observed in spring and summer compared to autumn and winter, most likely as a result of manure spreading. Ambient PM_{10} levels ranged from 9.6 – 54.0 µg/m^3 (week- average values; see Figure 3) with a median PM_{10} level of 18.9 µg/m^3. Correlation between week-average NH_3 and PM_{10} levels was moderately strong (Pearson's r = 0.64). Smoothed plots indicated negative linear associations between all lung function variables and week- average NH_3 level prior to the lung function measurement (see Figure 4). Significant associations were found for FEV_1, FEV_1/FVC and MMEF, which are indicators of airway obstruction. Other lags for NH_3 (lag 0-2 days) resulted generally in weaker, but often statistically significant, associations (see Figure E3A online supplement). Adjustment for farm density around the home address did not change the association between lung function and NH_3 (see Figure 4). Spirometry results expressed as z-scores showed minimal differences compared to results presented as % predicted values (see online supplement Figure E4).

Smoothed plots showed similar negative linear associations between all lung function variables and week-average PM_{10} (See Figure E5 online supplement). However, in a two- pollutant model, only NH_3 remained associated with lung function (see Figure 4 and Figure E5 and E6 online supplement). Linear regression analyses showed that a change in week- average NH_3 levels between the P10 and P90 (25.1 µg/m^3) was associated with a difference in FEV_1 of -2.22 (95%CI -3.69 to -0.74), FVC: -1.07 (95%CI -2.33 to 0.20), FEV_1/FVC: -1.12 (95%CI -1.96 to -0.28) and MMEF: -5.67 (95%CI -8.80 to -2.55). Adjustment for lung function technician, influenza-season, humidity and temperature had minor effects on the association between week-average NH_3 and lung function (see sensitivity analyses Figure E6 online supplement). When analyses were restricted to non-atopic subjects, or ever smokers, associations became stronger. However, no significant interaction terms between atopy or smoking status and week-average NH_3 levels were observed. When atopy was defined as specific serum IgE to one or more common allergens (instead of the combination: specific serum IgE and/or total IgE > 100 IU/ml): associations

restricted to non-atopics were still stronger compared to the total population (results not shown). Minor changes were observed when COPD patients were removed.

DISCUSSION

This large-scale population-based study shows that emissions from livestock farms are associated with a reduced lung function level of inhabitants of a rural area with high livestock farming density. Associations were found between lung function and both spatial and temporal livestock exposure estimates. A spatial association was found between the number of livestock farms within 1,000 m and MMEF. A temporal association was found between FEV_1, FEV_1/FVC and MMEF - three indicators of airway obstruction - and week-average ambient NH_3 levels prior to the lung function measurements. Mutual adjustment of temporal and spatial effects did not change these associations. Our results indicate that the spatial association was especially apparent in non-atopic subjects, while the temporal association with NH_3 was observed in the whole population.

To our knowledge, this is the largest study on respiratory health of residents living in close proximity to livestock farms focused on lung function. The combination of both spatial and temporal variation in livestock farm exposure in association with lung function of residents from the general population has not been explored before. Another strength of the current study was the detailed medical information available for non-responders, enabling a detailed non-response analysis. We compared associations between respiratory endpoints using electronic medical records from general practitioners and self-reported respiratory conditions and livestock related exposure for different subpopulations. A comparison between characteristics of non-responders and responders of the questionnaire survey (source- population) was described before and showed differences in personal characteristics between both groups (20). However, both in the previous questionnaire survey and in the present study, selection bias did not seem to affect associations between different farm exposure estimates and prevalence of respiratory morbidity.

The Netherlands is a small country with a high population density in combination with a high livestock farm density. On an area of 41,000 km^2, 17 million people live together with 105
million chickens, 12 million pigs, 4 million cows, 0.5 million goats, and 1 million mink (31). We found an association between lung function and living in areas with more than 17 farms within a 1,000 m radius and designated such areas as ‘hotspots’. This cut-off value is based on a data-driven approach, based on a visual inspection of the smoothed plots. This spatial association is consistent with results from a cross-sectional study among residents from a rural
area in Germany (Lower Saxony Lung Study (17): more than 12 stables (4th quartile) within 500 m of the home address was associated with a lower FEV_1 (-7.4 % FEV_1, 95% CI: -14.4 to -0.4) compared to less than 5 stables within 500 m (1st quartile). Annual NH_3 concentrations were measured in the same study area in Germany and the number of farms within 500 m explained 28% of the variability of annual average

outdoor NH_3 concentrations (18). Subjects exposed to annual NH_3 levels of 19.7 µg/m^3 or higher, had a lower FEV_1 and FEV_1/FVC (-8.2 %FEV_1 and -3.3% FEV_1/FVC compared to subjects exposed to lower NH_3 levels). Both the Lower Saxony Lung study and the current study found indications for obstructive effects.

We found stronger associations when analyses were restricted to non-atopic participants. A significant interaction between atopy and living in a 'hotspot' was observed. Previous studies are inconsistent on the effect of farm exposure in non-atopic subjects. A study among children from farming and non-farming households found a negative (protective) association for atopic wheeze and endotoxin levels in matrass dust, whereas for non-atopic wheeze, there was a positive association (32). Other studies did not demonstrate differences between atopic and non-atopic subjects in the effect of endotoxin exposure on respiratory health (33, 34).
Contrary, previous studies among farmers showed stronger associations between endotoxin exposure and respiratory health effects for sensitized than for non-sensitized farmers (35, 36). In addition, a German study found a positive association between endotoxin levels and asthmatic symptoms among children with atopic parents while no association was found among children with non-atopic parents (19). Our study also suggested that some of the associations with 'hotspot' exposure were restricted to COPD patients, who may be especially susceptible to air pollutants (37). This finding is supported by a previous questionnaire study and analysis of medical records in the same study area, showing that COPD patients living in close proximity to livestock farms reported more wheezing and inhaled corticosteroids usage, and were more often treated for exacerbations (20, 22).

Livestock farm emissions are a complex mixture of bio-aerosols, gases and vapors, many of which are respiratory irritants. One etiologically plausible candidate is endotoxin. Increased endotoxin concentrations have been measured around farms (10, 11). In addition, a larger spatial variation in endotoxin compared to PM_{10} concentrations was observed in ambient air measurements in the same study area and endotoxin concentrations were more strongly predicted by livestock-related characteristics in the environment (38). Previous studies among residents have also found associations between H_2S and eye irritation and respiratory symptoms (14). Guidry *et al.* found spatial and temporal associations between size and distance of upwind livestock farms and H_2S levels (8). Due to low concentrations of H_2S and strong correlation with other pollutants, Guidry *et al*. argued that health effects can probably not be attributed to H_2S alone, but to a mixture of which H_2S is part of.

As individuals were subjected to pulmonary function tests on one occasion, but over a study period of a year (between March 2014 and February 2015), we adjusted for week-average air pollution concentrations prior to each individual's lung function test (39). An association was found between both week-average PM_{10} and NH_3 levels prior to spirometry and lung function. In a model with mutual adjustment for NH_3 and PM_{10}, the NH_3 association remained (minor change in size of effect) but the association with PM_{10} was no longer observed. Livestock production is the major contributor to ambient NH_3 levels. We evaluated potential confounding by correlated air pollutants and other time-variant variables such as seasonal effects, but we do not

expect that temporal variation in ambient NH_3 levels is associated with other (occupational) exposures or usage of cleaning products. Therefore, we did not consider these variables as potential confounders.

The temporal association with NH_3 levels corresponds to results of Loftus and others who conducted a panel study among 51 children with asthma living in an agricultural region of Washington State (12), where ammonia levels in ambient air were strongly associated with proximity to farms. Even though Loftus *et al.* used a different study design (panel study with repeated measurements) and a different study population compared to our study, both studies found an association between lung function and agricultural air pollution. A 3.8% (95% CI 0.2-7.3) decrease of FEV_1 was observed per interquartile range (IQR; 25 µg/m^3) increase in previous day NH_3 concentration (12). They found a smaller association with $PM_{2.5}$: FEV_1 decreased by 0.9% (95%CI 1.8-0.0) for an IQR increase of previous day $PM_{2.5}$ levels (7.9 µg/m^3) (13).

As NH_3 is mostly locally generated (40, 41), this suggests that the temporal associations with lung function were mostly driven by locally generated pollutants, represented by NH_3. Considering the ambient NH_3 levels during the study period (median 16.3 µg/m^3), it is unlikely that NH_3 has a direct effect on respiratory health of residents. The threshold limit value for ammonia in an occupational setting is 25 ppm (1,800 µg/m^3) (42). However, a study among farmers showed a decrease in FEV_1 at levels above 7.5 ppm (43). Nonetheless, it is more plausible that ambient NH_3 levels serve as a marker for airborne emissions from livestock farms and agricultural activities and that the observed decrease in lung function results from exposure to one or more co-pollutants, including microbial agents such as endotoxins (9). Another explanation would be that ambient NH_3 concentrations are associated with Secondary Inorganic Aerosol (SIA) formation. NH_3 reacts in the atmosphere with nitrogen oxides and sulfur dioxide to form solid (particulate) ammonium sulfates and nitrates which are part of the $PM_{2.5}$ fraction and can penetrate deeply into the lung (4). Secondary particle formation takes time, however, and without further studies of the local atmospheric chemistry we cannot support the likelihood of these transformations happening locally. A recent study from Barcelona suggested that significant SIA formation may take place already on an urban spatial scale (44). More detailed characterization of livestock associated environmental exposures, including bio-aerosol analysis and SIA formation, are needed.

