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Respiratory health effects of livestock farm emissions in neighbouring residents

Respiratoire gezondheidseffecten van de emissies van veehouderijen bij omwonenden
(met een samenvatting in het Nederlands)

Proefschrift

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Contents

Chapter 1

Introduction

This thesis presents research on living near livestock farms and associated respiratory health effects in local residents. The research was conducted as part of the Livestock Farming and Neighbouring Residents' Health Study (in Dutch: Veehouderij en Gezondheid Omwonenden, the VGO study). First, this introductory chapter will give a short overview of livestock farming in the Netherlands, farm emissions, and potential health risks for neighbouring residents. Then, adverse respiratory health effects due to occupational exposure in farmers will be discussed and an overview will be given of the scientific literature on respiratory health of residents living in close proximity to livestock farms. The knowledge gaps and research needs identified from the previous studies in neighbouring residents lead to the aim of the research described in this thesis. Thereafter, a short description will be given of the VGO study design, followed by an outline of this thesis.

Livestock farming in the Netherlands

The Netherlands has one of the highest population densities and also one of the highest farm and farm animal densities worldwide. On a surface area of 41,000 km^2, 17 million people live together with 107 million chickens, 12 million pigs, 4 million cows and 1.5 million goats and sheep[1]. In total 355,000 houses and 27,000 livestock farms are located within 250 m of each other[2]. During the last decades, the total number of farms has been declining but the number of farm animals is, depending on the species, either stable or still increasing[3]. The number of large-scale, intensive animal farms is growing.

Recent studies have highlighted the large contribution of agriculture to particulate matter (PM) air pollution[4,5]. Livestock farms diminish the air quality in surrounding areas by emitting particulate matter (PM), (parts of) microorganisms, allergens and (malodorous) gases such as ammonia (NH_3) and hydrogen sulfide (H_2S)[6]. The main sources of primary, directly emitted PM consist of organic material such as manure, uric acid, feathers, bedding material, animal feed, skin flakes, hair, wood shavings, straw and silage[7]. Emission from precursor gasses that form secondary inorganic aerosols contributes highly to atmospheric $PM_{2.5}$ concentrations. Ammonia is such a precursor gas, it is formed by enzymes in animal waste and reacts in the atmosphere to form ammonium nitrate and sulphate aerosols.

People living at short distances from livestock farms are potentially exposed to air pollutants from livestock farms and may be at risk for negative health effects. Previous studies have measured elevated endotoxin levels – the major component of the outer membrane of Gram-negative bacteria – were elevated up to 200 meters downwind from farms[8-10]. A study in the Netherlands has estimated that in areas with a high farm density, farms contribute 16-21% to the atmospheric PM concentrations[11]. The annual mean ammonia level in the Netherlands was 7 μg/m^3, but in areas with a high livestock farm density it was substantially higher (11.9 -39.9 μg/m^3)[11]. Potential health risks for people living in surrounding areas include emerging zoonotic diseases such as avian influenza and Q-fever, and infection with antimicrobial resistant bacteria, but also respiratory and cardiovascular health effects due to air pollutant emissions[6,12-18]. This thesis focusses on respiratory health risks for people living in close proximity to livestock farms.

Respiratory health risks among farmers

Most information on potentially adverse health effects from animal farm exposures comes from working populations. Donham and co-workers were the first to systematically study respiratory health of workers in swine confinement buildings in the 70s[19]. Since that time numerous studies have investigated respiratory symptoms and pulmonary function in livestock farm workers[20-22]. Working in agriculture – and especially when working in livestock farms – poses a serious risk for development of respiratory diseases. It was estimated that 20-40% of farm workers in beef or pork production (based on studies from the United States of America (USA) and Europe) report respiratory symptoms such as wheeze, cough and dyspnoea[20]. Among European farmers, pig farmers most frequently reported work-related respiratory symptoms[23] compared to cattle-, poultry- and sheep farmers. A dose-response association was observed between the number of daily working hours in stables and respiratory symptoms among pig and poultry farmers[23]. These symptoms are frequently nonspecific and might reflect acute airway irritation as well as symptoms associated with respiratory diseases. Previous studies have demonstrated short-term lung function decline (after a work shift)[24], increase of bronchial responsiveness[25] and an accelerated decline in lung function over a period of several years[26,27]. Asthma, chronic obstructive pulmonary disease (COPD, defined as self-reported chronic bronchitis or (fixed) airway obstruction) and long-term lung function decline (long-term exposure) have all been reported in relation to livestock farming[20,28-32]. The resultant respiratory disease is mostly non-IgE mediated and marked by neutrophilic influx[33].

To better understand the pathophysiology of these negative effects of livestock farm exposures, experimental studies have exposed healthy non-farming subjects to swine or poultry dust in livestock stables for a couple of hours.

These studies were designed to mimic the first exposure of new workers to the indoor livestock farming environment and the resulting acute airway and systemic responses. Exposure to a swine confinement building led to an inflammatory reaction and caused various symptoms like fever, headache and malaise, upper and lower airway inflammation and increased exhaled nitric oxide and bronchial responsiveness to methacholine[34-36].

Increased levels of neutrophils, lymphocytes, macrophages, eosinophils and pro-inflammatory cytokines like interleukin 1, 6 and 8 (IL-1, IL-6 and IL-8) and tumour necrosis factor-α were observed in bronchoalveolar and nasal lavage fluid after exposure to pig stables[35,37]. These experiments indicate that exposure to swine dust is capable of causing intense upper and lower airway inflammation. Symptoms like fever, headache, joint pain and general malaise could be explained by a systemic response at very high exposure levels as demonstrated by increased levels (IL-6) and TNFα in peripheral blood[38]. Experimental exposure of naive subjects in poultry confinement buildings leads to a similar inflammatory reaction[39]. Naive volunteers had a stronger airway reaction to acute exposure to a swine facility compared to swine farmers, indicating adaptation in chronically exposed farmers[40,41]. Non-naive volunteers who had previously worked with swine but not worked with pigs during previous years, showed a reduction in serum TNFα after 3 hours of swine dust exposure[42]. TNFα remained decreased until two weeks after exposure. TNFα is a cytokine involved in systemic inflammation, a decrease in TNFα indicates immune suppression.

Paradoxically, it has also been suggested that exposure to a farming environment might protect against the development of atopy and atopic asthma[23]. Children growing up on farms are less likely to develop allergic diseases than children living in the same area but with non-farming parents[43,44]. This effect is still detectable in adulthood[45-48]. A few studies suggest that not only exposure during early life is protecting, but occupational farm exposure during adulthood may also provide long-lasting protection against atopic sensitization[49-52]. It has been postulated that exposure to a greater microbial diversity during early life, but possibly also during adulthood, prevents the development of allergic diseases[53,54]. Previous studies showed that farming exposures were associated with a decreased risk of atopic asthma, while the same exposures were associated with an increased risk of non-atopic asthma[32,55,56]. Although this appears paradoxical, these opposite effects may be explained by the potential underlying mechanisms[32,57]. As explained above, it is well known that microbial exposures such as endotoxin, can induce neutrophilic airway inflammation which may lead to reversible airway obstruction, a phenotype compatible with non-atopic asthma. On the other hand, the same microbial exposures have also been suggested to inhibit IgE production by suppressing Th2 driven immune responses, explaining the decreased risk of atopic asthma.

It is not completely understood which agents are responsible for respiratory health effects among farmers. The air inside livestock houses contains high levels of organic and inorganic dusts, bacteria, endotoxin, spores and toxic gases such as ammonia and hydrogen sulphide (H_2S). Levels of these agents inside animal houses are substantially higher compared to ambient levels[58]. For example, in Dutch pig farms endotoxin concentrations of 1300 EU/m^3 inside stables were measured, while downwind of farms concentrations of maximal 10 EU/m^3 were measured[10]. Most occupational studies measured only a few agents. A large occupational study among 4,735 Norwegian farmers measured personal exposure to a range of agents: dust, fungal spores, actinomycete spores, endotoxins, bacteria, storage mites, (1→3)-β-D-glucans, fungal antigens, organic dust, inorganic dust, silica, ammonia, and hydrogen sulphide[27]. Airborne concentrations of most agents were predictors of respiratory morbidity. However, many agents were strongly correlated with each other, leading to multicollinearity in regression models. As a result, studying the independent effects of specific agents was not possible. Endotoxin, ammonia and dust have been associated with respiratory symptoms in farmers[24,29,32,59]. However, exposure to biological agents -especially endotoxin - play a major role in the explanation of work-related respiratory symptoms in farms[6,28].

Respiratory health of residents living in close proximity to livestock farms

Table 1.1 gives an overview of the scientific literature on respiratory health of residents living in close proximity of livestock farms that was available before the start of the VGO study (2012). There is a large heterogeneity in methodology in terms of study design, study populations, and exposure and health outcome assessment. Although most studies show some negative effects, not all results are consistent. The first health studies among residents of livestock farms were conducted in the USA. Residents living in communities with large hog operations reported increased occurrence of symptoms such as headaches, respiratory symptoms and irritation of nose and eyes, compared to residents of communities with no intensive livestock operations[60]. Two studies conducted in North Carolina and Iowa reported a higher prevalence of

wheezing and physician-diagnosed asthma among children and adolescents attending schools near confined swine-feeding operations[61,62]. Conversely, another study from North Carolina found a lower asthma prevalence in schoolchildren associated with higher community-level livestock farm exposures[63]. The previously mentioned studies rely on group-level exposures which could give rise to an ecological fallacy. Inferences from studies on the effect of livestock farm exposures assessed at the individual level are likely to be more valid. In a cross-sectional study in 565 children from Iowa, a higher relative environmental exposure to animal feeding operations was associated with asthma outcomes[64]. A panel study among 101 non-smoking adults in North Carolina showed that acute physical symptoms, particularly upper respiratory symptoms and irritation of nose and eyes, were associated with air pollutant concentrations near hog operations[65]. Participants were asked to sit outside their homes twice a day for 10 minutes, and had to report symptoms and measure their lung function. During the same period, continuous air pollution monitors measured levels of H_2S, endotoxin, PM_{10}, $PM_{2.5}$ and $PM_{2.5\text{-}10}$. On days with raised levels of these pollutants, the number of individuals reporting physical symptoms increased. Also, a 10 $\mu g/m^3$ increase in mean 12-hour $PM_{2.5}$ was associated with a decline in FEV_1. The fact that individuals were asked to sit outside and report symptoms makes this study sensitive for reporting bias and creates issues related to generalizability.
Studies from Europe have been conducted in Germany and the Netherlands. A large cross-sectional study was conducted in four rural German towns (Lower Saxony Lung Study) among 6,937 adults[66,67]. Respiratory health was assessed with a questionnaire and was also objectively measured in a part of the study population (n=2,478, 36%) by pulmonary function testing and bronchial challenges. Subjects residing in a neighbourhood with more than 12 stables within 500m from their home address reported more often wheezing without a cold and had a lower forced expiratory volume in 1s (FEV_1) (-7%) compared to a reference group[67]. Moreover, subjects who were exposed to higher annual ammonia levels were more likely to be sensitised to ubiquitous allergens and showed a significantly lower FEV_1 (-8%) compared to a control reference group[66]. In the same area, a cross-sectional study among 3,867 children found an association between endotoxin exposure levels based on a dispersion model and asthmatic symptoms, but only among children with atopic parents[68].

Table 1.1 Studies on respiratory health of residents living in close proximity to livestock farms published before the start of the VGO study (2012).

Country	Study population	Study design	Health effects	Ref.
Exposure assessed on group level				
North Carolina, USA	155 subjects were interviewed from 3 rural communities	Ecological study	Residents in the vicinity of the hog operation reported more (respiratory) symptoms as compared to residents of the community with no intensive livestock operations.	Wing *et al.* (2000)[60]
North Carolina, USA	128,568 children (7^{th} and 8^{th} grade)	Ecological study	Asthma prevalence was inversely associated with the number of farms in a county, beef cattle and acreage of hay.	Elliot *et al.* (2004)[63]
Iowa, USA	61 children from a school near a pig farm, and 248 from a control school	Cross-sectional	Children on a school <1km located to a pig farm had a significantly higher asthma prevalence compared to controls (>10 km of livestock farm).	Sigurdarson *et al.* (2006)[61]
North Carolina, USA	58,169 adolescents attending public schools	Cross-sectional	For students who reported allergies, the prevalence of wheezing was higher at schools that were located within 3 miles of a pig operation.	Mirabelli *et al.* (2006)[62]
Exposure assessed on individual level				
Lower Saxony, Germany	3,867 5-6 year old children	Cross-sectional	Only for children with atopic parents, higher endotoxin levels were associated with increased asthmatic symptoms.	Hoopman *et al.* (2006)[68]
Lower Saxony, Germany	2,425 non-farming adults completed a questionnaire and 1,076 participated to a medical survey	Cross-sectional	The number of animal houses within 500m was a predictor of self-reported wheeze and decreased FEV_1%. Respiratory symptoms and asthma was associated with increasing self-reported odor annoyance.	Radon *et al.* (2007)[67]
Lower Saxony, Germany	457 non-farming subjects	Cross-sectional	Higher annual ammonia exposure was associated with increased risk of sensitization against ubiquitous allergens and a decrease in FEV_1%.	Schulze *et al.* (2011)[66]
North Carolina, USA	101 non-smoking adults	Longitudinal panel study	10 $\mu g/m^3$ increase in mean 12-h $PM_{2.5}$ was associated with an increased log odds of wheezing and a decline of FEV_1.	Schinasi *et al.* (2011)[65]
The Netherlands	22,406 children and 70, 142 adults	Cross-sectional	GO-diagnosed (electronic medical records) pneumonia was associated with presence of a poultry farm within 1 km and with a high number of goats within 5 km.	Smit *et al.* (2012)[14]
The Netherlands	22,406 children and 70, 142 adults	Cross-sectional	Modelled PM_{10} exposure was inversely associated with GP-diagnosed (electronic medical records) asthma, COPD and allergic rhinitis. Living in close proximity to mink farms was positively associated with asthma and allergic rhinitis.	Smit *et al.* (2014)[69]*

*This study (part of the Intensive Livestock Farming and Health study[10]) was conducted in the Netherlands between 2009 and 2011, before the VGO study started.

Between 2009 and 2011, the Intensive Livestock Farming and Health study (in Dutch: Intensieve Veehouderij en Gezondheid, the IVG study), a first explorative study on the association between living in close proximity of intensive livestock farms and various health effects was conducted in the Netherlands[10]. Information on health status was collected via Electronical Medical Records (EMR) of their general practitioners (GP). Elevated levels of micro-organisms and endotoxin were found in proximity of livestock farms.
These endotoxin levels could potentially affect the health of susceptible subjects such as patients with asthma or COPD. However, somewhat surprisingly, indicators of air pollution from livestock farms were associated with a *lower* prevalence of asthma, allergic rhinitis and COPD[69]. Living in close proximity to goat and poultry farms was identified as a risk factor for GP-diagnosed pneumonia among adults[14].