Spatial exposure variables were based on participants' home address, but since most people do not spend 24 h a day at home, this may lead to exposure misclassification. However, in Europe, adults spend the majority of their time indoors at home (56–66%) (45), which suggests that home address might be a reasonable proxy for individual exposure. In addition, we did not take into account the influence of wind direction or wind speed on exposure. In the Netherlands, winds are slightly more often from the south-west, but south-westerly winds are also associated with less stable weather conditions favoring larger dispersion of pollutant emissions. As a result, there is usually not much difference between concentrations measured in different directions from a source. Both limitations will introduce non-differential exposure misclassification, leading to an underestimation of the effect of farm

exposure. A more comprehensive method to estimate spatial exposure – for example with dispersion modelling or even an actual intensive air measurement network – will increase precision of spatial exposure. We have used central site monitoring data to represent temporal NH_3 exposure, which is the usual approach in studies investigating acute effects of air pollution (37).
Validation studies have reported moderately high correlations between temporal variations in ambient outdoor concentrations at the home address and at central monitoring sites for both particulate matter and gaseous air pollutants (46, 47).

In conclusion, air pollutant emissions from livestock farms are associated with a reduced lung function level in non-farming residents of a rural area in the Netherlands. Further research into the impact of emissions from livestock farms, especially on respiratory health of susceptible subgroups e.g. children, elderly and respiratory disease patients, is warranted.

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TABLES AND FIGURES

Table 1. General characteristics of the study population.

Characteristics	**Total population (n=2,494)**
Age, years	54.7 ± 11.0
Female	1363 (54.6%)
BMI*	27.1 ± 4.3
Neversmoker	1122 (45.0%)
Eversmoker	1112 (44.6%)
Currentsmoker	252 (10.1%)
Packyears†	18.1 ± 17.9
Education level,low	607 (24.3%)
Education level, medium	1112 (44.6%)
Education level,high	746 (29.9%)
Farmchildhood	837 (33.6%)
Grown up in study area	1871 (75.0%)
Lung functioncharacteristics	
Pre-BD measurement % predicted (n=2,308)ǂ	
FEV_1	99.0 ± 15.4
FVC	102.9 ± 13.0
FEV_1/FVC	95.7 ± 8.7
MMEF	93.5 ± 32.8
Post-BD measurement % predicted (n=2,169)ǂ	
FEV_1	102.0 ± 14.5
FVC	102.9 ± 12.6
FEV_1/FVC	98.7 ± 8.4
MMEF	105.9 ± 35.1
Health characteristics (n=2,037ǂ, and subjects with atopy measurement n=2,443)	
Reversibility§	83 (4.1%)
COPD ‖	222 (10.9%)
Atopy	727 (29.8%)

Data are presented as mean ±SD or n (%).
*BMI: body mass index = mass (kg)/(height (m))^2
† Mean pack years for subjects who eversmoked.
ǂ Lung function measurements with quality C or better. Reversibility and COPD were analyzed in subjects with a pre AND post measurement with a sufficient quality (n=2,037).
§ Reversibility: a post-bronchodilator measurement with > 12% increase or > 400 ml
" COPD: a post bronchodilator (BD) measurement of FEV_1/FVC below the LLN AND/OR a post-BD measurement of FEV_1/FVC
<0.70 (GOLD).

Table 2. Comparison of characteristics of consenting versus non-consenting subjects

	Agreed to be contacted for follow-up study			Participated in medical examination		
	Yes	No	Adjusted OR (95% CI)	Yes	No	Adjusted OR (95% CI)
Subjects n	8714	5449		2494	4686	
Age, mean years (SD)*	51.1 (12.9)	49.8 (13.9)	**1.07 (1.04-1.10)**	54.7 (11.0)	49.1 (13.3)	**1.49 (1.43-1.56)**
Female %	53.0	54.7	0.94 (0.88-1.00)	54.6	52.2	**1.20 (1.08-1.32)**
Never smoker %	45.5	49.1	1	45.0	46.4	1
Ever smoker %	38.8	31.4	**1.28 (1.18-1.38)**	44.6	35.7	**1.20 (1.08-1.33)**
Current smoker %	15.4	17.4	0.94 (0.86-1.04)	10.1	17.7	0.97 (0.86-1.08)
Self-reported morbidity						
Current asthma %	5.9	4.3	**1.46 (1.24-1.71)**	4.9	5.9	0.94 (0.75-1.18)
Ever asthma %	7.7	6.3	**1.29 (1.12-1.48)**	6.3	8.0	0.90 (0.74-1.10)
COPD %	4.7	4.0	1.14 (0.96-1.35)	5.1	4.3	1.03 (0.81-1.30)
Nasal allergies %	24.3	19.0	**1.42 (1.30-1.54)**	24.6	23.7	**1.15 (1.02-1.30)**
Morbidity based on EMR						
n subject complete EMR	6859	4390		1936	3443	
Asthma (ICPC R96) %	7.2	6.1	**1.21 (1.03-1.41)**	5.9	6.9	0.88 (0.69-1.12)
COPD (ICPC R91 and/or R95) %	3.7	3.3	1.05 (0.85-1.30)	3.6	3.4	0.80 (0.59-1.10)
Allergic Rhinitis (ICPC R97) %	7.1	5.4	**1.40 (1.19-1.65)**	6.9	6.9	1.18 (0.95-1.50)
Exposure						
Mean distance to the nearest farm						
Any farm in meters (SD)†	487 (277)	493 (271)	0.99 (0.98-1.00)	439 (257)	486 (278)	**0.94 (0.92-0.95)**
Pig farm in meters (SD) †	767 (369)	775 (765)	0.99 (0.98-1.00)	692 (346)	763 (354)	**0.94 (0.93-0.95)**
Poultry farm in meters (SD) †	955 (456)	967 (457)	**0.99 (0.99-1.00)**	874 (409)	909 (413)	**0.98 (0.97-0.99)**
Cattle farm in meters (SD) †	587 (315)	598 (311)	**0.99 (0.98-1.00)**	503 (273)	552 (284)	**0.94 (0.92-0.95)**
Goat farm in meters (SD) †	1648 (473)	1642 (474)	1.00 (0.99-1.01)	1602 (504)	1635 (481)	**0.98 (0.97-0.99)**
Mink farm in meters (SD) †	1848 (370)	1841 (381)	1.00 (0.99-1.01)	1794 (426)	1847 (370)	**0.96 (0.95-0.98)**
Mean number of livestock farms						
Within 500 m radius (SD)	1.5 (1.9)	1.4 (1.8)	**1.03 (1.01-1.05)**	1.8 (2.1)	1.5 (1.8)	**1.10 (1.07-1.13)**
Within 1000 m radius (SD)	7.9 (5.5)	7.9 (5.6)	1.00 (0.99-1.01)	9.3 (5.9)	8.1 (5.6)	**1.04 (1.03-1.05)**
Modeled fine dust emission						
Weighted fine dust emission from farms within 500 m median‡ (Q1-Q3: 4*10^-4 - 1.65 g*year^{-1}*m^{-2})	0.02	0.01	**1.01 (1.00-1.02)**	0.07	0.02	**1.04 (1.03-1.05)**
Weighted fine dust emission from farms within 1000 m median‡ (Q1-Q3: 0.22 – 4.36 g*year-1*m-2)	1.17	1.18	1.00 (0.99-1.02)	1.83	1.33	**1.08 (1.06-1.10)**

The likelihood of agreeing to follow-up / being a participant is modeled for different characteristics with logistic regression. OR (95% CI) were adjusted for age, gender and smoking habits. Bold type indicates statistical significance ($p < 0.05$). In total 14,163 subjects responded to the short questionnaire(20), of which 8,714 agreed to be contacted for a follow-up study and 5,449 disagreed to be contacted. In total 7,180 subjects were invited for a medical examination, of which 2,494 participated and 4,686 were invited but did not participate. EMR: Electronic Medical Records; ICPC: International Classification of Primary Care.*OR (95% CI) for an increase per 10 year.† OR(95% CI) for an increase per 100 m.‡ OR(95% CI) for an unit increase in log-transformed exposure.

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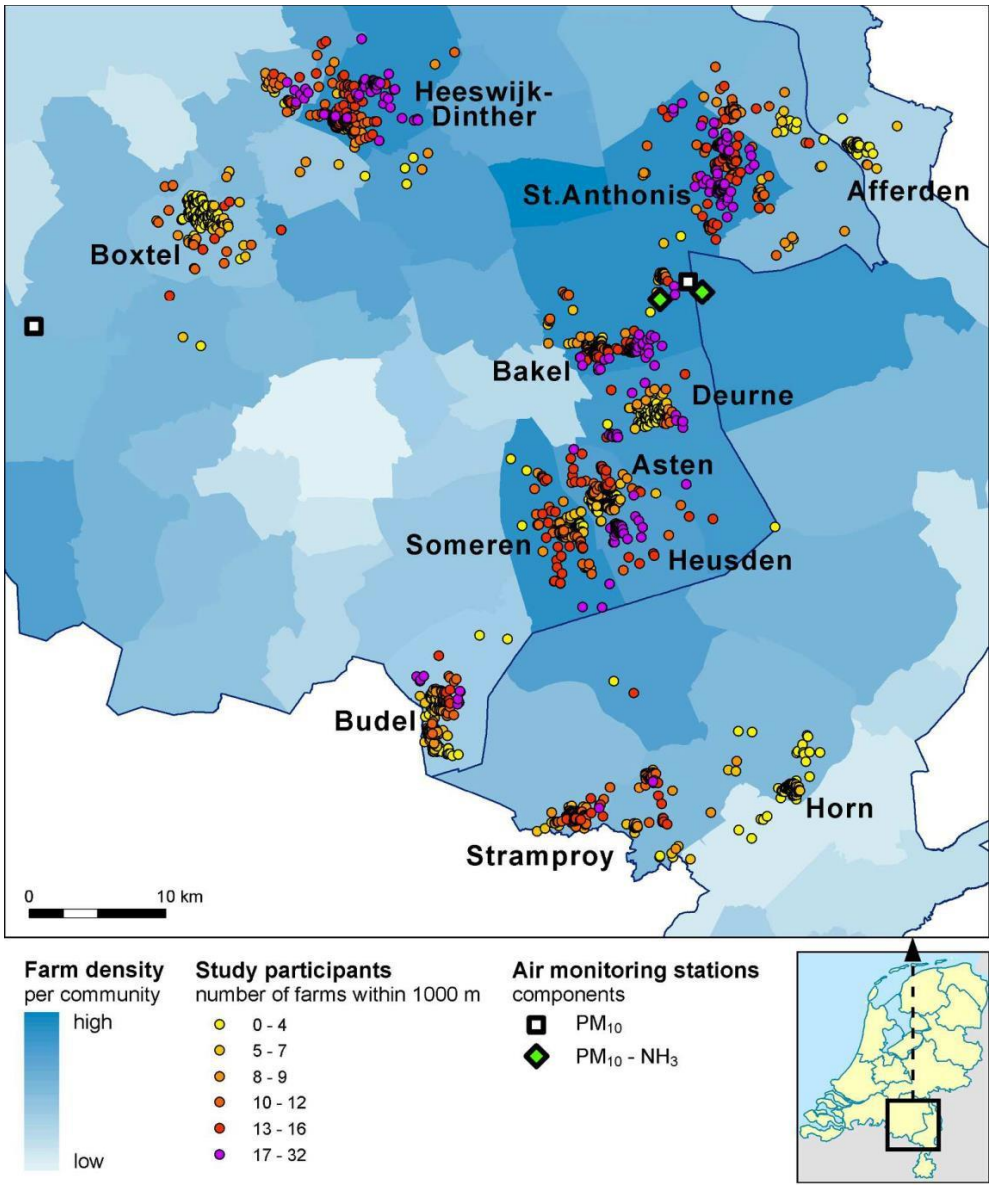
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10 km
Farm density
per community
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low
Study participants
number of farms within 1000 m
0 - 4
5 - 7
8 - 9
10 - 12
13 - 16
17 - 32
Air monitoring stations
components
PM10
PM10 - NH3

Figure 1. Map of the study area.

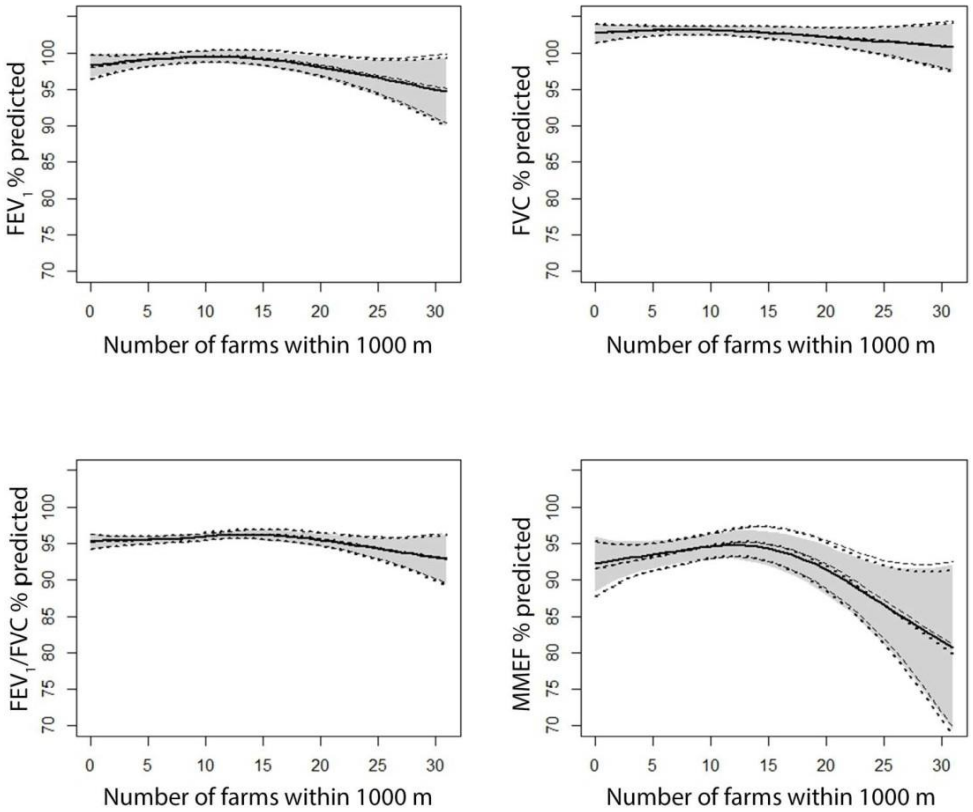

Figure 2. Association between the number of livestock farms within 1,000 m of the home address and lung function in 2,308 residents.

Smoothed plots show the association between the number of livestock farms within 1,000 m of the home address and lung function. P-values of the smooth terms are: FEV1:0.116; FVC:0.347; FEV1/FVC:0.114; MMEF: 0.045. Adjustment for age, gender and height was made by calculating % predicted spirometry variables based on GLI-reference values(27). Associations are also adjusted for smoking habits, being born in the study area and farm childhood. The striped lines show the results after further adjustment for week- average ambient NH3 (µg/m3) levels prior to the lung function test. P-values of the smooth terms after adjustment for week-average ambient NH3 levels are: FEV1: 0.142; FVC:0.355; FEV1/FVC:0.106; MMEF:0.051. The dotted lines show the models with further adjustment for week-average ambient PM10 (µg/m3) levels prior to the lung function test. P-values of the smooth terms after adjustment for week- average PM10 levels are: FEV1: 0.101; FVC:0.361; FEV1/FVC:0.081; MMEF:0.030.

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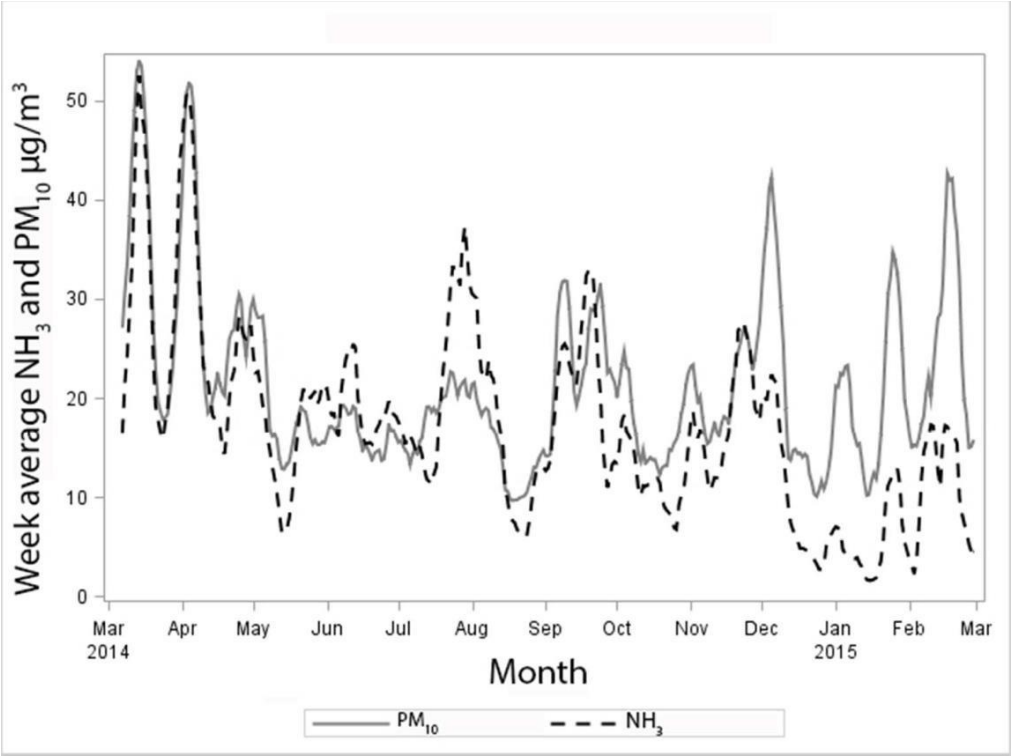

Figure 3. Week-average ambient NH3 and PM10 (µg/m3) levels in the study area.

Week-average ambient NH3 and PM10 concentrations were obtained from respectively two and four rural background monitoring stations located in the study area, which are part of the Dutch Air Quality Monitoring Network(28). Pearson's correlation between week-average ambient NH3 and PM10 levels was r =0.64.