Aim

It is well-established that farmers and farm workers have an increased risk of respiratory morbidity. On the other hand, exposure to a farming environment during childhood, but possibly also during adulthood, reduces the risk of atopic sensitization. The air inside livestock stables contains high levels of air pollutants that might lead to adverse respiratory health effects. People living in close proximity to livestock farms can potentially be exposed to these agents and may be at risk to develop adverse respiratory health effects. However, occupational health risks cannot directly be extrapolated to potential health risks for residents. The level of exposure to these agents is considerably higher for farmers compared to subjects living in close proximity to farms. Moreover, farmers belong to a healthy worker population, whereas the general population includes more individuals who are more susceptible for the effects of air pollutant emissions, such as the elderly, young children and subjects with chronic diseases. As detailed above, previous studies on respiratory health risks of neighbouring residents show a large heterogeneity in methodology, and not all results are consistent. Most studies were conducted in the USA, where farm characteristics and the management of manure differs from the situation in the Netherlands[70], which may result in different exposure levels and characteristics. The Netherlands has one of the highest population densities and one of the highest livestock farm densities worldwide. A considerable number of households are located in close proximity to livestock farms, which may be a potential health risk for residents. The aim of this thesis was to explore associations between air pollution from livestock farms and respiratory health of non-farming residents living in close proximity to farms in a rural area in the Netherlands.

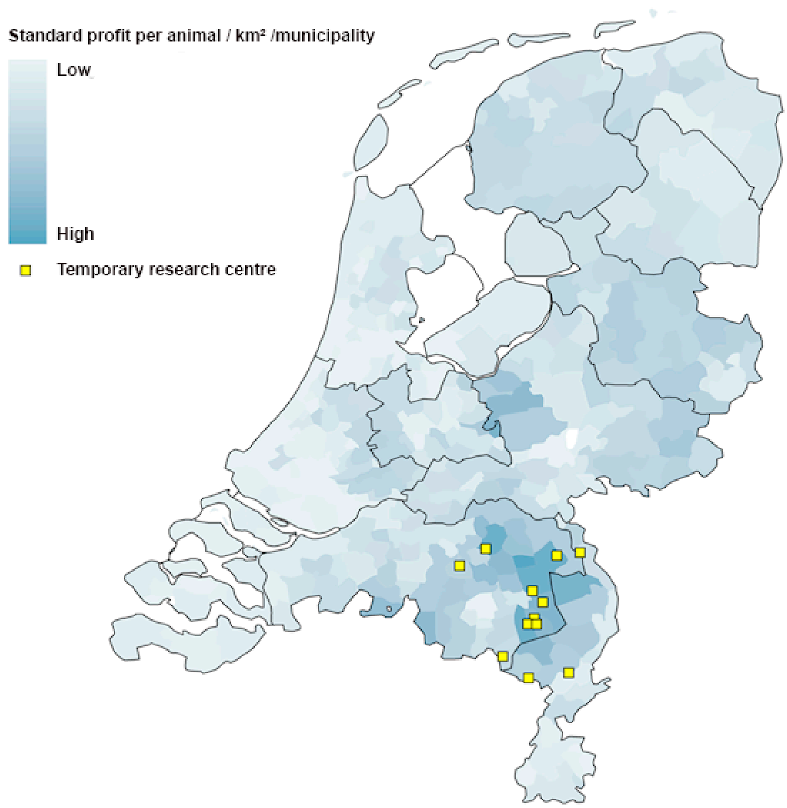
The VGO study

Between 2007 and 2010, the Netherlands experienced a Q-fever epidemic - a zoonotic bacterial infectious disease caused by *Coxiella burnetii* – of an unprecedented size with more than 4,000 notifications of human cases[13], of which an estimated 74 fatal. Infected dairy goat farms with high abortion rates due to *C. burnetii* infections were the most likely source. The most important risk factor for human Q fever appeared living close (<5 km) to an infected dairy goat farm[13]. Due to, amongst others - the increasing number of large–scale farms (‘mega-farms’) and the Q-fever outbreak, concerns about public health risks of exposure to emissions of livestock farms were raised, especially among general practitioners and people living in close proximity to farms. This was the reason for the Ministry of Economic Affairs (which includes Agriculture) and the Ministry of Health, Welfare and Sports to fund a first explorative study on potential health risks of livestock farm emissions among residents living in close proximity to farms.
The IVG study showed that indicators of air pollution from livestock farms were inversely associated with GP diagnosed asthma, allergic rhinitis and COPD[69], while living in close proximity to goat and poultry farms was identified as a risk factor for pneumonia among adults[14]. However, it was speculated that this association could (partly) be explained by the Q-fever epidemic which occurred during the same period. The IVG study results were a starting point for a more focused follow-up study – the VGO study. Whereas in the IVG study health outcomes were based on data from Electronic Medical Records of the participants’ GPs (over the year 2009), in the VGO study health outcomes were based on two additional data sources namely: self-reported questionnaire data and objectively measured data collected during a health examination survey. The VGO study focused not only on respiratory health outcomes but also on zoonotic infections such as hepatitis E, avian influenza and Q-fever, and carriage of antimicrobial resistant bacteria. Another part of the VGO study was focused on air measurements in the study area to gain more insight in spatial and temporal levels of air pollutants from livestock farms.

Study area

The study area is located in the eastern part of the province of Noord-Brabant and the northern part of the province of Limburg (see Figure 1.1). This is a rural area with the highest farm density of the Netherlands. Also, the intensity of the 2007-2010 Q-fever outbreak was particularly high in this area. Farms located in the study area differ in size, from small-scale farms with relatively few animals to large facilities, e.g. with more than 7,500 fattener pigs or more than 220,000 broilers. The province of Noord-Brabant and Limburg contain the highest number of these so-called 'large-scale farms' ('megastallen') of the Netherlands[3]. Cattle farms are most frequently present in the study area, followed by pig and poultry farms, and a lower number of goat and mink farms (see also Chapter 4 Table S4.1).

Figure 1.1 Study area of the VGO study.

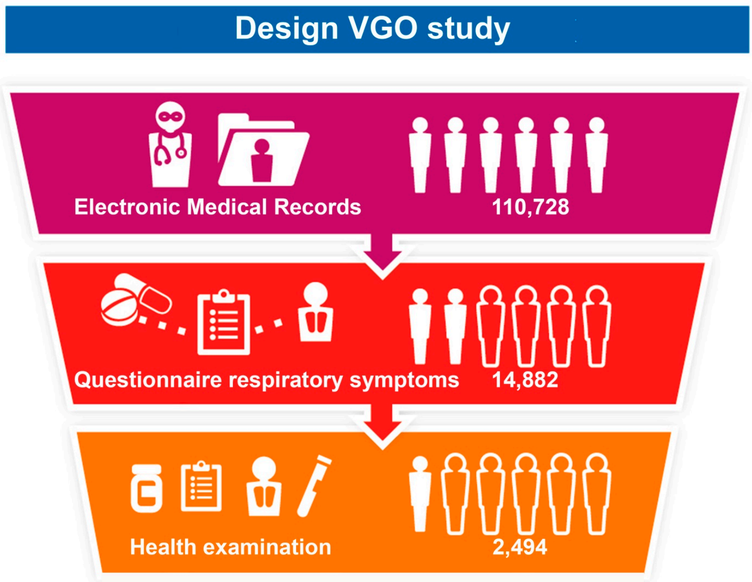


The map shows livestock farm animals density expressed as the standard profit per animal / km^2 /municipality. The yellow boxes represent the twelve temporary research centres.

Study design and study population

The study design is depicted in Figure 1.2. The VGO study includes a questionnaire survey among 14,882 respondents and health examination survey among 2,494 participants.

Figure 1.2 The design of the VGO study.



The explorative IVG study collected data in 27 practices of GPs located in the study area[10]. This resulted in data from Electronic Medical Records of 110,728 patients for the years 2009[69]. Of these 27 GP practices, 21 were willing to participate to the VGO study. The first part of the VGO study consisted of a questionnaire survey among patients from these GP practices. Patients were invited to participate to the study if they met the following inclusion criteria: 1) living in the eastern part of Noord-Brabant or the northern part of Limburg; 2) inhabitant of a municipality with <30,000 residents; and 3) aged 18-70 years. Of the eligible patients, one person per home address was randomly selected. In total 28,163 subjects received a questionnaire, and 14,882 responded (53.4%). The second part of the VGO study consisted of a health examination survey. Respondents to the questionnaire who gave consent for a follow-up study, and who were not working or living on a farm were eligible (n=8,714). Based on their home addresses, twelve temporary research centres were established (see Figure 1.1 yellow boxes). Between March 2014 en February 2015, all participants living within a distance of approximately 10 km of a temporary research centre (n=7,180) were invited to the nearest research centre for a health examination which resulted in 2,494 participants (34.7% response). The health examination consisted of the completion of a second and more extended questionnaire, length and weight measurements, a lung function measurement (pre- and post-bronchodilator spirometry) and collection of serum. The following samples were also collected but were not used for the studies reported in this thesis: EDTA-blood, nasal and buccal cells, a nasal swab and a faecal sample.

Exposure to livestock farms

Information on farm characteristics in the study area was derived from the provincial databases of mandatory environmental licences for keeping livestock in 2012. These databases contain data on number and type of animals, geographic coordinates of farms and estimated fine dust emissions from each farm per year on the basis of farm type and number of animals. Addresses of subjects were geocoded. The Euclidian distance to livestock farms was computed for each participant of the VGO study using a geographic information system (ArcGis 10.1; Esri, Redlands, CA, USA).

Thesis outline

In **Chapter 2** we describe the epidemiological analysis of self-reported respiratory symptoms in relation to proximity to livestock farms among residents who participated to the first part of the VGO study. Analyses were conducted on 12,117 responders, after excluding farmers and subjects who were living > 2 years at their current home address. In **Chapter 3** we compare the COPD prevalence in participants of the health examination based on four different operational definitions and their level of agreement. We used spirometry results, questionnaire data and data from electronic medical records to define COPD. In addition, we studied associations between COPD definitions and risk factors. The aim of **Chapter 4** was to explore associations between both spatial and temporal variation in air pollutant emissions from livestock farms and lung function in 2,308 VGO participants. In **Chapter 5** we describe the associations between residential proximity to livestock farms and atopy, while taking into account farm exposures during childhood. In **Chapter 6** we developed an attitude score that measures attitude towards livestock farming in the residential environment. We explored determinants that are associated with this attitude score and assessed potential confounding and effect modification by attitude score on the relationship between self-reported health outcomes and livestock farm exposure. In **Chapter 7**, the main findings of this thesis are summarized and reflected upon in a broader context.

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Chapter 2

Increased respiratory symptoms in COPD patients living in the vicinity of livestock farms

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Abstract

Several studies have investigated the effect of livestock farm emissions on respiratory health of local residents but results are inconsistent. This study aims to explore associations between the presence of livestock farms and respiratory health in a high density livestock farming area in the Netherlands. We focused especially on associations between farm exposures and respiratory symptoms within subgroups of potentially susceptible patients with a pre-existing lung disease.

In total, 14,875 adults (response 53.4%) completed a questionnaire concerning respiratory health, smoking habits, and personal characteristics. Different indicators of livestock farm exposures relative to the home address were computed using a geographic information system.

Prevalence of COPD and asthma was lower among residents living within 100m of a farm (OR 0.47 (0.24-0.91), 0.65 (0.45-0.93) respectively). However, >11 farms in 1000m compared to <4 farms in 1000m (4th quartile vs. 1st quartile) was associated with wheezing among COPD patients (OR 1.71 (1.01-2.89)). Using general practitioners' electronic medical records, we demonstrated that selection bias did not affect the observed associations.

Our data suggest a protective effect of livestock farm emissions on respiratory health of residents. Nonetheless, COPD patients living near livestock farms reported more respiratory symptoms, suggesting an increased risk of exacerbations.

Introduction

Intensive livestock production is associated with environmental impacts and public health issues on a global scale[1]. Concerns about emerging antibiotic resistance and outbreaks of zoonotic diseases, such as avian influenza[2] and Q-fever[3] have called attention to various human health risks that may result from livestock farms near residential areas. Neighbouring residents can potentially be exposed to dust, infectious agents, microbial toxic agents (endotoxins), allergens, and irritant gases such as ammonia and hydrogen sulfide emitted by livestock farms[1]. Various studies have measured elevated levels of livestock farm related agents in the vicinity of stables, especially downwind[4-8].

Exposure to endotoxins, cell wall fragments of Gram-negative bacteria, has been associated with pro-inflammatory responses and adverse respiratory health effects[9]. Paradoxically, a farm childhood is associated with a lower prevalence of asthma and atopy[10,11]. Higher and more diverse environmental exposures to microbial components seem to play a role in this protective effect on IgE-mediated asthma and allergies[12,13].

Two ecological studies reported a higher prevalence of wheezing and physician diagnosed asthma among children and adolescents attending schools near confined swine feeding operations[14,15]. However, Elliot *et al.* found a lower frequency of asthma in school children associated with higher community-level livestock farm exposures[16]. Studies on the effect of livestock farm exposures assessed at the individual level are scarce. In a cross-sectional study in 565 children from Iowa, a higher environmental exposure to animal feeding operations was associated with asthma outcomes[17]. A panel study among 101 non-smoking adults in North Carolina showed that self-reported hog odor, and measured air pollutants were associated with acute physical symptoms[18]. In a rural area of Germany, living within 500m of more than 12 animal houses was a predictor of self-reported wheeze and decreased FEV_1[19]. Conversely, a Dutch study found mostly inverse associations between the presence of livestock near the home address and asthma, allergic rhinitis and COPD based on 92,548 electronic medical records (EMR) from general practitioners (GPs)[20]. However, a comparison of the EMR of patients in rural Dutch areas with high and low densities of livestock farms suggested more airway infections, cough and pneumonia among asthma and COPD patients in areas with high livestock densities, which could be indicative of an increased risk of exacerbations[8]. Indeed, patients with pre-existing respiratory diseases seem to respond with a greater intensity to air pollution from livestock farms in experimental studies[21,22]. Therefore, we hypothesize that livestock farm emissions may especially affect potentially susceptible patients with a pre-existing lung-disease.