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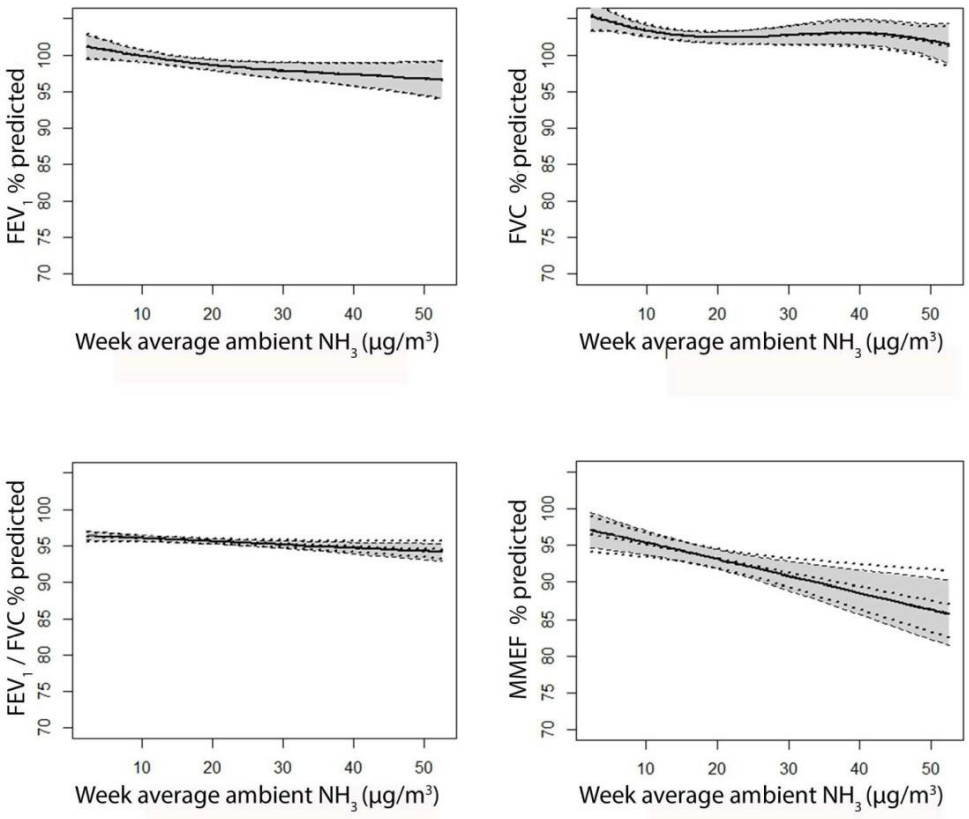

Figure 4. Associations between ambient NH3 (µg/m3) in the week before the lung function test and lung function in 2,308 residents.

Smoothed plots show the association between the week-average ambient NH3 (µg/m3) levels prior to the lung function test and lung function. P-values of the smooth terms are: FEV1: 0.012; FVC:0.098; FEV1/FVC:0.009; MMEF:<0.001. Adjustment for age, gender and height was made by calculating % predicted spirometry variables based on GLI-reference values(27). Associations are also adjusted for smoking habits, being born in the study area and farm childhood. The striped lines show the results after further adjustment for spatial exposure: n farms within 1,000 m of the home address. P-values of the smooth terms after adjustment for n farms within 1,000 m are: FEV1: 0.015; FVC:0.107; FEV1/FVC:0.008; MMEF:<0.001. The dotted lines show the models for further adjustment for week-average ambient PM10 (µg/m3) levels prior to the lung function test. P-values of the smooth terms after adjustment for week-average PM10 are: FEV1: 0.101; FVC:0.133; FEV1/FVC:0.196; MMEF:0.070.

179x161mm (300 x 300 DPI)

ONLINE SUPPLEMENT

Borlée, F., Yzermans, C.J., Aalders, B., Rooijackers, J., Krop, E., Maassen, C.B.M., Schellevis, F., Brunekreef, B., Heederik, D., Smit, L.A.M. Air pollution from livestock farms is associated with airway obstruction in neighboring residents. American Journal of Respiratory and Critical Care Medicine: 2017, 196(9), 1152-1161

Air pollution from livestock farms is associated with airway obstruction in neighboring residents

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METHODS

Study population and study design

The study was conducted in the eastern part of the province of Noord-Brabant and the northern part of Limburg, an area in the South of the Netherlands which is characterized by a high density of livestock farms. The study population originates from participants of a questionnaire survey previously described by Borlée et al[1]. In short, in 2012, a questionnaire survey was conducted among patients of 21 general practitioner practices. Patients were invited if they met the following criteria: 1) living in the eastern part of Noord-Brabant or the Northern part of Limburg; 2) inhabitant of a municipality of < 30,000 inhabitants and 3) aged between 18 and 70 years. Of the eligible patients, one person per home address was randomly selected. In total, 14,163 (53.4% response) adults responded. Questionnaire participants who gave consent for further contact for a follow-up study, and who were not working or living on a farm were eligible for a medical survey (n=8,714). Based on their home addresses, twelve temporary research centers were established. Between March 2014 and February 2015, all participants living within a distance of approximately 10 km of a temporary research center (n=7,180) were invited to the nearest research center for medical examination which resulted in 2,494 participants (response 34.7%). The medical examination consisted of a second and more extended questionnaire, length and weight measurements, a lung function measurement (pre- and post-bronchodilator (BD) spirometry) and collection of serum.
The study protocol (13/533) was approved by the Medical Ethical Committee of the University Medical Centre Utrecht. All 2,494 subjects signed informed consent. Patients' privacy was ensured by keeping medical information and address records separated at all times by using a Trusted Third Party.

Medical examination

Patient characteristics were collected with an extended questionnaire. The questionnaire comprised amongst others items on symptoms and diseases, smoking habits, education and profession. Atopy was defined as the presence of specific serum IgE antibodies to one or more common allergens and/or a total IgE higher than 100 IU/ml. Specific IgE to common allergens (house dust mite, grass, cat and dog) and total IgE levels were determined in serum with enzyme immunoassays as described before [2].

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Pre- and post-BD spirometry was conducted according to European Respiratory Society (ERS) guidelines and the European Community Respiratory Health Survey III (ECRHS-III) [3]. Pre-BD spirometry reflects the lung function of the study population without any effect of lung medication. Post-BD spirometry was used to assess reversibility and fixed lung obstruction. Participants stopped using inhalers and oral lung medication 4 and 8 hours prior to the lung function test, respectively. An EasyOne Spirometer (NDD Medical Technologies, Inc.) was used which measures flow and volume by ultra-sound transit time. After the pre-BD test, four puffs of short-acting beta-agonist (salbutamol, 100 µg per puff) were administered to the participant using a standard spacer. The post-BD measurement was performed at least 15 minutes after the lastly administered puff. To increase the quality of the spirometry data, we attempted to obtain four acceptable spirograms (pre- and post) per subject. The quality of all lung function curves were manually reviewed in NDD software by a specialist. The three best curves were selected or ranked manually when the best curves that were chosen by the NDD software program were not the best curves based on predefined ATS/ERS and NDD criteria (19). In total 97.8% of the participants who conducted a lung function test had a pre- and/or post-BD measurement with a quality of C or higher (quality C: at least two reproducible curves or reproducibility within 200 ml). Particulate air pollution is associated with worsening in asthma control and decreases in parameters of both large airways and small airways [4].
Large airway function is assessed with parameters: Forced Expiratory Volume in 1 second (FEV_1), Forced Vital Capacity (FVC) and FEV_1/FVC), small airway function is assessed with Maximum Mid-Expiratory Flow (MMEF). FEV_1, FVC, FEV_1/FVC and MMEF were converted into the percent of the predicted values based on the GLI-2012 reference equations (% predicted values)[5]. COPD was defined as a post-BD measurement of FEV1/FVC below the lower limit of normal AND/OR a post-BD measurement of FEV1/FVC <0.70 (GOLD) and 2) [6].

Spatial livestock farm exposure proxies

Spatial livestock farm exposure proxies were computed for each subject. We used data from the provincial databases of mandatory licenses for keeping livestock in 2012 which contained data on geographic coordinates of farms, number and type of animals, and estimated fine dust emissions from each farm per year on the basis of farm type and number of animals.
Geographic coordinates of the farms were based on the centroid of the barn(s). Table E1 shows an overview of the number of farms and farm animals present in the study area. Livestock farm proximity to the home address (geographic coordinates based on centroids of the home) was determined using a geographic information system (ArcGis 10.1; Esri, Redlands, CA, USA). The following livestock farm exposure proxies were studied for each subject: 1) total number of farms within 500 and 1000 m; 2) distance (m) to the nearest farm (general and specific animal types: pigs, poultry, cattle, goats and mink); 3) inverse-distance weighted fine dust emissions from all farms within 500 m and 1000 m as described previously[7]. In short, modelled fine dust (PM_{10}, g per year) for each farm in the study area was available from the license database. Emission from the farm was calculated by summing

the products of estimated PM_{10} emission factors (g per year per animal), and the number of animals per stable. To allow for atmospheric dispersion and dilution of PM_{10}, weighted dust emissions from all farms within 500 m and 1000 m from the home address was calculated by summing the products of the squared inverse of the distance between a farm and home address and the farm's fine dust emission (PM_{10}, g per year per m^2).

Temporal livestock farm exposure proxies

Week-average ambient NH_3 and PM_{10} levels prior to spirometry were calculated for each subject. Average daily ambient NH_3 and PM_{10} concentrations were obtained from respectively two (station 131 and 244), and four (station 131, 230, 243, 244) rural background monitoring stations located in the study area, which are part of the Dutch Air Quality Monitoring Network[8]. Week-average ambient NH_3 an PM10 concentrations were calculated by taking the average concentration measured by the respectively two and four monitoring stations.
Correlation between the monitoring stations was strong (Pearson's correlation NH_3 r = 0.69 and PM_{10} r $\geq$ 0.88). Correlation between week-average ambient NH_3 and PM_{10} levels was moderately strong (Pearson's r = 0.64).

Other data

Meteorological data

Meteorological data was obtained from a weather station from the Royal Netherlands Meteorological Institute which was centrally located in the study area (location Volkel)[9]. Week-average relative humidity and temperature (°C) prior to the lung function measurement were calculated.

Traffic-related air pollution data

For each subject exposure to NO_2, particulate matter (PM) with diameter of less than 2.5µm ($PM_{2.5}$) and $PM_{2.5}$ absorbance (soot) at home addresses were estimated using land-use regression models from the ESCAPE project (European Study of Cohorts for Air Pollution Effects) [10,11].