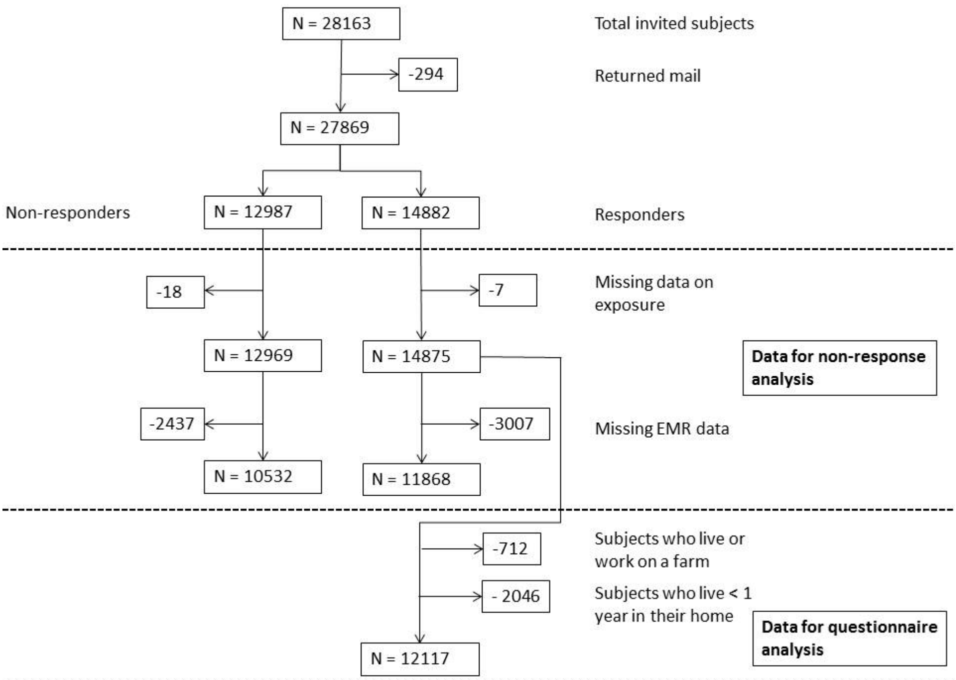
In conclusion, the number of studies on the effect of (individually estimated) livestock farm exposure on respiratory health of local residents are limited and results are inconsistent. We carried out a survey, based on a large sample size, with a validated and widely used questionnaire to assess respiratory health which enabled us to explore respiratory diseases and self-reported respiratory symptoms. Our aim was to 1) investigate associations between livestock farm exposures and respiratory health in residents, and 2) focus especially on associations between livestock farm exposures and self-reported respiratory symptoms within subgroups of potentially susceptible patients. Since subjects were recruited via GPs, we had the unique opportunity to investigate selective response by comparing the EMR of responders and non-responders. This research is part of the VGO study (Dutch acronym for Farming and Neighbouring Residents' Health).

Methods

Study population

A cross-sectional study was conducted among residents living in the eastern part of the province of Noord-Brabant and the northern part of the province of Limburg, a highly populated rural area with a high density of livestock farms in the Netherlands. In the Netherlands, every resident is obligated to be on the list of just one GP, who acts as a gatekeeper to secondary care. Therefore, all Dutch inhabitants can be reached by using the patient lists of GPs. Residents were selected by a two-stage selection procedure. First, GPs located in the study area in 2012 were selected on pre-defined registration quality criteria as described earlier[23] and were asked to collaborate in the study. In total 24 GPs met these criteria and 21 agreed to participate. In the second-stage of the selection procedure, patients from the selected GPs were invited for the study when they met the following inclusion criteria: 1) living in the eastern part of Noord-Brabant or the northern part of Limburg, 2) inhabitant of a municipality with <30,000 residents, 3) aged between 18 – 70 years. From the eligible patients, one person per home address was randomly selected. In total, 28,163 subjects received a questionnaire. The questionnaires were accompanied by a letter from the GP that stated the name and birthday of the selected subject to ensure that the selected person would complete the questionnaire. Figure 2.1 shows a flow chart of the selection procedure of the study population. Questionnaires for 294 subjects were undeliverable, and were subtracted from the total number of invited patients. The total number of responders was 14,882, resulting in a response of 53.4%. Analyses were conducted on 12,117 responders, after excluding farmers (those who reported to be living or working on a farm), and subjects who lived less than 1 year on their home address, since we assumed that their exposure period was too short.

Figure 2.1 Flow chart of the data collection.



Data for the non-response analysis included subjects with data available on exposure. When comparing Electronic Medical Records (EMR), only subjects were included with data available on EMR. Data for the questionnaire analysis contained subjects with data available on exposure, who were not living or working on a farm and who lived longer than 1 year in their current home.

Questionnaire

The questionnaires were sent in November 2012. After two weeks, a reminder was sent. The two-page questionnaire contained questions on respiratory health, smoking habits, age, gender, whether subjects were living or working on a farm, and the number of years living in their current home. Questions on respiratory health were adopted from the European Community Respiratory Health Survey-III (ECRHS-III) screening questionnaire[24] (see Supplementary Table S2.1).

Exposures from livestock farms

Exposure to livestock farms was computed for each subject. Information on farm characteristics in the study area was derived from the provincial databases of mandatory environmental licenses for keeping livestock in 2012. These databases contain data on number and type of animals, geographic coordinates of farms and estimated fine dust emissions from each farm per year on the basis of farm type and number of animals. Addresses of subjects were geocoded. Distances between the home address and all livestock farms within 500m and 1000m radius were determined using a geographic information system (ArcGis 10.1, Esri, Redlands, CA). The following farm exposure variables were studied for each subject: 1) distance (m) to the nearest farm (continuous variable and quartiles); 2) total number of farms within 500m en 1000m (quartiles), 3) presence of a specific livestock farm type within 500m and 1000m (pigs, poultry, cattle, goats and mink) (binary variables), 4) inverse-distance weighted fine dust emissions from all farms within 500m and 1000m (continuous variables) as described previously[20], and in the Supplementary Methods S2.1.

Non-response analysis

To study potential selection bias, age, gender, morbidity data and farm exposure estimates of responders and non-responders were compared in subjects with EMR and exposure data available (see Figure 2.1). EMR were available through the GPs who all participated in the NIVEL Primary Care Database (PCD) and agreed to participate in the study[25]. EMR contain data at the patient level. The International Classification of Primary Care (ICPC)[26] was used to define asthma, COPD and allergic rhinitis for responders and non-responders. Chronic diseases asthma (R96) and COPD (R91 or R95), were defined as at least one or more -ICPC code was found in 2010-2012. Allergic rhinitis (R97) was defined if one or more ICPC code was found in 2011-2012.

Data analysis

Data were analysed using SAS 9.4 (SAS Institute Inc., Cary, NC) and R 3.0.2.

First, we investigated selective response to the questionnaire, and to what extent risk estimates were biased as a result of self-selection. Multiple logistic regression was used to analyse whether a response to the questionnaire (dependent variable) was associated with livestock farm exposure estimates and morbidity data based on EMR, with adjustment for age and gender (independent variables). Furthermore, we compared associations between different exposure estimates and asthma, COPD and nasal allergies (from EMRs) in the total “source” population, in all responders (including farmers) and in responders excluding farmers.

Logistic regression models were used to explore associations between farm exposure estimates (independent variables) and self-reported COPD, current asthma and nasal allergies (dependent variables) in responders. Analyses were adjusted for age, gender and smoking habits (never smoker, ex-smoker, current smoker). The presence of a farm animal species was adjusted for the presence of other farm animal species. We expected a negligible effect of the GP on self-reported outcomes, and therefore we decided not to adjust for clustering across practice. Moreover, results obtained by generalised estimating equations (exchangeable correlation, clustering by practice) were very similar to analyses in which was not adjusted for.

Analyses on current asthma were stratified by nasal allergies (as a proxy for atopy) to assess the effect of exposure on ‘atopic’ and ‘non-atopic’ asthma[27]. Separate analyses were done for susceptible subgroups: individuals with self-reported COPD, current asthma, or nasal allergies. Additionally, associations between farm exposure estimates and the use of inhaled corticosteroids (ICS) in COPD patients were studied. COPD is a progressive illness that develops most often in people who are 40 years or older. We assume that reported COPD diagnosis is more reliable for patients who report an age of 40 years or older at diagnosis. In a sensitivity analysis, only subjects who were ≥40 years of age at diagnosis of COPD were included.

The shape of the relationships between wheeze and exposure variables within susceptible subgroups was studied by means of a penalized regression spline using the (default) “thin plate” basis as implemented in the R package mgcv. Selection of smoothing parameters was based on the Un-Biased Risk Estimator (UBRE) criterion (a scaled version of the AIC).

Ethical aspects

Patients' privacy was ensured as described earlier[20]. In short, medical information and address records were kept separated at all times by using a Trusted Third Party. The VGO study protocol was approved by the Medical Ethical Committee of the University Medical Centre Utrecht.

Results

Non-response analysis

Characteristics of responders and non-responders are summarized in Table 2.1. Responders were older than non-responders (mean age 50.4 vs. 42.8 years) and women were more often willing to participate. Responders were living closer to livestock farms than non-responders (mean distance to the nearest farm 475m vs. 498m), the mean number of farms within 1000m was higher for responders (8.1 vs. 7.4), and responders were more often living near specific farm animals. Although the prevalence of GP-registered COPD was slightly higher among responders, an inverse association was found with being a responder (OR 0.81 (95%CI: 0.69-0.96)) after adjustment for age and gender. GP-registered allergic rhinitis was positively associated with being a responder after adjusting for age and gender (OR 1.28 (95%CI: 1.14-1.44)). Overall, selection bias did not seem to affect associations between different farm exposure estimates and morbidity based on EMR data (Supplementary Table S2.2). All associations in the total invited population ("source" population) and in the responder populations (including and excluding farmers) showed a similar magnitude, with overlapping confidence intervals, and had a similar direction.

Table 2.1 Comparison of characteristics of responders and non-responders on exposure variables and Electronic Medical Records. The likelihood of being a responder is modelled for different characteristics with logistic regression.

Responders n = 14875, non-responders n = 12969	**Responders**	**Non-responders**	**OR (CI) unadjusted**	**OR (CI) adjusted**
Age, mean years (SD) ∞	50.4 (13.3)	42.8 (13.6)	1.51 (1.48-1.53)	1.52 (1.49-1.54)
Gender, % female	53.2	45.4	1.48 (1.41-1.55)	1.54 (1.47-1.62)
Exposure				
Distance to the nearest farm				
Mean distance to the nearest farm in meters (SD) Ω	475 (281)	498 (287)	0.97 (0.96-0.98)	0.97 (0.96-0.98)
Number of livestock farms				
Mean number of livestock farms within 500 m radius (SD)	1.5 (1.9)	1.4 (1.8)	1.03 (1.02-1.05)	1.04 (1.02-1.05)
Mean number of livestock farms within 1000 m radius (SD)	8.1 (5.7)	7.4 (5.4)	1.02 (1.02-1.03)	1.02 (1.02-1.03)
Presence of farm animals within 500 m				
Pigs %	25.1	23.0	1.12 (1.06-1.19)	1.09 (1.02-1.17)
Poultry %	15.7	14.9	1.06 (0.99-1.13)	1.03 (0.95-1.11)
Cattle %	44.5	41.3	1.14 (1.09-1.19)	1.10 (1.04-1.16)
Goats %	1.7	1.3	1.27 (1.05-1.55)	1.16 (0.94-1.44)
Mink %	1.7	1.6	1.06 (0.88-1.27)	1.03 (0.85-1.25)
Pigs %	74.7	71.6	1.17 (1.11-1.24)	1.11 (1.04-1.19)
Poultry %	59.0	55.5	1.15 (1.10-1.21)	1.09 (1.04-1.16)
Cattle %	89.2	87.4	1.20 (1.12-1.29)	1.06 (0.97-1.16)
Goats %	10.1	8.1	1.27 (1.17-1.38)	1.23 (1.12-1.34)
Mink %	7.1	6.9	1.04 (0.95-1.14)	0.99 (0.89-1.09)
Modelled fine dust emission				
Weighted fine dust emission from farms within 500 m, median*	0.03	0.01	1.13 (1.08-1.17)	1.14 (1.09-1.20)
Weighted fine dust emission from farms within 1000 m, median*	1.30	1.10	1.10 (1.08-1.13)	1.12 (1.09-1.15)
Electronic Medical Records				
(Subjects included with complete EMR data, responders n=11868, non-responders n=10532)				
Asthma % (R96)	6.8	6.6	1.03 (0.93-1.15)	1.06 (0.95-1.18)
COPD % (R95 or R91)	3.4	2.7	1.30 (1.11-1.52)	0.81 (0.69-0.96)
Allergic rhinitis % (R97)	6.3	5.8	1.08 (0.97-1.21)	1.28 (1.14-1.44)

OR and 95% CI were adjusted for age and gender. The presence of farm animals was also adjusted for the presence of other types of farm animals. Bold type indicates statistical significance ($p<0.05$). ∞ OR and 95% CI for an increase per 10 years. Ω OR and 95% CI for an increase per 100 meter. *OR and 95% CI for an IQR increase in log-transformed exposure. IQR for ln (fine dust g/y/m²) for farms within 500 m=7.54 corresponding to a 1881-fold increase (exp. 7.54) for non-transformed values and IQR for ln (fine dust g/y/m²) for farms within 1000 m=3.08 corresponding to a 22-fold increase for non-transformed values.

Associations between livestock farm exposures with respiratory outcomes

The prevalence of self-reported asthma, COPD and nasal allergies (Table 2.2) were higher than based on EMR (Table 2.1). Associations between the covariates (age, gender, and smoking) and COPD, asthma and nasal allergies showed expected patterns (Table2.2). Several indicators of livestock farm exposures were inversely associated with current asthma and COPD, minor associations were found with nasal allergies (Table 2.2). Adjustment for age, gender, smoking and the presence of specific farm animals did not change the results (unadjusted data not shown). Participants living very close to a farm (<290m, Q4) had significantly lower odds for current asthma, COPD and nasal allergies compared to participants living at more than 640m (Q1) from the nearest farm. A statistically significant test-for-trend was found between the quartiles of the minimal distance to the nearest farms and current asthma, COPD and nasal allergies. The presence of a livestock farm within 100m of the home address was significantly negatively associated with COPD (OR 0.71 (95%CI: 0.51-0.98)) and current asthma (OR 0.65 (95%CI: 0.45-0.93)). Analysis of specific animals around the home address showed inverse associations between the presence of pigs within 500m and the presence of goats within 1000m and current asthma.

When analyses of current asthma were stratified by nasal allergies (as a proxy for atopy) a positive association was found for presence of poultry at 500m and 'atopic asthma' (Supplementary Table S2.3). However, the presence of mink at 500m and goats at 1000m showed negative associations with 'atopic asthma'. 'Non-atopic asthma' was only significantly negatively associated with the presence of pigs within 500m.

Table 2.2 Associations of livestock farm exposures and COPD, current asthma and nasal allergies in 12117 questionnaire responders.

	COPD Prevalence 4.6% (n=553)	**Current asthma Prevalence 11.3% (n=1,365)**	**Nasal allergies Prevalence 23.2% (n=2,778)**
	OR (95% CI)	**OR (95% CI)**	**OR (95% CI)**
Covariates			
Age (increase per 10 years)	**1.56 (1.44-1.70)**	**1.06 (1.02-1.12)**	**0.84 (0.81-0.87)**
Gender (ref. is male)	1.05 (0.88-1.25)	**1.15 (1.03-1.29)**	1.10 (1.00-1.20)
Ex-smoker	**1.63 (1.32-2.01)**	**1.16 (1.01-1.31)**	**0.82 (0.74-0.90)**
Current smoker	**2.30 (1.78-2.90)**	1.18 (1.00-1.39)	**0.62 (0.54-0.71)**
Exposure			
Presence of livestock farms (yes or no)			
Within 100 m	**0.47 (0.24-0.91)**	**0.65 (0.45-0.93)**	0.78 (0.61-1.00)
Within 500 m	0.91 (0.77-1.09)	0.96 (0.85-1.07)	0.99 (0.91-1.08)
Within 1000 m	**0.71 (0.51-0.98)**	0.84 (0.67-1.06)	0.92 (0.76-1.10)
Presence of farm animals in 500 m (yes or no)			
Pigs	1.01 (0.80-1.26)	**0.84 (0.72-0.97)**	1.02 (0.91-1.13)
Poultry	1.04 (0.80-1.35)	1.17 (0.99-1.38)	1.01 (0.89-1.14)
Cattle	0.91 (0.76-1.11)	0.98 (0.87-1.11)	0.93 (0.85-1.02)
Goats	1.07 (0.51-2.26)	0.90 (0.54-1.48)	1.10 (0.77-1.57)
Mink	0.59 (0.24-1.45)	0.68 (0.39-1.18)	1.34 (0.96-1.88)
Presence of farm animals in 1000 m (yes or no)			
Pigs	0.97 (0.77-1.21)	0.94 (0.81-1.09)	1.03 (0.92-1.15)
Poultry	0.87 (0.72-1.05)	1.02 (0.90-1.15)	0.94 (0.85-1.03)
Cattle	1.01 (0.75-1.37)	1.03 (0.84-1.26)	0.94 (0.81-1.10)
Goats	0.96 (0.69-1.32)	**0.79 (0.64-0.98)**	0.95 (0.82-1.11)
Mink	0.95 (0.66-1.36)	0.99 (0.78-1.25)	0.90 (0.75-1.08)
Distance to the nearest farm (quartiles)			
>640 m	1	1	1
450 - 640 m	**0.76 (0.60-0.97)**	0.87 (0.75-1.03)	1.03 (0.91-1.17)
290 - 450 m	0.92 (0.73-1.16)	0.86 (0.73-1.00)	1.02 (0.91-1.15)
<290 m	**0.71 (0.56-0.91)**	**0.83 (0.71-0.98)**	**0.87 (0.77-0.98)**
Test for trend	**0.03**	**0.03**	**0.03**
Number of livestock farms in 1000 m (quartiles)			
<4	1	1	1
4 to 7	0.96 (0.76-1.21)	1.02 (0.87-1.19)	0.97 (0.87-1.10)
7 to 11	0.91 (0.72-1.15)	0.97 (0.83-1.13)	0.94 (0.84-1.06)
>11	0.89 (0.69-1.15)	0.95 (0.80-1.12)	0.93 (0.82-1.05)
Test for trend	0.33	0.45	0.19
Modelled fine dust emission from farms			
Log weighted fine dust emission from farms within 500 m*	0.92 (0.78-1.09)	0.96 (0.86-1.07)	0.98 (0.91-1.07)
Log weighted fine dust emission from farms within 1000 m*	0.93 (0.85-1.01)	0.96 (0.90-1.02)	0.98 (0.93-1.02)

All responders with complete exposure data were included, who do not live or work on a farm and who have lived more than 1 year in their home (n=12,117, see Figure 2.1). All OR and 95% CI were adjusted for age, gender and smoking habits. The presence of a type of farm animal is adjusted for the presence of other types of farm animals. Bold type indicates statistical significance ($p<0.05$). # Analyses on influence of covariates on COPD, current asthma and nasal allergies were mutually adjusted. *OR and 95% CI for an IQR increase in log-transformed exposure, IQR for ln (fine dust g/y/m²) for farms within 500m=7.54 corresponding to a 1881-fold increase (exp. 7.54) for non-transformed values and IQR for ln (fine dust g/y/m²) for farms within 1000m=3.08 corresponding to a 22-fold increase for non-transformed values.

Associations of livestock farm exposure and respiratory symptoms within susceptible subgroups

Wheezing among COPD patients was positively associated with several indicators of livestock farm exposures (Table 2.3). Living at 290-450m (Q3) and 450-640m (Q2) from the nearest farm compared to living at more than 640m (Q1, reference) from a farm, was significantly associated with current wheeze within COPD patients (Q3: OR 1.65 (95%CI: 1.05–2.59), Q2: OR 2.17 (95%CI: 1.32-3.57)). The spline in Figure 2.2 illustrates a non-linear association between a decreasing probability of wheezing when living at 500 meters or further from a farm. Living in an area with a high density of livestock farms (>11 farms within 1000m Q4) was also associated with more wheezing within COPD patients (OR 1.71 (95%CI: 1.01-2.89), Table2.3). No associations were observed between livestock farm exposure variables and wheezing within current asthma- and nasal allergy patients.

The presence of at least one cattle farm within 500m was significantly associated with an increased OR for usage of ICS (OR 1.50 (95%CI: 1.01-2.23)) (Supplementary Table S2.4). In addition, a positive, non-significant association with usage of ICS was found among COPD patients with more than 11 farms within 1000m of the home address (Q4) compared with less than 4 farms (Q1), but a significant association was seen with more than 12 farms within 1000m (OR 1.71 (95%CI: 1.08–2.71), result not shown).

Sensitivity analyses

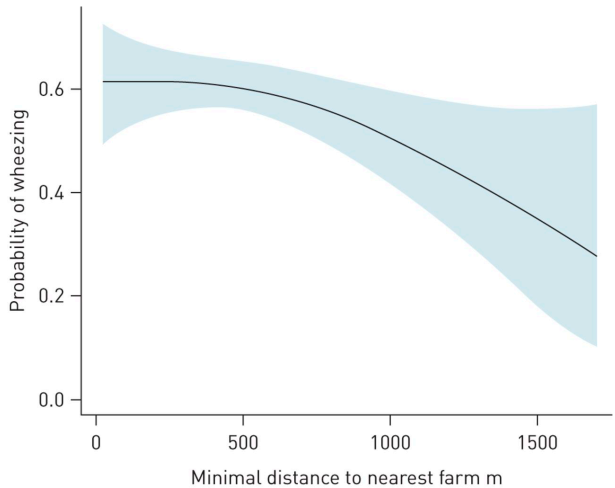
Sensitivity analyses of COPD patients aged 40 years or older at diagnosis (62% of COPD patients, n=344) showed no significant associations between livestock farm exposures and COPD prevalence (Supplementary Table S2.5). However, distance to the nearest farm was still significantly associated with wheezing among COPD patients (290-450m (Q3): OR 2.18 (95%CI: 1.21-3.93) and 450-640m (Q2): OR 1.89 (95%CI: 1.03-3.47). The presence of more than 11 farms within 1000m (Q4) was also still associated with wheezing among COPD patients (OR 2.88 (95%CI: 1.36-6.11)). The association between the presence of cattle between 500m and usage of ICS among COPD patients was attenuated. However, the positive association between more than 11 farms within 1000m and the usage of ICS among COPD patients became significant (OR 2.15 (95%CI: 1.02-4.53)).

Table 2.3 Association between livestock farm exposures and current wheeze within subgroups of patients (based on questionnaire).

	Wheezing or whistling on chest last 12 months in individuals with:		
	COPD (n=322)	**Current asthma (n=748)**	**Nasal allergies (n=670)**
Exposure	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
Presence of livestock farms			
Within 100m	0.93 (0.25-3.54)	0.61 (0.30-1.23)	0.80 (0.46-1.37)
Within 500m	1.35 (0.96-1.91)	0.93 (0.74-1.15)	0.89 (0.74-1.06)
Within 1000m	1.62 (0.87-3.02)	1.17 (0.76-1.80)	1.07 (0.74-1.55)
Presence of farm animals in 500 m (yes or no)			
Pigs	1.05 (0.66-1.68)	1.08 (0.81-1.44)	0.90 (0.72-1.14)
Poultry	1.30 (0.76-2.23)	1.17 (0.86-1.61)	1.27 (0.99-1.63)
Cattle	1.46 (0.99-2.16)	0.84 (0.67-1.07)	0.88 (0.73-1.07)
Goats	0.69 (0.14-3.40)	0.79 (0.30-2.10)	0.80 (0.38-1.71)
Mink	2.68 (0.27-26.29)	2.05 (0.63-6.65)	0.71 (0.34-1.47)
Presence of farm animals in 1000 m (yes or no)			
Pigs	0.97 (0.60-1.56)	1.04 (0.78-1.39)	0.89 (0.70-1.12)
Poultry	0.96 (0.66-1.39)	0.94 (0.74-1.19)	0.95 (0.79-1.16)
Cattle	1.19 (0.63-2.24)	0.93 (0.62-1.39)	1.02 (0.74-1.39)
Goats	0.99 (0.52-1.88)	1.16 (0.76-1.76)	0.83 (0.60-1.15)
Mink	1.09 (0.53-2.28)	0.88 (0.56-1.39)	1.19 (0.83-1.71)
Distance to the nearest farm (quartiles)			
>640 m	1	1	1
450 - 640 m	**2.17 (1.32-3.57)**	0.94 (0.69-1.27)	1.14 (0.89-1.46)
290 - 450 m	**1.65 (1.05-2.59)**	0.88 (0.65-1.19)	0.89 (0.70-1.14)
<290 m	1.45 (0.90-2.35)	0.91 (0.67-1.23)	0.99 (0.77-1.27)
Test for trend	0.14	0.48	0.46
Number of livestock farms in 1000 m (quartiles)			
<4	1	1	1
4 to 7	0.96 (0.61-1.51)	0.76 (0.57-1.02)	0.96 (0.76-1.22)
7 to 11	1.01 (0.64-1.59)	0.81 (0.60-1.08)	0.83 (0.65-1.05)
>11	**1.71 (1.01-2.89)**	0.93 (0.67-1.28)	0.90 (0.70-1.15)
Test for trend	0.09	0.58	0.20
Fine dust emission from farms			
Log weighted fine dust emission from farms within 500m*	1.03 (0.99-1.08)	1.00 (0.97-1.03)	0.99 (0.97-1.01)
Log weighted fine dust emission from farms within 1000m*	1.01 (0.96-1.07)	0.99 (0.96-1.03)	0.98 (0.95-1.01)

OR and 95% CI were adjusted for age, gender and smoking habits. The presence of a type of farm animal is adjusted for the presence of other types of farm animals. Bold type indicates statistical significance ($p<0.05$). *OR and 95% CI for an IQR increase in log-transformed exposure, IQR for ln (fine dust g/y/m²) for farms within 500m=7.54 corresponding to a 1881-fold increase (exp. 7.54) for non-transformed values and IQR for ln (fine dust g/y/m²) for farms within 1000m=3.08 corresponding to a 22-fold increase for non-transformed values.

Figure 2.2 Smoothed plots representing associations of the minimal distance to the nearest farm and wheezing within COPD patients, significance of smooth terms (p=0.06).



Discussion

We found inverse associations between the proximity to livestock farms and self-reported asthma, COPD, and allergic rhinitis among neighbouring residents. This suggests a protective effect of livestock farm exposures on respiratory health. However, current wheezing and usage of ICS among COPD patients were positively associated with indicators of livestock farm exposures as well. This may indicate an increased risk of exacerbations among COPD patients who have a higher exposure to livestock farm emissions.

The inverse associations between livestock farm exposures and respiratory diseases in neighbouring residents confirm the study by Smit *et al.*[20] in 92,548 individuals in the same area using GP EMRs instead of questionnaires. Most studies on proximity to livestock farms show adverse respiratory health effects among neighbouring residents[14-19]. Several studies[15,16,18,28] come from North Carolina (USA) where industrial hog farms cause widespread pollution. Farm characteristics and the management of manure in North Carolina[29] differs from our study area, which may result in different exposures. Furthermore, hog farms in North Carolina are clustered in low-income minority communities. Therefore, the results of these studies may not be directly comparable with ours.

An explanation for the protective effect of farms could be migration of people with respiratory health problems from rural areas to more urbanized areas. However, we found that asthma and COPD patients living in close proximity to farms deregistrated less frequently from the GP-registers (based on EMR data over four years (2009-2012)) than asthmatics and COPD patients who live far away from farms. Moreover, asthma and COPD patients showed a similar relation between distance from livestock farms and the number of years they had lived at their present address in comparison to the non-patient population (Supplementary methods S2.2 and S2.3, and Supplementary Figure S2.1). Together these analyses do not give any indication that selective migration due to respiratory health status might explain the associations observed in this study.

Higher and more diverse environmental exposures to microbial components have been attributed to a protective effect on IgE mediated allergies and asthma in childhood[11,30]. The inverse association between livestock farm exposures and COPD prevalence was observed before by Smit *et al.* who used EMR data[20]. We found moderate agreement (kappa: 0,58 (0,54-0,62)) between self-reported COPD and COPD based on EMR. Although there are some inconsistencies between both COPD definitions, the associations were similar in terms of magnitude and direction. The observed protective effect on COPD is not easily explained. We did not have information on potentially confounding farm exposures in childhood, which could partly explain the observed protective effect on asthma and allergy, and possibly COPD. However, several studies have shown that farm exposures during adulthood may also protect against atopy and allergic asthma[31-33]. In the present study we had to rely on self-reported nasal allergy as a proxy for atopy, which might explain why we did not observe pronounced differences in associations between farm exposures and 'atopic' and 'non-atopic' asthma. The second aim of the present study was to explore respiratory symptoms in susceptible subgroups. We found support for the hypothesis that patients with a chronic lung disease may be more susceptible for livestock farm exposures. Increased symptom reporting associated with several indicators of livestock farm exposures in COPD patients could indicate an elevated risk of exacerbations. We did not find this in adult asthma or nasal allergy patients. In occupational settings, an increased risk of COPD is reported for livestock farmers compared with crop farmers[34], and dust and endotoxin exposure showed a dose–response relationship with COPD in never-smoking animal farmers[35]. Farmers are exposed to much higher levels than non-farming residents, since exposure levels inside stables are considerably higher than outside. However, elevated levels of PM10 and microbial agents such as endotoxin emitted from stables have been measured 200-250 m downwind of livestock farms[7,8]. Therefore, increased farm-related air pollution could lead to airway inflammation in neighbouring COPD patients, and might explain the associations we found with wheezing and usage of ICS.