Self-reported respiratory health

Information on self-reported respiratory health, age, gender and smoking habits was assessed with the first (short) questionnaire collected in November 2012 as described previously[1]. The definitions used for self-reported respiratory morbidity are presented in Table E2.

Electronic Medical Records

Electronic Medical Records (EMR) were available through the General Practitioners who all participated in the Netherlands Institute for Health Services Research (NIVEL) Primary Care Database[12]. The practice had to meet the following EMR quality criteria: 1) record diagnostic information in the patients' EMR using the International Classification of Primary Care ICPC,
2) assign ICPC-codes to at least 70% of the morbidity records, and 3) record morbidity data at least 46 weeks per year. In addition, patients had to be registered at the GP for at least three-quarters of the year 2012. All subjects included in the data analysis gave written permission to link their study data to their EMR. For the majority of the source population, EMR data were available that met the quality criteria.

Statistical analysis

Potential selection bias was studied by comparing characteristics of the source population *versus* subjects who agreed to be contacted for a follow-up study, and participants of the medical examination *versus* subjects who were invited but who did not participate. The likelihood of agreeing to follow-up, or being a participant was modeled for different characteristics with logistic regression. In order to study the effect of potential selection bias, we compared associations between different exposure estimates and respiratory health (self- reported) in different subpopulations. Both non-response analyses were adjusted for age, gender and smoking habits.
The relationship between the four pre-BD spirometry variables and livestock farm exposure variables (both spatial and temporal) were studied using a penalized regression spline using the (default) "thin plate" basis as implemented in the mgcv (mixed generalized additive model computation vehicle) R package. Based on the results of the spline analyses, exposure cut-off values were created. Multiple linear regression analyses was used to study the created exposure cut-off values and pre-BD spirometry variables. Potential confounders were selected beforehand. Associations between the four pre-BD spirometry variables and potential confounders were studied with multivariable linear regression modelling following a forward stepwise procedure based on improvement of Akaike's Information Criterion (AIC).
Consequently, all models were adjusted for smoking habits (ever smoked and number of pack years (number of pack years was mean-centered for ex- and current smokers)), farm childhood, and growing up in the study area.
For analyses with spirometry and average daily ambient NH_3 and PM_{10} concentrations, lagged relationships ranging from 0, 1 and 2 days and week-average prior to the spirometry measurement were investigated with multiple linear regression analyses. Analyses of varying lag days of ambient NH_3 and PM_{10} levels showed that week-average NH_3 and PM_{10} levels prior to spirometry were most strongly associated with lung function variables (see Figure E3A and B online supplement). Week-average NH_3 and PM_{10} levels were therefore chosen as proxies for temporal livestock exposure.
Sensitivity analyses were conducted to investigate the effect of fieldworker, influenza-season (month with influenza epidemic > 50 patients per 10.000 patients[13]), relative humidity and temperature. In addition, analyses were stratified

for atopy, COPD, asthma, and smoking habits and interaction between these groups and exposure variables were tested.

Data were analyzed using SAS 9.4 (SAS Institute Inc. Cary, NC, USA) and R version 3.02 (www.r-project.org). Figures were created with SigmaPlot 13.

RESULTS

Non-response analyses

Subjects who agreed to participate were slightly older (mean age 51.1 *vs.* 49.8 years) and more often ever smokers (38.8% *vs.* 31.4%), compared to subjects who disagreed to be contacted for a follow-up study (Table 2 main text). They had more often asthma and nasal allergies both based on self-reported data and EMR data. Indicators of exposure to livestock farms were slightly higher among subjects who agreed to follow-up compared to subjects who disagreed: they lived closer to poultry and cattle farms, the number of farms and the weighted fine dust emission within 500 m was slightly higher. Subjects who participated in the medical examination were older (mean age 54.7 *vs.* 49.1 years), more often female (54.6% *vs.* 52.2%) and more often former smokers (44.6% vs. 35.7%) compared to subjects who were invited but did not participate. Only prevalence of self-reported nasal allergies was higher among participants. Participants lived closer to livestock farms compared to subject who were invited but who did not participate. Also the number of farms and modeled fine dust emission was higher within 500 and 1000 meter of the homes address. Overall, selection bias did not seem to affect associations between farm exposure estimates and respiratory morbidity based on self-reported data (online supplementary table E3). Associations in the source population, the population who agreed to follow-up, total invited population for medical examination and participants in the medical examination showed a similar direction and magnitude with overlapping confidence intervals.

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ONLINE FIGURES AND TABLES

Table E1. Overview of the number of farms and farm animals in the study area.

			Number of animals inquartiles			
	N farms	**Mean nr ofanimals per farm**	**Q1**	**Q2**	**Q3**	**Q4**
Total pig farms	2569	2659	26-912	913-1892	1893-3692	3693-22392
Piglets	56	4117	77-1008	1009-2827	2828-5774	5775-22392
Breeding boars	14	231	30-96	97-224	225-270	271-920
Sows	988	988	28-1017	1018-1857	1858-3525	3526-17512
Fattening pigs	1511	2659	26-821	822-1920	1921-3804	3805-20616
Total poultry farms	768	76718	371-18000	18001-45070	45071-105500	105501-813312
Laying hens	357	98679	375-25115	25116-60000	60001-132380	132381-813312
Broiler chickens	354	63879	1467-20000	20001-40000	40001-93000	93001-381700
Other poultry	57	18908	371-5750	5751-15000	15001-27000	27001-73250
Total cattle farms	4043	205	6-80	81-145	146-240	241-4849
Dairy cattle	3469	168	6-80	81-140	141-219	220-3485
Beef cattle	574	430	8-80	81-228	229-600	601-4849
Total goat farms	72	1681	60-781	782-1514	1515-2369	2370-5700
Total mink farms	110	5700	900-2999	3000-4972	4973-8250	8251-17950

Main farm categories as registered in the livestock database are presented. The minimum amount of animals that has to be registered in the licence database is: 50 goats, 250 chickens, 25 pigs and 5 cows. The study area was defined as

the eastern part of the province of Noord-Brabant and the northern part of the province of Limburg (postcode starting with 52-60), and municipalities with more than 30,000 inhabitants were excluded.

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Table E2. Definitions of self-reported respiratory morbidity based on questions adopted from the ECRHS-III screening questionnaire[14].

Self-reported respiratory morbidity	**Definition**
Current asthma (described before by de Marco et al.[15])	Self-reported ever asthma AND either one or more asthma-like symptoms (wheezing/whistling in the chest, chest tightness, shortness of breath at rest/following strenuous activity/at night-time or asthma attacks) AND/OR use of inhaled or oral medication for breathing problems in the last year
Ever asthma	A positive answer to the following question: 'Did you ever had asthma?'
COPD	A positive answer to the following question: 'Have you ever been told by a doctor that you had chronic obstructive pulmonary disease or emphysema?'
Nasal allergies	A positive answer to the following question: 'Do you have any nasal allergies including 'hay fever'?'

Table E3. Associations between different farm exposure estimates and indicators of respiratory health compared between different population subsets to study potential selection bias.

		Source population (n=14,163)	**Subjects who agreed to follow-up (n=8,714)**	**Subjects invited for medical examination (n=7,180)**	**Subjects who participated in medical examination (n=2,494)**
		OR (95% CI) adjusted	**OR (95% CI) adjusted**	**OR (95% CI) adjusted**	**OR (95% CI) adjusted**
Current asthma	**Presence of livestock farms (yes or no)**				
	Within 100 m	0.63 (0.40-1.01)	0.60 (0.34-1.05)	0.64 (0.36-1.15)	0.96 (0.44-2.10)
	Within 500 m	**0.83 (0.72-0.97)**	**0.82 (0.69-0.98)**	0.88 (0.72-1.08)	0.94 (0.64-1.38)
	Within 1000 m	0.77 (0.58-1.02)	0.85 (0.60-1.21)	0.81 (0.53-1.23)	0.66 (0.30-1.47)
	Presence of farm animals within 500 m (yes or no)				
	Pig	0.84 (0.70-1.01)	0.84 (0.68-1.04)	0.89 (0.70-1.13)	0.94 (0.63-1.41)
	Poultry	0.94 (0.76-1.17)	0.88 (0.68-1.14)	0.89 (0.67-1.18)	0.94 (0.58-1.55)
	Cattle	**0.83 (0.71-0.96)**	**0.78 (0.65-0.94)**	0.87 (0.71-1.07)	0.89 (0.61-1.28)
	Goat	0.91 (0.55-1.52)	0.57 (0.27-1.23)	0.55 (0.24-1.26)	1.44 (0.57-3.64)
	Mink	1.04 (0.56-1.92)	0.73 (0.30-1.80)	0.65 (0.24-1.78)	0.43 (0.06-3.13)
	Minimal distance to the nearest farm				
	Distance per 100 m	**1.05 (1.02-1.08)**	**1.04 (1.01-1.07)**	**1.04 (1.00-1.08)**	1.05 (0.98-1.12)
	Number of farms				
	Within 500 m	**0.94 (0.90-0.98)**	**0.93 (0.88-0.98)**	0.96 (0.90-1.01)	0.98 (0.89-1.07)
	Within 1000 m	**0.98 (0.96-0.99)**	**0.98 (0.96-0.99)**	**0.98 (0.96-1.00)**	0.97 (0.94-1.01)
Ever asthma	**Presence of livestock farms (yes or no)**				
	Within 100 m	0.71 (0.48-1.04)	0.68 (0.42-1.08)	0.73 (0.45-1.18)	0.95 (0.48-1.92)
	Within 500 m	0.91 (0.79-1.03)	0.91 (0.77-1.07)	0.96 (0.80-1.15)	0.90 (0.64-1.26)