Furthermore, increased morbidity in individuals with COPD near livestock farms might be explained by environmental exposures to pathogenic micro-organisms from livestock farms, leading to exacerbations with an infectious etiology[36]. However, infections with specific zoonotic pathogens were not very common in patients with community acquired pneumonia living in an area with many livestock farms[37]. Non-pathogenic micro-organisms might contribute through their toxins, such as endotoxins, resulting in inflammatory responses[21]. Moreover, Dickson *et al.*[38] suggested that exacerbations of COPD are occasions of respiratory dysbiosis: disorder and dysregulation of the microbial ecosystem of the respiratory tract, coupled with a dysregulated host immune response. It could be speculated that changes in the lung microbiome as a result of long-term environmental exposures could play a role in these adverse respiratory effects as well.

The medical information available for non-responders is a unique feature of the VGO study, and enabled us to compare characteristics of non-responders and responders. We were able to demonstrate that selection bias did not affect associations between farm exposures and respiratory disease, which was available for invited subjects through the EMR. The prevalence of self-reported asthma, COPD and nasal allergies (Table 2.2) was higher than based on EMR. Especially the prevalence of current asthma was higher than one might expect in a rural setting. Both methods have their advantages and disadvantages, but estimation of respiratory symptom prevalence by the ECRHS questionnaire is a more commonly used method in epidemiological studies. Misclassification of individual exposure estimates is likely to be limited because we used information on livestock farm licenses from the same year in which the questionnaires were collected. We did not take into account the influence of wind direction on exposure. The average wind direction in the Netherlands is South-West, but the wind speed and direction varies across the whole year. Therefore we do not expect that wind direction will greatly influence these results. More refined exposure assessment approaches are under development, which take into account meteorological circumstances, and will be deployed in subsequent phases of this study.

Nonetheless, individual exposure estimates were calculated based on the home address and most people do not spend 24 hours a day at home. However, in Europe, adults spent the majority of their time indoors at home (56%-66%)[39]. Therefore the home address should be a good and convenient predictor to estimate exposure. It could be argued that an analysis in retired participants would lead to more accurate exposure estimates, leading to less attenuation bias. However, a sensitivity analysis in those older than 65 years of age did not yield different odds ratios (data not shown).

This is an explorative study, involving multiple exposure variables. The results should be interpreted with caution, given the number of tests performed. Nevertheless, the observed positive and negative trends seem to be consistent across several exposure variables.

In conclusion, we found an inverse association between different indicators of livestock farm exposure from livestock farms and self-reported current asthma and COPD among neighbouring residents. This suggests a protective effect from livestock farm emissions, possibly explained by higher and more diverse environmental exposures to microbial components. However, current wheezing and usage of ICS among COPD patients was positively associated with several indicators of livestock farm exposures as well, suggesting an increased risk of exacerbations in a susceptible group.

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Supplementary tables and figures

Table S2.1 Definition of respiratory symptoms based on questions adopted from the ECRHSIII screening questionnaire. Respiratory symptoms were defined according to the ECRHS definition*.

Symptom	Definition symptom
Current asthma	A positive answer to at least one of the questions: Have you had an attack of asthma in the last 12 months? • Have you been woken by an attack of shortness of breath at any time in the last 12 months? • Are you currently taking any medicine (including inhalers, aerosols or tablets) for 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’?
Wheeze	A positive answer to the following question: • Have you had wheezing or whistling in your chest at any time in the last 12 months?
Usage of inhaled corticosteroids	A positive answer to the following question: • In the last 12 months, have you regularly (on most days) taken budesonide, fluticason, beclomethason, ciclesonide or any other corticosteroid inhaler?

*ECRHS: European Community Respiratory Health Survey: http://www.ecrhs.org/

Table S2.2 Comparison of associations between different farm exposure estimates and asthma, COPD and allergic rhinitis based on electronic medical records (EMR) in the total invited "source" population, responders including farmers, and responders excluding farmers.

		Total population (n=22377)		Responders incl. farmers (n=11862)		Responders excl. Farmers (n=11244)	
Outcome	**Exposure variable**	**OR (95% CI) Unadjusted**	**OR (95% CI) Adjusted**	**OR (95% CI) Unadjusted**	**OR (95% CI) Adjusted**	**OR (95% CI) Unadjusted**	**OR (95% CI) Adjusted**
Asthma (R96)	**Presence of livestock farms**						
	Within 100m	0.62 (0.39-1.00)	0.79 (0.63-1.00)	0.91 (0.68-1.22)	0.90 (0.67-1.21)	0.78 (0.51-1.20)	0.78 (0.51-1.19)
	Within 500m	**0.89 (0.80-0.99)**	**0.89 (0.80-0.99)**	0.91 (0.79-1.05)	0.91 (0.79-1.05)	0.90 (0.77-1.04)	0.90 (0.77-1.04)
	Within 1000m	**0.79 (0.64-0.98)**	**0.80 (0.64-0.99)**	0.78 (0.57-1.06)	0.78 (0.57-1.06)	0.79 (0.58-1.08)	0.79 (0.58-1.09)
	Distance to the nearest farm (quartiles)						
	>640m	1	1	1	1	1	1
	450-640m	0.97 (0.84-1.12)	0.99 (0.86-1.15)	0.95 (0.78-1.16)	0.95 (0.78-1.16)	0.94 (0.77-1.15)	0.94 (0.77-1.15)
	290-450m	**0.85 (0.73-0.98)**	**0.85 (0.73-0.99)**	0.85 (0.69-1.04)	0.84 (0.69-1.04)	0.84 (0.69-1.04)	0.84 (0.68-1.03)
	<290m	**0.86 (0.74-0.99)**	0.87 (0.75-1.00)	**0.81 (0.67-0.99)**	**0.81 (0.67-0.99)**	**0.79 (0.64-0.97)**	**0.79 (0.64-0.97)**
	Number of livestock farms within 1000m (quartiles)						
	<4	1	1	1	1	1	1
	4 to 7	0.97 (0.84-1.11)	0.99 (0.86-1.13)	1.04 (0.85-1.25)	1.04 (0.86-1.26)	1.04 (0.85-1.26)	1.05 (0.86-1.27)
	7 to 11	**0.85 (0.74-0.99)**	**0.85 (0.74-0.99)**	**0.76 (0.61-0.93)**	**0.76 (0.61-0.93)**	**0.77 (0.62-0.95)**	**0.77 (0.62-0.95)**
	>11	0.90 (0.78-1.05)	0.91 (0.79-1.05)	0.96 (0.79-1.17)	0.96 (0.79-1.16)	0.94 (0.77-1.15)	0.94 (0.76-1.15)
COPD (R95 or R91)	**Presence of livestock farms**						
	Within 100m	**0.66 (0.45-0.95)**	0.73 (0.50-1.07)	**0.55 (0.33-0.92)**	0.68 (0.41-1.13)	0.86 (0.49-1.50)	0.90 (0.51-1.60)
	Within 500m	**0.77 (0.66-0.90)**	**0.79 (0.68-0.93)**	**0.74 (0.61-0.90)**	**0.79 (0.65-0.97)**	**0.76 (0.62-0.93)**	**0.80 (0.65-0.98)**
	Within 1000m	1.02 (0.72-1.44)	0.95 (0.66-1.35)	0.78 (0.51-1.18)	0.75 (0.49-1.16)	0.80 (0.52-1.21)	0.76 (0.49-1.17)
	Distance to the nearest farm (quartiles)						
	>640m	1	1	1	1	1	1
	450-640m	**0.79 (0.65-0.98)**	0.81 (0.66-1.00)	0.81 (0.66-1.00)	0.86 (0.66-1.13)	0.84 (0.64-1.10)	0.86 (0.66-1.14)
	290-450m	**0.81 (0.66-0.99)**	0.84 (0.68-1.04)	0.84 (0.68-1.04)	0.84 (0.64-1.11)	0.79 (0.60-1.03)	0.84 (0.63-1.11)
	<290m	**0.63 (0.51-0.78)**	**0.64 (0.51-0.80)**	**0.64 (0.51-0.80)**	**0.69 (0.52-0.91)**	**0.68 (0.51-0.90)**	**0.70 (0.52-0.93)**
	Number of livestock farms within 1000m (quartiles)						
	<4	1	1	1	1	1	1
	4 to 7	0.98 (0.81-1.19)	0.96 (0.79-1.18)	0.93 (0.72-1.21)	0.92 (0.71-1.20)	0.94 (0.72-1.23)	0.93 (0.71-1.21)
	7 to 11	0.83 (0.67-1.02)	0.81 (0.65-1.01)	0.83 (0.63-1.08)	0.84 (0.64-1.11)	0.85 (0.65-1.12)	0.85 (0.65-1.13)
	>11	**0.69 (0.56-0.87)**	**0.72 (0.57-0.90)**	**0.68 (0.51-0.91)**	0.78 (0.58-1.04)	**0.71 (0.53-0.95)**	0.79 (0.58-1.06)

Table S2.2 (continued)

		Total population (n=22377)		Responders incl. farmers (n=11862)		Responders excl. Farmers (n=11244)	
Outcome	**Exposure variable**	**OR (95% CI) Unadjusted**	**OR (95% CI) Adjusted**	**OR (95% CI) Unadjusted**	**OR (95% CI) Adjusted**	**OR (95% CI) Unadjusted**	**OR (95% CI) Adjusted**
Allergic rhinitis (R97)	**Presence of livestock farms**						
	Within 100m	**0.69 (0.54-0.90)**	**0.69 (0.53-0.89)**	0.73 (0.52-1.01)	**0.69 (0.49-0.97)**	0.94 (0.63-1.41)	0.93 (0.62-1.40)
	Within 500m	0.95 (0.85-1.06)	0.94 (0.84-1.05)	0.97 (0.84-1.13)	0.96 (0.82-1.11)	1.01 (0.87-1.18)	1.00 (0.86-1.16)
	within 1000m	0.89 (0.70-1.13)	0.93 (0.73-1.18)	0.79 (0.57-1.09)	0.81 (0.59-1.12)	0.81 (0.59-1.12)	0.83 (0.60-1.15)
	Distance to the nearest farm (quartiles)						
	>640m	1	1	1	1	1	1
	450-640m	1.12 (0.97-1.30)	1.13 (0.97-1.31)	**1.24 (1.01-1.53)**	**1.25 (1.01-1.53)**	**1.25 (1.02-1.54)**	**1.25 (1.02-1.54)**
	290-450m	0.92 (0.79-1.08)	0.90 (0.77-1.05)	0.91 (0.73-1.13)	0.90 (0.72-1.12)	0.91 (0.73-1.14)	0.90 (0.72-1.12)
	<290m	0.87 (0.75-1.01)	0.88 (0.75-1.03)	0.96 (0.78-1.18)	0.95 (0.77-1.17)	1.05 (0.85-1.30)	1.05 (0.85-1.30)
	Number of livestock farms within 1000 m (quartiles)						
	<4	1	1	1	1	1	1
	4 to 7	0.95 (0.82-1.10)	0.95 (0.82-1.10)	0.85 (0.69-1.04)	0.86 (0.70-1.05)	0.87 (0.71-1.07)	0.88 (0.71-1.08)
	7 to 11	0.91 (0.78-1.06)	0.93 (0.80-1.09)	0.83 (0.67-1.02)	0.83 (0.67-1.02)	0.83 (0.67-1.03)	0.83 (0.67-1.03)
	>11	1.02 (0.88-1.18)	1.01 (0.87-1.17)	0.95 (0.78-1.16)	0.92 (0.75-1.12)	1.03 (0.84-1.26)	1.00 (0.82-1.23)

OR and 95% CI were adjusted for age and gender. Bold type indicates statistical significance ($p<0.05$). Farmers were defined as working or living on a farm.

Table S2.3 Associations between livestock farm exposures and wheezing within asthma patients stratified by nasal allergies. The combination nasal allergy with current asthma could be an indication for atopic asthma.

Exposure	Nasal allergy Current asthma (n=612) OR (95% CI)	No nasal allergy Current asthma (n=732) OR (95% CI)
Presence of livestock farms		
Within 100m	0.62 (0.34-1.13)	0.73 (0.46-1.16)
Within 500m	0.96 (0.80-1.16)	0.95 (0.81-1.10)
Within 1000m	0.86 (0.60-1.23)	0.86 (0.63-1.17)
Presence of farm animals in 500m (yes or no)		
Pigs	0.90 (0.71-1.14)	**0.78 (0.64-0.96)**
Poultry	**1.46 (1.14-1.88)**	0.97 (0.77-1.22)
Cattle	0.95 (0.78-1.15)	1.03 (0.88-1.22)
Goats	0.48 (0.20-1.17)	1.34 (0.72-2.51)
Mink	**0.31 (0.11-0.87)**	0.98 (0.51-1.88)
Presence of farm animals in 1000m (yes or no)		
Pigs	0.96 (0.75-1.22)	0.91 (0.75-1.11)
Poultry	1.06 (0.87-1.29)	1.01 (0.85-1.19)
Cattle	1.00 (0.72-1.38)	1.10 (0.83-1.45)
Goats	**0.69 (0.49-0.98)**	0.88 (0.66-1.16)
Mink	0.76 (0.50-1.14)	1.19 (0.88-1.59)
Distance to the nearest farm (quartiles)		
>640 m	1	1
450 - 640m	**0.74 (0.58-0.96)**	0.97 (0.79-1.20)
290 - 450m	0.84 (0.66-1.08)	0.85 (0.69-1.06)
<290 m	0.83 (0.64-1.07)	0.89 (0.72-1.10)
Test for trend (p-value)	0.2496	0.1554
Number of livestock farms in 1000m (quartiles)		
<4	1	1
4 to 7	1.00 (0.78-1.27)	1.04 (0.84-1.28)
7 to 11	0.86 (0.67-1.10)	1.09 (0.88-1.33)
>11	0.87 (0.67-1.13)	1.05 (0.84-1.31)
Test for trend (p-value)	0.1713	0.5523
Fine dust emission from farms		
Log weighted fine dust emission from farms within 500m*	1.00 (0.84-1.18)	0.94 (0.81-1.08)
Log weighted fine dust emission from farms within 1000m*	0.93 (0.85-1.03)	0.99 (0.91-1.07)

OR and 95% CI were adjusted for gender, age and smoking habits. The presence of a type of farm animal is adjusted for the presence of other types of farm animals. Bold type indicates statistical significance ($p<0.05$). *OR and 95% CI for an IQR increase in log-transformed exposure, IQR for ln (fine dust g/y/m²) for farms within 500m=7.54 corresponding to a 1881-fold increase (exp. 7.54) for non-transformed values and IQR for ln (fine dust g/y/m²) for farms within 1000m=3.08 corresponding to a 22-fold increase for non-transformed values.