	Within 1000 m	0.80 (0.62-1.03)	0.95 (0.69-1.33)	0.90 (0.61-1.33)	0.73 (0.35-1.55)
	Presence of farm animals within 500 m (yes or no)				
	Pig	0.89 (0.76-1.03)	0.92 (0.76-1.11)	0.96 (0.78-1.18)	0.94 (0.66-1.35)
	Poultry	1.00 (0.84-1.20)	0.92 (0.73-1.15)	0.95 (0.74-1.21)	0.96 (0.62-1.49)
	Cattle	0.90 (0.79-1.03)	0.88 (0.75-1.03)	0.95 (0.79-1.13)	0.90 (0.65-1.26)
	Goat	1.01 (0.66-1.54)	0.82 (0.47-1.46)	0.83 (0.46-1.51)	1.32 (0.56-3.10)
	Mink	1.11 (0.66-1.85)	0.89 (0.43-1.85)	0.86 (0.40-1.86)	0.32 (0.04-2.35)
	Minimal distance to the nearest farm				
	Distance per 100 m	**1.03 (1.01-1.05)**	1.02 (0.99-1.05)	1.02 (0.99-1.05)	1.04 (0.98-1.11)
	Number of farms				
	Within 500 m	0.97 (0.94-1.01)	0.96 (0.92-1.00)	0.98 (0.94-1.03)	0.96 (0.89-1.04)
	Within 1000 m	**0.99 (0.98-1.00)**	**0.98 (0.97-1.00)**	0.99 (0.97-1.00)	**0.97 (0.94-1.00)**

Continuation Table E3)

		Source population (n=14,163)	**Subjects who agreed to follow-up (n=8,714)**	**Subjects invited for medical examination (n=7,180)**	**Subjects who participated in medical examination (n=2,494)**
		OR (95% CI) adjusted	**OR (95% CI) adjusted**	**OR (95% CI) adjusted**	**OR (95% CI) adjusted**
COPD	**Presence of livestock farms (yes or no)**				
	Within 100 m	**0.56 (0.32-0.98)**	0.66 (0.36-1.22)	0.60 (0.30-1.17)	0.48 (0.17-1.31)
	Within 500 m	0.92 (0.78-1.09)	0.95 (0.77-1.16)	0.99 (0.79-1.25)	0.79 (0.55-1.14)
	Within 1000 m	0.74 (0.54-1.01)	0.98 (0.65-1.49)	0.97 (0.59-1.59)	0.45 (0.23-0.87)
	Presence of farm animals within 500 m (yes or no)				
	Pig	0.97 (0.80-1.18)	0.97 (0.76-1.23)	1.00 (0.77-1.30)	0.84 (0.56-1.27)
	Poultry	1.11 (0.88-1.39)	1.13 (0.85-1.49)	1.17 (0.87-1.58)	1.38 (0.89-2.15)
	Cattle	0.90 (0.76-1.06)	0.87 (0.71-1.07)	0.91 (0.73-1.14)	0.78 (0.55-1.12)
	Goat	0.99 (0.58-1.68)	0.79 (0.39-1.63)	0.64 (0.28-1.46)	1.21 (0.47-3.08)
	Mink	0.55 (0.22-1.35)	0.33 (0.08-1.33)	0.35 (0.09-1.42)	0.30 (0.04-2.20)
	Minimal distance to the nearest farm				
	Distance per 100 m	**1.04 (1.01-1.07)**	1.02 (0.98-1.05)	1.02 (0.98-1.06)	1.09 (1.02-1.16)
	Number of farms				
	Within 500 m	0.99 (0.94-1.03)	0.98 (0.92-1.04)	0.99 (0.93-1.05)	0.97 (0.89-1.06)
	Within 1000 m	0.99 (0.98-1.01)	1.00 (0.98-1.01)	1.00 (0.98-1.02)	0.98 (0.95-1.01)
Nasal allergies	**Presence of livestock farms (yes or no)**				
	Within 100 m	**0.76 (0.61-0.95)**	0.80 (0.62-1.05)	0.84 (0.64-1.11)	**0.65 (0.43-1.00)**
	Within 500 m	0.97 (0.90-1.05)	0.97 (0.88-1.08)	0.98 (0.88-1.10)	0.90 (0.74-1.09)
	Within 1000 m	0.90 (0.76-1.06)	0.93 (0.76-1.14)	0.98 (0.77-1.26)	0.93 (0.59-1.47)
	Presence of farm animals within 500 m (yes or no)				
	Pig	0.98 (0.89-1.07)	0.96 (0.85-1.07)	0.94 (0.83-1.06)	0.98 (0.80-1.20)
	Poultry	0.97 (0.87-1.09)	0.95 (0.83-1.09)	1.01 (0.87-1.17)	1.03 (0.81-1.31)
	Cattle	0.93 (0.86-1.01)	0.95 (0.86-1.05)	0.96 (0.86-1.07)	0.89 (0.74-1.07)
	Goat	1.05 (0.81-1.36)	1.02 (0.74-1.41)	1.07 (0.77-1.49)	0.99 (0.58-1.67)

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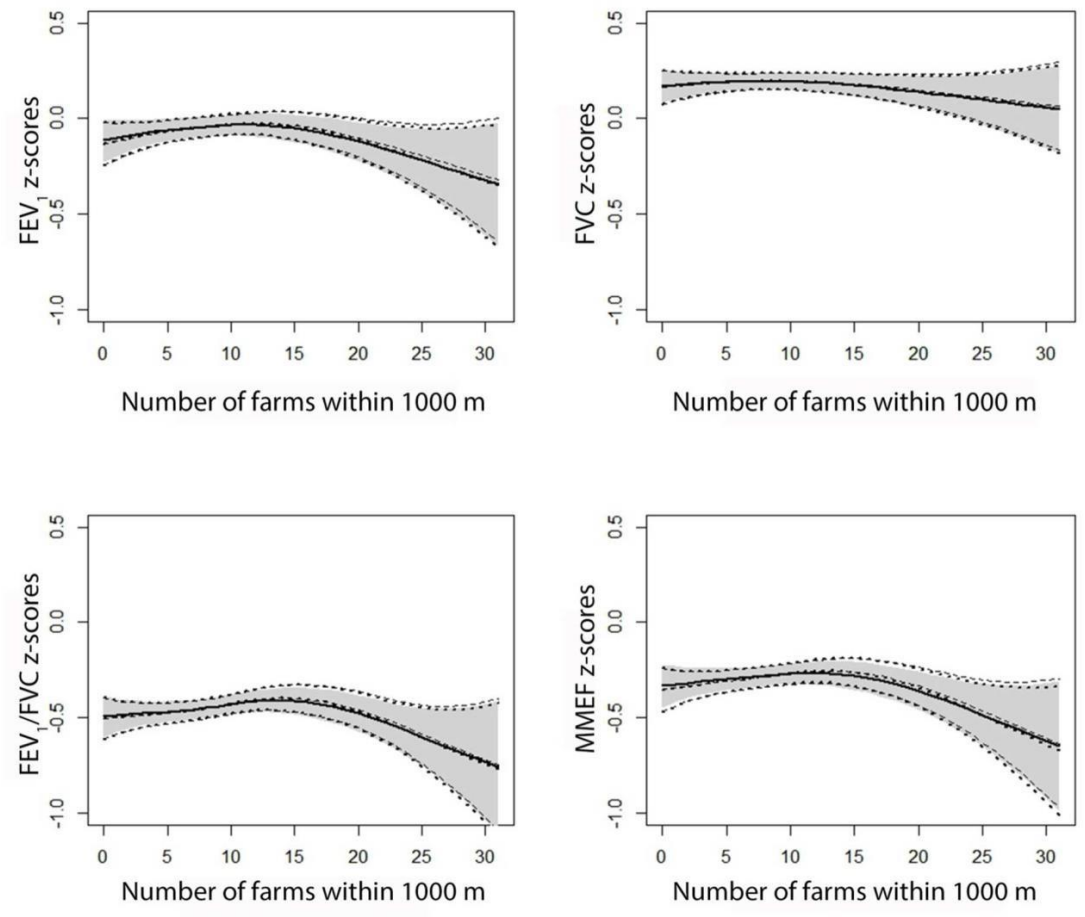
	Mink	1.29 (0.94-1.77)	**1.59 (1.08-2.35)**	**1.71 (1.15-2.55)**	1.17 (0.62-2.23)
	Minimal distance to the nearest farm				
	Distance per 100 m	**1.02 (1.00-1.03)**	1.02 (1.00-1.04)	1.01 (0.99-1.03)	1.03 (0.99-1.06)
	Number of farms				
	Within 500 m	0.98 (0.96-1.01)	0.98 (0.95-1.01)	0.99 (0.96-1.01)	0.99 (0.95-1.04)
	Within 1000 m	0.99 (0.99-1.00)	0.99 (0.98-1.00)	0.99 (0.98-1.00)	1.00 (0.98-1.01)

OR and 95% CI were adjusted for age, gender and smoking habits. Bold type indicates statistical significance ($p < 0.05$). Information on self-reported respiratory health, age, gender and smoking habits was assessed with the first questionnaire collected in November 2012 as described previously[1]. The definitions used for self-reported respiratory morbidity are presented in table E2.

Figure E1. Association between the number of livestock farms within 1,000 m of the home address and lung function expressed as z-scores in 2,308 residents.