Table S2.4 Associations between livestock farm exposures and use of inhaled corticosteroids within COPD patients.

Use of ICS in the last 12 months in individuals with:	
Exposure	**COPD** **OR (95% CI)**
Presence of livestock farms	
Within 100m	1.57 (0.38-6.46)
Within 500m	1.32 (0.93-1.89)
Within 1000m	1.21 (0.62-2.35)
Presence of farm animals in 500m (yes or no)	
Pigs	1.20 (0.74-1.92)
Poultry	0.73 (0.43-1.25)
Cattle	**1.50 (1.01-2.23)**
Goats	0.84 (0.17-4.20)
Mink	3.28 (0.33-32.83)
Presence of farm animals in 1000m (yes or no)	
Pigs	0.87 (0.54-1.42)
Poultry	1.26 (0.86-1.85)
Cattle	1.05 (0.54-2.04)
Goats	0.93 (0.49-1.77)
Mink	0.85 (0.41-1.74)
Distance to the nearest farm(quartiles)	
>640m	
450 - 640m	1.06 (0.64-1.73)
290 - 450m	1.37 (0.86-2.18)
<290m	1.12 (0.68-1.84)
Test for trend	0.40
Number of livestock farms in 1000m(quartiles)	
<4	
4 to 7	0.90 (0.57-1.44)
7 to 11	0.95 (0.59-1.54)
>11	1.64 (0.97-2.76)
Test for trend	0.11
Fine dust emission from farms	
Log weighted fine dust emission from farms within 500m*	1.26 (0.90-1.77)
Log weighted fine dust emission from farms within 1000m*	1.05 (0.88-1.26)

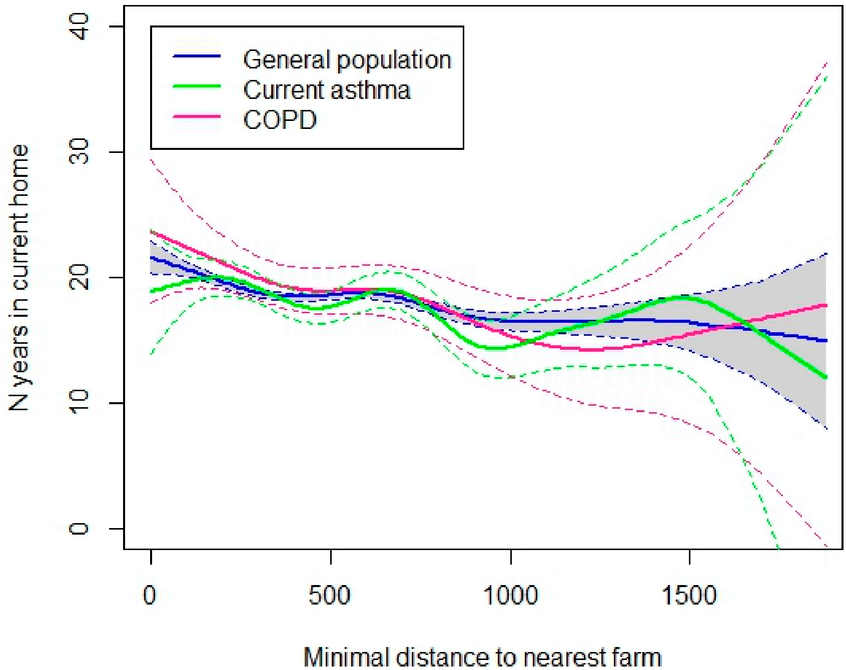
OR and 95% CI were adjusted for age, gender and smoking habits. The presence of a type of farm animal is adjusted for the presence of other types of farm animals. Bold type indicates statistical significance ($p<0.05$). *OR and 95% CI for an IQR increase in log-transformed exposure, IQR for ln (fine dust $g/y/m^2$) for farms within 500m=7.54 corresponding to a 1881-fold increase (exp. 7.54) for non-transformed values and IQR for ln (fine dust $g/y/m^2$) for farms within 1000m=3.08 corresponding to a 22-fold increase for non-transformed values.

Table S2.5 Sensitivity analysis for the age at diagnosis of COPD, only subjects were included who were 40 years or older at diagnosis of COPD.

		Wheezing or whistling on chest last 12 months	**Use of ICS in the last 12 months**
	COPD ≥40 year (n=344)	**COPD ≥40 year**	**COPD ≥40 year**
Exposure	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
Presence of livestock farms			
Within 100m	1.14 (0.23-5.65)	0.61 (0.30-1.23)	0.81 (0.38-1.71)
Within 500m	0.82 (0.54-1.24)	0.93 (0.74-1.15)	0.89 (0.71-1.11)
Within 1000m	1.45 (0.70-3.00)	1.17 (0.76-1.80)	0.93 (0.59-1.46)
Presence of farm animals in 500 m (yes or no)			
Pigs	0.87 (0.50-1.51)	1.03 (0.55-1.93)	1.37 (0.72-2.60)
Poultry	1.03 (0.55-1.91)	1.44 (0.69-2.99)	0.78 (0.38-1.60)
Cattle	0.90 (0.57-1.43)	1.42 (0.86-2.35)	1.18 (0.70-1.97)
Goats	1.31 (0.20-8.64)	1.75 (0.17-18.26)	0.93 (0.13-6.68)
Mink	n.e.	1.88 (0.18-19.21)	3.03 (0.30-30.89)
Presence of farm animals in 1000 m (yes or no)			
Pigs	0.84 (0.47-1.51)	1.15 (0.78-1.71)	0.87 (0.58-1.32)
Poultry	1.33 (0.85-2.09)	1.05 (0.75-1.46)	1.07 (0.75-1.53)
Cattle	1.27 (0.60-2.72)	0.81 (0.47-1.41)	0.71 (0.40-1.27)
Goats	1.07 (0.48-2.40)	1.35 (0.76-2.38)	0.92 (0.50-1.72)
Mink	1.09 (0.45-2.64)	0.61 (0.34-1.12)	0.92 (0.48-1.74)
Distance to the nearest farm			
>640 m	1	1	1
450 - 640m	0.93 (0.51-1.70)	**1.89 (1.03-3.47)**	0.96 (0.52-1.79)
290 - 450m	0.65 (0.38-1.11)	**2.18 (1.21-3.93)**	1.17 (0.65-2.12)
<290m	1.17 (0.64-2.11)	1.42 (0.78-2.58)	1.13 (0.60-2.11)
Test for trend	0.9024	0.0994	0.5853
Number of livestock farms in 1000 m			
<4	1	1	1
4 to 7	1.05 (0.61-1.81)	1.24 (0.70-2.18)	0.68 (0.38-1.23)
7 to 11	1.36 (0.78-2.39)	0.93 (0.53-1.64)	0.97 (0.53-1.77)
>11	0.93 (0.51-1.70)	**2.88 (1.36-6.11)**	**2.15 (1.02-4.53)**
Test for trend	0.8233	0.0541	0.0666
Fine dust emission from farms			
Log weighted fine dust emission from farms within 500m*	0.84 (0.57-1.25)	1.01 (0.82-1.23)	0.96 (0.77-1.18)
Log weighted fine dust emission from farms within 1000m*	1.06 (0.86-1.31)	0.98 (0.87-1.10)	0.97 (0.87-1.10)

OR and 95% CI were adjusted for gender, age and smoking habits. The presence of a type of farm animal is adjusted for the presence of other types of farm animals. Bold type indicates statistical significance ($p<0.05$). n.e. not estimable. *OR and 95% CI for an IQR increase in log-transformed exposure, IQR for ln (fine dust g/y/m²) for farms within 500m=7.54 corresponding to a 1881-fold increase (exp. 7.54) for non-transformed values and IQR for ln (fine dust g/y/m²) for farms within 1000m=3.08 corresponding to a 22-fold increase for non-transformed values.

Figure S2.1 Association between the number or years subjects live in their current home and the distance to the nearest farm stratified for the general population, current asthma patients and COPD patients. Significance of smooth terms general population (p<0.001), current asthma (p=0.008), COPD (p=0.04).



Methods S2.1. Fine dust emission estimates

The license database of the province contained modeled farm dust emission levels (PM10, g per year) for each farm. These emission levels were calculated by summing the products of estimated PM10 emission factors (g per year per animal), and the number of allowed animals per stable*. Weighted dust emissions from farms within 500 m and 1000 m from the home address were calculated by summing the products of the squared inverse of the distance between a farm and a home address and the farm's fine dust emission (PM10, g per year per m^2).

* Reference: Hofschreuder, P. ,Aarnink, A.J.A., Ogink, N.W.M., Measurement protocol for emissions of fine dust from animal housings, Wageningen : Animal Sciences Group, 2007

Methods S2.2. Migration out of the study area: Analysis 1.

Deregistration from GP

Possible migration out of the study area due to health problems, was investigated by comparing whether the number of deregistrations from GPs for asthma and COPD patients differed between the patients who live in close proximity to livestock farms and patients who live further away. In the Netherlands, every resident is obligated to be on the list of just one GP. Therefore deregistration is a good indication for migration. Databases of all participating GPs was used, and the number of asthma and COPD patients in 2009 in Q1 (< 290 m to nearest farm) and Q4 (>640 m to nearest farm) was compared with the number of patients in both groups which were traced back in GP-databases of 2012. The number of deregistrations among asthma and COPD patients in Q1 was 29/1962 (1,48%) and in Q4 69/2741(2,5%). Multilevel analyses were conducted as the data was hierarchically structured (patients are nested within general practices). Analyses were adjusted for age (polynomial) and gender. The dependent variable was deregistration and the independent variable was the group variable Q1, where 0 refers to the patients who live far away from farms (Q4>640 m) and 1 refers to subject who live close to farms (Q1<290 m). This resulted in an inverse association with deregistration from the GP and living in close proximity to farms (OR 0.55 (0.34-0.89). This indicates that asthma and COPD patients living in close proximity to farms migrate less often out of the area than asthma and COPD patients living far away from farms.

Methods S2.3. Migration out of the study area: Analysis 2.

Number of years in current home and the distance to the nearest farm

The association between the number of years subjects live in their current home and the distance to the nearest farm was investigated. The number of years living in the current home can be seen as a proxy for migration; the longer subjects live in their home, the less migration. In order to assess whether health status influences this association, the analyses were stratified for the general population, current asthma patients and COPD patients. All analysis were adjusted for age. Generalized additive models (smoothing) were conducted by using ‘gam’ in R. The shape of the relationships between the number of years subjects live in their current home and the distance to the nearest farm was studied by means of a penalized regression spline using the (default) “thin plate” basis as implemented in the R package mgcv. Selection of smoothing parameters was based on the Un-Biased Risk Estimator

(UBRE) criterion (a scaled version of the AIC). The estimated smooth curve was obtained by plotting predicted responses across a range of exposure values, while fixing other covariates in the model to average levels. Supplementary Figure S2.1 shows age-adjusted spline plots for the three strata, which indicate an overall decreasing trend for the number of years in the current home and a larger distance to the nearest farm. Although the interpretation of this trend is limited by the cross-sectional nature of the analysis, it is important to notice that the same trends are seen for the general population, and for COPD patients and current asthma patients.

Chapter 3

Spirometry, questionnaire and Electronic Medical Record based COPD in a population survey: comparing prevalence, level of agreement and associations with potential risk factors

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Abstract

COPD-diagnosis is confirmed by post-bronchodilator (BD) spirometry. However, epidemiological studies often rely on pre-BD spirometry, self-reports, or medical records. This population-based study aims to determine COPD-prevalence based on four different operational definitions and their level of agreement, and to compare associations between COPD-definitions and risk factors.

COPD-prevalence in 1,793 adults from the general Dutch population (aged 18-70 years) was assessed based on self-reported data, Electronic Medical Records (EMR), and post-BD spirometry: using the FEV_1/FVC below the lower limit of normal (LLN) and GOLD fixed cut-off ($FEV_1/FVC < 0.70$). Using spirometry as a reference, sensitivity was calculated for self-reported and EMR-based COPD. Associations between COPD and known risk factors were assessed with logistic regression. Data were collected as part of the cross-sectional VGO study (Livestock Farming and Neighboring Residents' Health Study).

The highest prevalence was found based on spirometry (GOLD: 10.9%, LLN: 5.9%), followed by self-report (4.6%) and EMR (2.9%). Self-reported or EMR-based COPD identified less than 30% of all COPD-cases based on spirometry. The direction of association between known risk factors and COPD was similar across the four definitions, however, magnitude and significance varied. Especially indicators of allergy were more strongly associated with self-reported COPD compared to the other definitions.

COPD-prevalence varied depending on the used definition. A substantial number of subjects with spirometry-based COPD cannot be identified with questionnaires or medical records which can cause underestimation of COPD-prevalence. The influence of the different COPD-definitions on associations with known risk factors was limited.

Introduction

Chronic obstructive pulmonary disease (COPD) is a leading cause of mortality and morbidity worldwide and expected to increase in the coming decades[1]. Epidemiological studies estimating COPD prevalence show remarkable variation due to differences in measurement methodology[2]. Halbert *et al.* conducted a meta-analysis to quantify the global prevalence of COPD[2]. Objective definitions based on spirometry tended to produce higher prevalence estimates than patient reported diagnosis and physician diagnosis (9.2% versus 4.9% versus 5.2%, respectively). This likely reflects the underestimation and under-diagnosis of the disease prevalence[3]. COPD based on post-bronchodilator (BD) spirometry is therefore preferred in epidemiological studies and very common. Objective measurements are also preferred because they are not influenced by symptom-perception, recall-bias and access to health care[4]. However, the advantage of self-reports or medical records are the relatively low costs, allowing large sample sizes and "big data" analysis.

Studies comparing COPD-prevalence based on different data sources in the same population also found that the definitions used to assess COPD greatly influence prevalence estimates[5-10]. A study from de Marco *et al.* showed that the effect of risk factors for the development of COPD, such as gender, age and Body Mass Index (BMI), may also depend on the definition used[11]. However, most of these studies were conducted in patient populations[7,9,10]. In the few studies that compared COPD-definitions in the general population, only pre-BD spirometry results were available[5,6,11]. To our knowledge, this is the first population-based study that compares post-BD spirometry-based COPD with COPD-prevalence based on other data sources.

For spirometry-based COPD, the recommended cut-off for the Forced Expiratory Volume in 1 second (FEV_1)/ Forced Vital Capacity (FVC) is the lower limit of normal (LLN) based on the Global Lung Initiative-2012 (GLI) reference equations that take into account sex, age, and height (12,13). Another commonly used cut-off point for COPD is the ratio between post-BD FEV_1 and FVC <0.70 (Global Initiative for Chronic Obstructive Lung Disease (GOLD))[1]. This GOLD-definition has been criticized since the FEV_1/FVC ratio generally decreases with age which results in over-diagnosis in elderly and under-diagnosis in younger people[14,15].

A comparison of different definitions for determining COPD-prevalence will give more insight into the possible effects of using various COPD-definitions on prevalence estimates and their associations with potential risk factors.

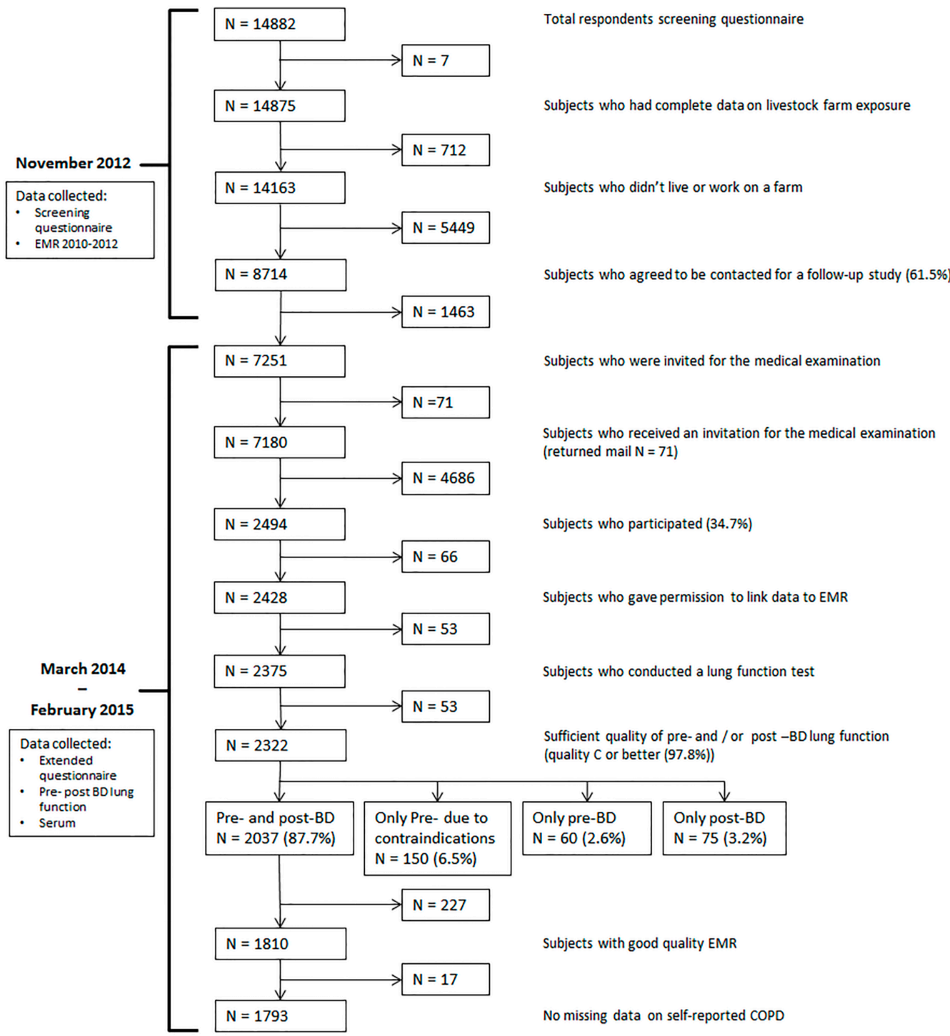
The objectives of this study are: 1) to compare COPD-prevalence and the level of agreement based on four different operational definitions: self-reported COPD, COPD based on general practitioners' (GP) Electronic Medical Records (EMR) and COPD based on post-BD spirometry: LLN and GOLD-definition; 2) to compare associations between COPD (four operational definitions) and potential risk factors and severity measures; and 3) to analyze COPD-prevalence based on pre-BD spirometry and to assess whether associations with potential risk factors are different from COPD based on post-BD spirometry.

Materials and methods

Study population

Data of the present study are derived from the cross-sectional VGO study (Dutch acronym for Livestock Farming and Neighboring Residents' Health), which aims to investigate health of residents living in the vicinity of livestock farms. In 2012, a questionnaire survey was conducted among 14,163 adults from the general population (aged 18-70 years) in the south of the Netherlands. Recruitment and inclusion criteria have been described previously by Borlée *et al.*[16]. 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-BD spirometry), collection of serum, EDTA-blood, nasal and buccal cells, and a nasal swab. In addition, fecal samples were taken by the participants at home and sent to the laboratory by mail. In this study we conducted analyses on subjects with a pre- and post-BD measurement with a sufficient quality (quality C or better), with good quality EMR available and with non-missing self-reported COPD (n=1,793) (see Figure 3.1 for a flow chart of the study population).

Figure 3.1 Flow chart of the data collection.



Analyses are conducted on subjects with a pre-and post-bronchodilator (BD) measurement with a sufficient quality, with Electronic Medical Records (EMR) of good quality and with non-missing self-reported COPD.

Ethical aspects

The VGO study protocol was approved by the Medical Ethical Committee of the University Medical Centre Utrecht (protocol number 13/533). 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.

Data sources and COPD-definitions

Self-reported data

Self-reported COPD was defined as a positive answer to the question: 'Have you ever been told by a doctor that you had chronic obstructive pulmonary disease or emphysema?' Questionnaire data on respiratory health was assessed with the first questionnaire collected in November 2012 as described previously[16]. This was a two-page questionnaire with questions on respiratory health adopted from the European Community Respiratory Health Survey-III (ECRHS-III) screening questionnaire[17].

Electronic Medical Records

EMR-based COPD was defined as: ICPC-code R91 (Chronic bronchitis) or R95 (Emphysema/COPD) recorded in 2010-2012. EMR data were available through the GPs who all participated in the Netherlands Institute for Health Services Research (NIVEL) Primary Care Database[18]. 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 (4), 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.

Spirometry

Two COPD-definitions were used based on spirometry data 1) a post-BD measurement of FEV_1/FVC below the LLN, and 2) a post-BD measurement of FEV_1/FVC <0.70 (GOLD). LLN was calculated with GLI-reference values based on age, gender and height (13). Pre- and post-BD spirometry was conducted according to European Respiratory Society (ERS) guidelines and the European Community Respiratory Health Survey III (ECRHS-III)[19]. 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[20]. 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) (N=2,322/2,375, respectively see Figure 3.1).

Potential risk factors and severity measures of COPD

Patient characteristics and severity measures of COPD were collected with the second, more extended, questionnaire which subjects completed before the medical examination. The questionnaire comprised amongst others items on symptoms and diseases, smoking habits, education and profession. Body Mass Index (BMI, kg/m^2) was assessed during the medical examination. 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[21]. To gain more insight into asthma-COPD overlap, we included self-reported current asthma as a potential risk factor. Self-reported current asthma was defined as: 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) or use of inhaled or oral medication for breathing problems in the last year (described before by de Marco *et al.*[22]. Three severity measures for COPD were computed for all participants: GOLD grades[1], self-reported health status, and the Clinical COPD Questionnaire (CCQ)-score[23]. The CCQ-score is developed to identify activity limitations and emotional dysfunction of COPD patients.

Statistical analysis

We conducted a detailed non-response analysis in order to detect possible selection bias. Characteristics of different population subsets were compared (see Figure 3.1 and Table 3.1). The likelihood of agreeing to follow-up, or being a participant was modeled for different characteristics with logistic regression and adjusted for age, gender and smoking habits. In order to study the effect of potential selection bias, we compared the association between self-reported COPD and risk factors among different populations subsets (see Table S3.1).

Table 3.1 Comparison of characteristics of subjects who agreed and disagreed to be contacted for a follow-up study, and subjects who participated and did not participate to the medical examination.

	Agreed to follow-up	Disagreed to follow-up	Adjusted OR (95% CI)	Participated	Invited, but did not participate	Adjusted OR (95% CI)
Subjects n	8,714	5,449		2,494	4,686	
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 (0.87-1.00)	54.6	52.2	**1.20 (1.08-1.32)**
Never smoker	45.5	49.1	1	45.0	46.4	1
Former 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 (0.85-1.04)	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)
COPD	4.7	4.0	1.14 (0.96-1.35)	5.1	4.3	1.03 (0.81-1.30)
Morbidity based on EMR						
Subjects included with good quality EMR data n	6,689	4,253		1,906	3,359	
Asthma (ICPC R96)%	7.2	6.2	**1.19 (1.02-1.39)**	5.9	7.0	0.87 (0.68-1.11)
COPD (ICPC R95 or R091)	3.7	3.3	1.04 (0.84-1.29)	3.7	3.5	0.82 (0.60-1.11)

Data are presented as mean ±SD or %, unless otherwise stated. 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 ($p < 0.05$). ICPC: International Classification of Primary Care. *OR(95% CI) for an increase per 10 year.

Agreement between the presence of COPD based on the three different data sources was determined by calculating Cohen's kappa. Using the results of the post-BD spirometry as reference standards for COPD, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for self-reported and EMR-reported COPD were calculated.

The association between each potential risk factor or severity measure with COPD was assessed by means of multiple logistic regression analysis. All analyses were adjusted for age (as a continuous variable), gender and smoking habits. To include both the qualitative effect of smoking status and the quantitative effect of smoking exposure, we included ever smoking and pack-years of smoking together as confounders[24]. Sensitivity analyses were conducted: 1) with COPD based on pre-BD measurements; 2) on subjects aged ≥40 years, since COPD diagnosis is more reliable in older patients[25,26]; and 3) after excluding subjects with self-reported current asthma.

Data were analyzed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

Non-response analysis

Subjects who agreed to be contacted for a follow-up study were slightly older (mean age 51.1 vs. 49.8 years), were more often former smokers (38.8% vs. 31.4%) and had more often asthma (both self-reported and EMR-based asthma) compared to subjects who disagreed (Table 3.1). 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. Selection bias did not seem to affect associations between potential risk factors and self-reported COPD in different population subsets (Table S3.1).

COPD-prevalence and the level of agreement

The highest COPD-prevalence was found based on spirometry using the GOLD-definition (10.9%), followed by LLN-definition (5.9%), self-report (4.6%) and EMR (2.9%) (see Table 3.2).

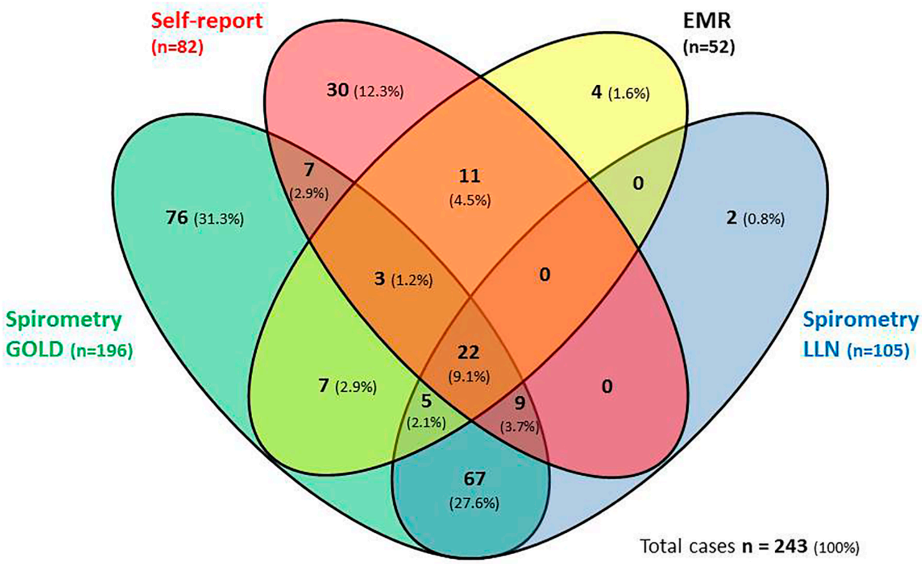
Table 3.2 Prevalence and lung function characteristics for four different definitions of COPD, based on three sources: self-reported data, GP Electronic Medical Records, and spirometry.

	Self-reported questionnaire	Electronic Medical Records	Spirometry LLN	Spirometry GOLD
COPD-definition	**A positive answer to the following question: Have you ever been told by a doctor that you had chronic obstructive pulmonary disease or emphysema?**	**ICPC R91(Chronic bronchitis) or R95 (Emphysema/COPD) found in years 2010-2012**	**A post-bronchodilator measurement of FEV_1/FVC lower than lower limit of normal**	**A post-bronchodilator measurement of $FEV_1/FVC < 0.70$**
N (%)	82 (4.6%)	52 (2.9%)	105 (5.9%)	196 (10.9%)
Age	61.7 ± 8.7	63.4 ± 7.2	59.5 ± 9.3	62.1 ± 7.2
Female, n (%)	35 (42.7%)	26 (50.0%)	44 (41.9%)	67 (34.2%)
Never smoker, n (%)	25 (30.5%)	7 (13.5%)	16 (15.3%)	33 (16.8%)
Former smoker, n (%)	44 (53.7%)	32 (61.5%)	51 (48.6%)	104 (53.1%)
Current smoker, n (%)	13 (15.9%)	13 (25.0%)	38 (36.2%)	59 (30.1%)
Lung function pre-measurement				
FEV_1 % predicted	79.5 ± 22.5	69.9 ± 21.8	73.6 ± 18.8	81.1 ± 18.2
FVC % predicted	96.0 ± 15.7	91.6 ± 15.8	98.3 ± 18.2	101.1 ± 16.5
FEV_1/FVC % predicted	81.8 ± 16.3	75.3 ± 16.8	74.0 ± 10.8	79.4 ± 10.1
MMEF % predicted	58.6 ± 35.9	44.6 ± 29.7	38.1 ± 16.1	48.3 ± 19.0
Lung function post-measurement				
FEV1 % predicted	83.9 ± 21.7	74.1 ± 21.1	77.9 ± 17.8	84.9 ± 17.1
FVC % predicted	98.1 ± 15.0	94.1 ± 15.6	101.2 ± 17.0	103.3 ± 15.6
FEV_1/FVC % predicted	84.8 ± 16.6	78.1 ± 17.2	76.4 ± 10.3	81.5 ± 9.5
MMEF % predicted	66.4 ± 38.8	49.5 ± 31.7	42.4 ± 15.8	52.1 ± 17.6

Data are presented as mean ±SD, unless otherwise stated. Pre- and post-bronchodilator lung function variables are presented as % predicted values compared with GLI-2012 reference[13] values based on age, gender and height. In total, 1793 subjects were included who had a pre- and post-BD measurement with a quality of C or higher, with Electronic Medical Records (EMR) of good quality and with non-missing self-reported COPD. FEV_1:Forced Expiratory Volume in 1 sec, FVC: Forced Vital Capacity , FEV_1/FVC: Tiffeneau-index, MMEF: Maximum Mid-Expiratory Flow. ICPC: International Classification of Primary Care.