Smoothed plots show the association between the number of livestock farms within 1,000 m of the home address and lung function. P-values of the smooth terms are: FEV_1:0.108; FVC:0.402; FEV_1/FVC:0.140; MMEF: 0.073. Adjustment for age, gender and height was made by calculating % predicted spirometry variables based on GLI-reference values [5]. Associations are also adjusted for smoking habits, being born in the study area and farm childhood. The striped lines show the results after further adjustment for week-average ambient NH (µg/m^3) levels prior to the lung function test. P-values of the
smooth terms after adjustment for week-average ambient NH_3 levels are: FEV_1: 0.115; FVC:0.476; FEV_1/FVC:0.115; MMEF:0.111. The dotted lines show the models with further adjustment for week-average ambient PM (µg/m^3) levels

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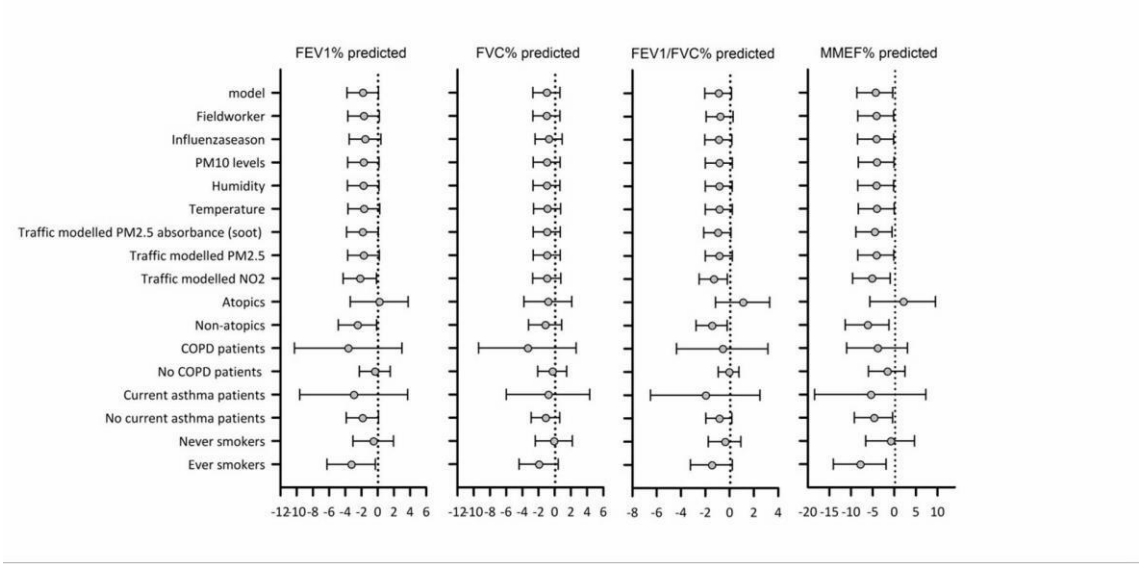
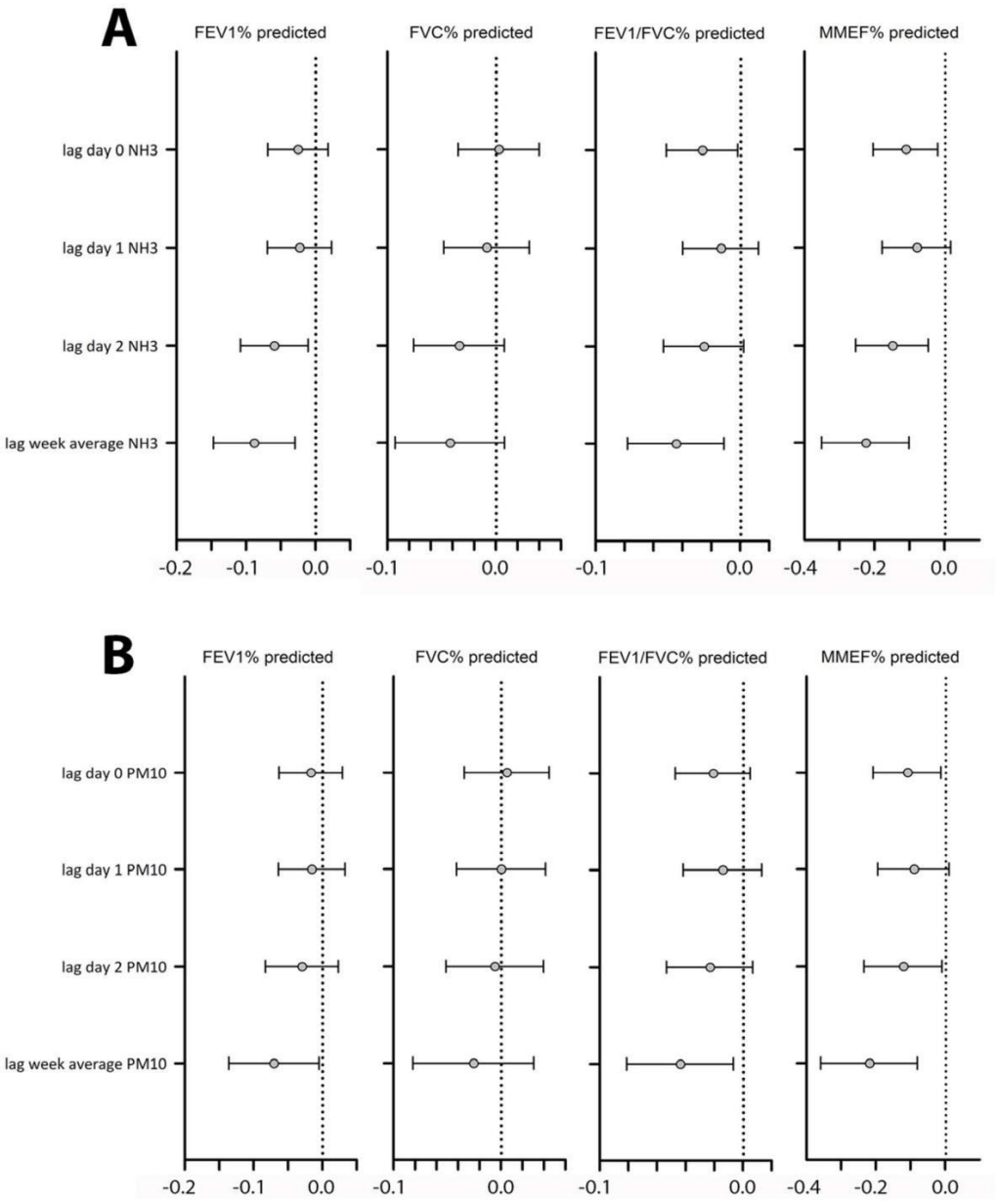
prior to the lung function test. P-values of the smooth terms after adjustment for week-average PM_{10} levels are: FEV_1: 0.085; FVC:0.411; FEV_1/FVC:0.089; MMEF:0.068.

Figure E2. Sensitivity analyses on 'Hotspot model', i.e. ≥17 farms versus <17 farms within 1,000 m.



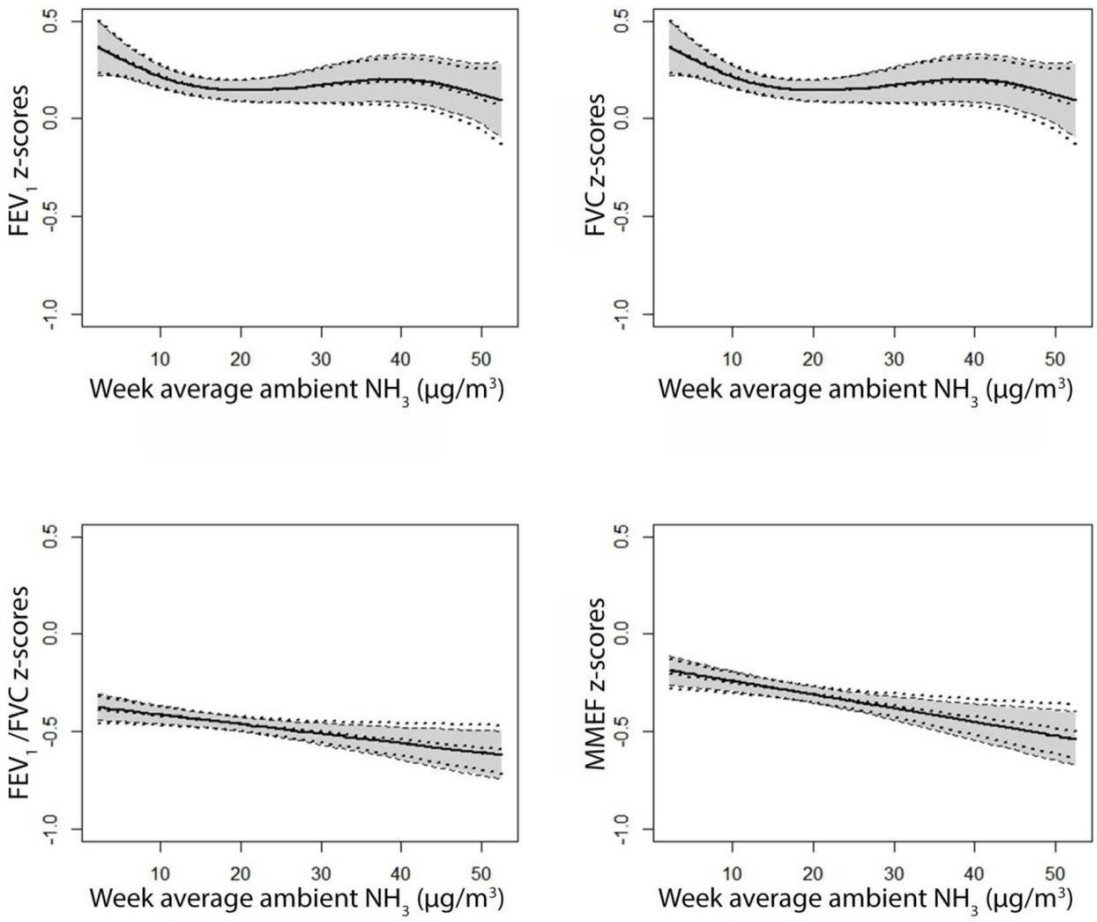
The association between living in a 'hotspot' and lung function variables, and additional sensitivity analyses (additional adjustment and analyses in subgroups) were analyzed with linear regression analyses. Adjustment for age, gender and height was made by calculating % predicted spirometry variables based on GLI-reference values[5]. All associations are also adjusted for smoking habits, being born in the study area and farm childhood. Living in a 'hotspot' ('model') was associated with a change in FEV_1 of -1.86 (95%CI -3.80 to 0.09), FVC: -1.03 (95%CI -2.70 to 0.64), FEV_1/FVC: -0.94 (95%CI -2.05 to -0.16)
and MMEF%: -4.50 (95%CI -8.64 to -0.36). A significant interaction term was observed between atopy and 'hotspot' in models with lung function variables FEV_1/FVC (p=0.03) and MMEF (p=0.02).

Figure E3. Association between lung function variables and ambient NH_3 and PM_{10} levels on multiple lag days.



Associations are presented for a 1 µg/m^3 increase of NH (A) or PM (B).

Figure E4. Associations between ambient NH_3 (µg/m^3) in the week before the lung function test and lung function expressed as z-scores in 2308 residents.

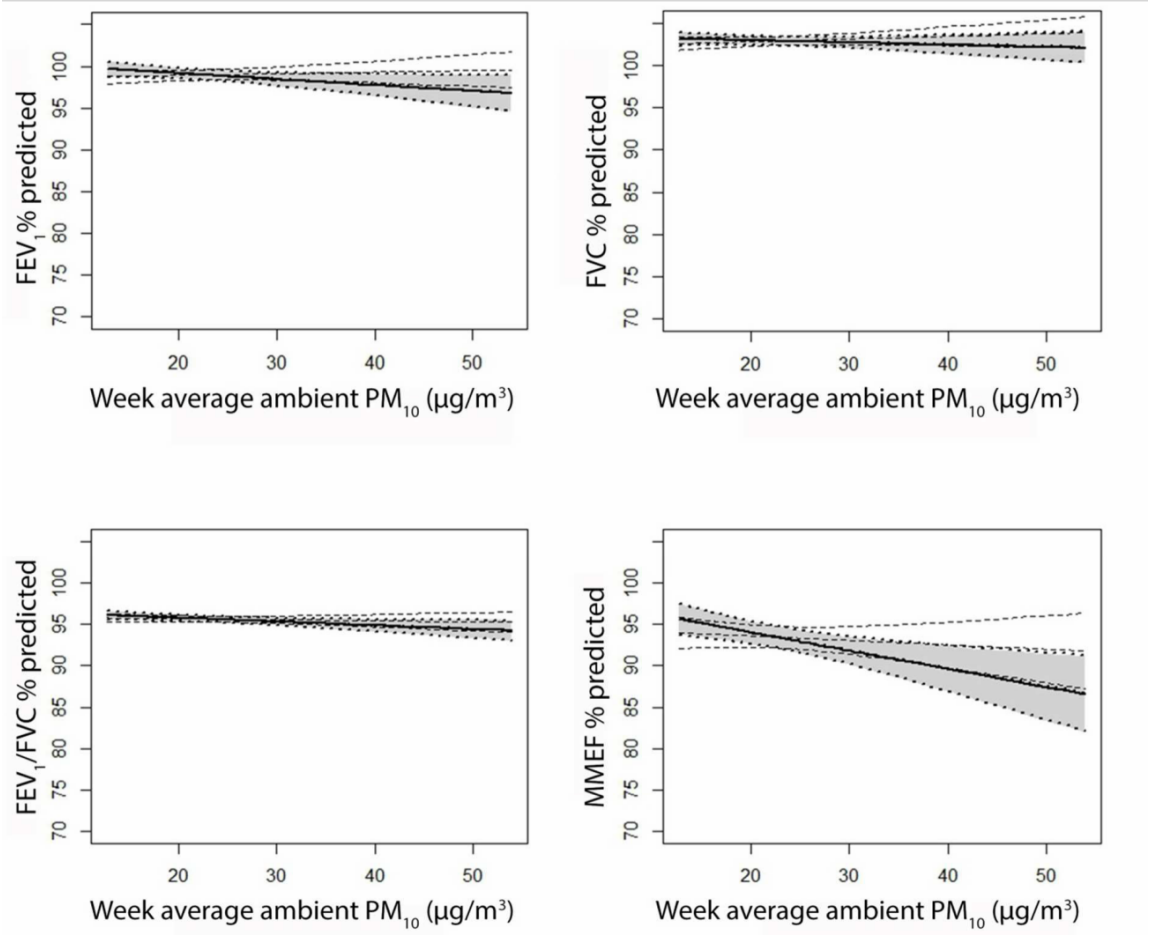


Smoothed plots show the association between the week-average ambient NH_3 (µg/m^3) levels prior to the lung function test and lung function. P-values of the smooth terms are: FEV_1: 0.012; FVC:0.062; FEV_1/FVC:0.007; MMEF:<0.001. Adjustment for age, gender and height was made by calculating % predicted spirometry variables based on GLI-reference values[5].

Associations are also adjusted for smoking habits, being born in the study area and farm childhood. The striped lines show the results after further adjustment for spatial exposure: n farms within 1,000 m of the home address. P-values of the

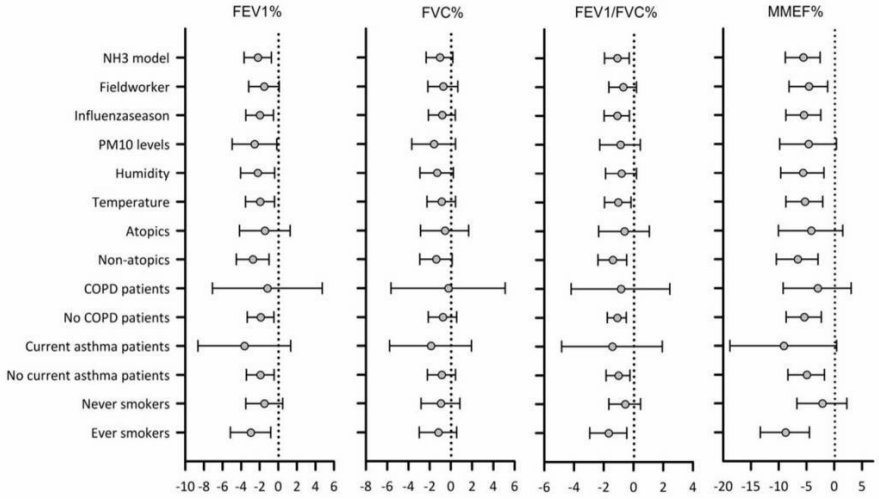
smooth terms after adjustment for n farms within 1,000 m are: FEV_1: 0.142; FVC:0.074; FEV_1/FVC:0.007; MMEF:<0.001. The dotted lines show the models for further adjustment for week-average ambient PM (µg/m^3) levels prior to the lung function test. P-values of the smooth terms after adjustment for week-average PM_{10} are: FEV_1: 0.040; FVC:0.082; FEV_1/FVC:0.167; MMEF:0.073.

Figure E5. Associations between ambient PM_{10} (µg/m^3) in the week before the lung function test and lung function in 2,308 residents.



Smoothed plots show the association between the week-average ambient PM (µg/m^3) levels prior to the lung function test and lung function. P-values of the smooth terms are: FEV_1: 0.035; FVC:0.371; FEV_1/FVC:0.021; MMEF:0.002. Adjustment for age, gender and height was made by calculating % predicted spirometry variables based on GLI-reference values[5]. Associations are also adjusted for smoking habits, being born in the study area and farm childhood. The dotted lines show the results after further adjustment for spatial exposure: n farms within 1,000 m of the home address. P-values of the smooth terms after adjustment for n farms within 1,000 m are: FEV_1:0.043; FVC:0.440; FEV_1/FVC:0.020; MMEF:0.003. The striped lines show the models for further adjustment for week-average ambient NH (µg/m^3) prior to the lung function test. P-values of the smooth terms after adjustment for week-average NH_3 are: FEV_1: 0.718; FVC:0.500; FEV_1/FVC:0.694; MMEF:0.645.

Figure E6. Sensitivity analyses on NH_3 model.



The association between week-average ambient NH_3 levels and lung function, and additional sensitivity analyses (additional adjustment and analyses in subgroups) were analyzed with linear regression analyses. Adjustment for age, gender and height was made by calculating % predicted spirometry variables based on GLI-reference values[5]. All associations are also
adjusted for smoking habits, being born in the study area and farm childhood. Results of the NH_3 model are expressed per P90-P10 increase in week-average NH_3 (P90-P10 NH_3 = 25.1 $\mu g/m^3$). NH_3 ('NH model') was associated with a change in
FEV_1 of -2.22 (95%CI -3.69 to -0.74), FVC: -1.07 (95%CI -2.33 to 0.20), FEV_1/FVC: -1.12 (95%CI -1.96 to -0.28) and MMEF: -
5.67 (95%CI -8.80 to -2.55). No significant interactions between specific subgroups and week-average ambient NH_3 levels were observed.