In total 243 COPD cases were ascertained by at least one definition. The overlap between COPD-prevalence based on the four different definitions was low (see Figure 3.2). Only 9.1% of COPD cases (n=22) had COPD according to all four definitions. A substantial part (59.7%) was only ascertained by spirometry: 27.6% by both the LLN and the GOLD-definition, 31.3% by the GOLD-definition alone and 0.8% by the LLN-definition alone. In total, 73.2% (145/198) of spirometry-based COPD was not identified by self-reported and or EMR-based data.

Figure 3.1 Comparison of COPD prevalence based on four different definitions, presented in n cases and in % of total identified cases.



In total 243 COPD cases were ascertained by at least one definition. In total, 1793 subjects who had a pre- and post-BD lung function measurement with a sufficient quality (C or better), Electronic Medical Records (EMR) of good quality, and without missing data on self-reported COPD (see Figure 3.1) were included. Self-report = self-reported data based on the ECRHSIII screening questionnaire, EMR=Electronic Medical Records, spirometry LLN=post-bronchodilator measurement of FEV_1/FVC lower than FEV_1/FVC-lower limits of normal, spirometry GOLD=post-bronchodilator measurement of FEV_1/FVC<0.70.

The highest agreement, expressed as Cohen's kappa (27), was found between COPD based on the two spirometry definitions (κ=0.65), followed by a moderate agreement between self-reported and EMR-based COPD (κ=0.52) (Table 3.3). Agreement between spirometry-based COPD compared with self-reported or EMR-based COPD was fair (LLN-definition: self-report: κ=0.30, EMR: κ=0.31, GOLD-definition: self-report: κ=0.25, EMR: κ=0.26).

Self-reported or EMR-based COPD identified less than 30% of all subjects with spirometry-based COPD (sensitivity varied between 0.19 and 0.30, specificity: 0.97 – 0.99) (Table 3.3).

As expected, since LLN is a subset of the GOLD definition, the proportion of subjects with self-reported or EMR-based COPD confirmed by spirometry-based COPD (PPV) was higher when compared with the GOLD-definition (PPV self-report: 0.50, PPV EMR: 0.71) than with the LLN-definition (PPV self-report: 0.38, PPV EMR: 0.52).

Table 3.3 Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of COPD based on self-reported data and based on Electronic Medical Records compared with COPD based on spirometry –LLN and GOLD-definition. Agreement between the three different data sources was determined with Cohen's Kappa.

	COPD-LLN		**COPD-GOLD**	
	Self-report	**EMR**	**Self-report**	**EMR**
Sensitivity	0.30	0.26	0.21	0.19
Specificity	0.97	0.99	0.97	0.99
PPV	0.38	0.52	0.50	0.71
NPV	0.96	0.96	0.91	0.91
Cohen's Kappa (95% CI)	0.30 (0.21-0.39)	0.31 (0.22-0.41)	0.25 (0.18-0.32)	0.26 (0.19-0.34)

The agreement between GOLD-definition cases and LLN-definition cases was κ=0.65 (0.60-0.72). Agreement between self-reported COPD and EMR-based COPD was (κ=0.52 (0.42-0.62)). Self-report = self-reported data based on the ECRHSIII screenings questionnaire, EMR=Electronic Medical Records, COPD-LLN=COPD LLN-definition based on post-bronchodilator measurement, COPD-GOLD=COPD GOLD-definition based on post-bronchodilator measurement. The reference value was based on spirometry (LLN and GOLD). The different definitions for COPD are presented in Table 3.2.

Associations between COPD-definitions and potential risk factors and severity measures

Overall, the direction of associations was consistent across all four COPD-definitions (Table 3.4). A low BMI (<20 vs. 20-25) and pack years of smoking were significant risk factors for each COPD-definition with comparable magnitude. However, the magnitude and significance of other associations varied between the definitions.

In particular, the association of age and gender with COPD varied according to the definition used. Age was significantly positively associated with COPD, except when the LLN-definition was used. The negative association with female gender was only statistically significant when the GOLD-definition was used, whereas the EMR-based definition showed a non-significant positive association. The positive association between self-reported allergy and COPD was only significant when using self-reported COPD or EMR-based COPD.

Table 3.4 Associations of patients' characteristics and severity measures with four different definitions of COPD.

		COPD			
	Total population	**Self-report**	**EMR**	**Spirometry LLN**	**Spirometry GOLD**
N (%)	1,793 (100%)	82 (4.6%)	52 (2.9%)	105 (5.9%)	196 (10.9%)
Age (per 10 years), mean (SD)	56.2 ± 10.8	**1.72 (1.29-2.29)**	**2.38 (1.55-3.66)**	1.10 (0.87-1.39)	**1.81 (1.47-2.22)**
Female gender	56.0	0.68 (0.43-1.09)	1.20 (0.67-2.15)	0.74 (0.48-1.13)	**0.49 (0.35-0.68)**
Ever smoker	54.8	1.41 (0.85-2.32)	**4.18 (1.85-9.44)**	**4.10 (2.35-7.17)**	**3.50 (2.35-5.22)**
Pack years (per 10 years), Mean* (SD))	18.7 ± 18.4	**1.18 (1.05-1.32)**	**1.20 (1.06-1.35)**	**1.31 (1.19-1.44)**	**1.23 (1.13-1.35)**
Occupational exposure to vapors, gases, dust or fumes	32.3	1.12 (0.68-1.86)	1.49 (0.79-2.83)	1.05 (0.67-1.65)	1.17 (0.82-1.66)
BMI<20 (ref=BMI 20-25)	1.8	**7.27 (2.33-22.68)**	**9.57 (2.53-36.18)**	**6.44 (2.43-17.11)**	**2.91 (1.04-8.11)**
BMI>25 (ref=BMI 20-25)	65.5	0.86 (0.51-1.44)	0.77 (0.41-1.46)	**0.57 (0.36-0.90)**	**0.57 (0.40-0.82)**
High education level (ref=low/ medium)	29.5	0.63 (0.35-1.13)	**0.26 (0.09-0.73)**	**0.47 (0.27-0.83)**	**0.63 (0.42-0.94)**
Current asthma	4.3	**32.18 (17.3-59.9)**	**7.19 (3.24-15.96)**	**2.48 (1.18-5.19)**	**2.52 (1.33-4.79)**
Self-reported ever allergy	39.9	**3.23 (1.98-5.26)**	**1.87 (1.03-3.39)**	1.22 (0.79-1.88)	1.29 (0.91-1.81)
Atopy	28.2	**2.36 (1.47-3.78)**	1.82 (0.99-3.34)	1.30 (0.83-2.04)	1.04 (0.72-1.50)
≥1 positive specific IgE	20.1	**2.63 (1.58-4.38)**	1.75 (0.86-3.55)	1.46 (0.88-2.44)	1.29 (0.85-1.96)
Total IgE ≥100 IU/ml	16.4	**2.77 (1.68-4.55)**	**2.55 (1.35-4.80)**	**1.92 (1.20-3.08)**	1.37 (0.91-2.06)
GOLD-1 † (ref=FEV_1/FVC>0.7)	7.3	**2.65 (1.26-5.54)**	**4.34 (1.62-11.60)**	**NA**	**NA**
GOLD 2-4 † (ref=FEV_1/FVC>0.7)	3.6	**28.15 (14.3-55.3)**	**53.1 (24.1-117.1)**	**NA**	**NA**
CCQ-score ‡, mean (SD)	0.55 ± 0.57	**3.95 (2.92-5.33)**	**4.01 (2.80-5.75)**	**2.97 (2.25-3.92)**	**2.15 (1.68-2.76)**
Less than good self-reported health §	20.9	**6.23 (3.81-10.18)**	**6.66 (3.58-12.41)**	**2.29 (1.48-3.53)**	**1.55 (1.09-2.23)**

Data are presented as mean ±SD or %, unless otherwise stated. OR and 95% CI were adjusted for age, gender, ever smoking and pack years (number of pack years was mean-centered for ex- and current smokers). Bold type indicates statistical significance ($p<0.05$). Self-reported: self-reported data based on the ECRHSIII screening questionnaire, EMR: Electronic Medical Records, spirometry: post-bronchodilator lung function measurement. Used definitions for COPD based on different databases are presented in Table 3.2. NA: Not available, as no (GOLD) or very few (LLN) subjects with spirometry-based COPD had FEV_1/FVC>0.7. *Mean packyears are calculated for ex-smokers and current smokers. † GOLD-1: FEV_1/FVC<0.70 and FEV_1≥ 80% predicted, GOLD-2-4: FEV_1/FVC<0.7 and FEV1<80% predicted. ‡ Clinical COPD Questionnaire (CCQ)-score[23]. § Less than good self-reported health: bad/moderate/reasonable, reference category: good/excellent self-reported health.

When focusing on indicators for objectively measured allergy, we found strong positive associations between self-reported COPD and all three definitions of IgE sensitization (>1 positive specific IgE, total IgE≥100 IU/ml, and a combination of both). EMR-based COPD and COPD based on LLN-definition were only associated with total IgE≥100 IU/ml. Current asthma was positively associated with all four definitions, nonetheless, a substantially stronger association was observed with self-reported COPD. Indicators for COPD severity were positively associated with COPD regardless of the definition used, but stronger associations were observed with self-reported and EMR-based COPD.

Sensitivity analyses of the 1626 subjects aged ≥40 years showed a small increase in COPD-prevalence based on all four definitions (self-report: n=81 (5.0%), EMR: n=52 (3.2%), LLN: n=103 (5.7%), GOLD: n=196 (12.1%)) (Table S3.2). The associations between COPD and potential risk factors did not change. Analyses without patients with current asthma showed a lower prevalence of self-reported COPD (n=52 (3.0%) vs. n=82 (4.6%)), prevalence based on the other definitions did not show major changes (Table S3.3). A stronger association was observed between self-reported COPD and age and a low BMI. The association between self-reported COPD and self-reported allergy and indicators for objectively measured allergy became weaker.

Pre- versus post bronchodilator spirometry

COPD-prevalence increased when using pre-BD measurements (LLN pre: 9.1% *vs.* post: 5.9%, GOLD pre: 16.4% vs. post: 10.9%) (see Table 3.5). In general, similar associations with risk factors were identified by using pre- or post-BD spirometry, although associations were stronger and more often significant when COPD was based on post-measurements.

Table 3.5 Associations between spirometry-based COPD and potential risk factors and severity measures. COPD is defined on pre-and post-measurements and on LLN-definition and GOLD-definition.

	Spirometry LLN		Spirometry GOLD	
	Pre-measurement	**Post-measurement**	**Pre-measurement**	**Post-measurement**
N (%)	163 (9.1%)	105 (5.9%)	294 (16.4%)	196 (10.9%)
Age (per 10 years)	0.91 (0.77-1.07)	1.10 (0.87-1.39)	**1.41 (1.21-1.64)**	**1.81 (1.47-2.22)**
Female gender	0.77 (0.55-1.08)	0.74 (0.48-1.13)	**0.45 (0.35-0.60)**	**0.49 (0.35-0.68)**
Ever smoker	**2.75 (1.87-4.06)**	**4.10 (2.35-7.17)**	**2.40 (1.79-3.23)**	**3.50 (2.35-5.22)**
Pack years (per 10 years)	**1.25 (1.14-1.37)**	**1.31 (1.19-1.44)**	**1.21 (1.12-1.32)**	**1.23 (1.13-1.35)**
Occupational exposure to vapors, gases, dust or fumes	1.01 (0.69-1.47)	1.05 (0.67-1.65)	1.04 (0.77-1.40)	1.17 (0.82-1.66)
BMI < 20 (ref = BMI 20-25)	**3.54 (1.48-8.46)**	**6.44 (2.43-17.11)**	**4.27 (1.79-10.18)**	**2.91 (1.04-8.11)**
BMI > 25 (ref = BMI 20-25)	**0.61 (0.43-0.88)**	**0.57 (0.36-0.90)**	**0.68 (0.50-0.92)**	**0.57 (0.40-0.82)**
High education level (ref = low/ medium)	0.77 (0.52-1.14)	**0.47 (0.27-0.83)**	0.81 (0.59-1.11)	**0.63 (0.42-0.94)**
Current asthma	**2.93 (1.62-5.29)**	**2.48 (1.18-5.19)**	**2.97 (1.72-5.12)**	**2.52 (1.33-4.79)**
Self-reported ever allergy	1.15 (0.81-1.63)	1.22 (0.79-1.88)	1.07 (0.81-1.43)	1.29 (0.91-1.81)
Atopy	1.41 (0.98-2.01)	1.30 (0.83-2.04)	1.07 (0.79-1.45)	1.04 (0.72-1.50)
>1 positive for specific IgE	1.36 (0.90-2.05)	1.46 (0.88-2.44)	1.15 (0.82-1.63)	1.29 (0.85-1.96)
Total IgE ≥100 IU/ml	**1.95 (1.33-2.87)**	**1.92 (1.20-3.08)**	**1.53 (1.09-2.15)**	1.37 (0.91-2.06)
CCQ-score †, mean (SD)	**2.10 (1.65-2.68)**	**2.97 (2.25-3.92)**	**1.76 (1.42-2.19)**	**2.15 (1.68-2.76)**
Less than good self-reported health ‡	**1.86 (1.29-2.68)**	**2.29 (1.48-3.53)**	**1.44 (1.06-1.97)**	**1.55 (1.09-2.23)**

OR and 95% CI were adjusted for age, gender, ever smoking and pack years (number of pack years was mean-centered for ex- and current smokers). Bold type indicates statistical significance ($p<0.05$). Spirometry: pre and post-bronchodilator lung function measurement. Used definitions for COPD based on different databases are presented in Table 3.2. *Mean packyears are calculated for ex-smokers and current smokers. † Clinical COPD Questionnaire (CCQ)-score[23]. ‡Less than good self-reported health: bad/moderate/reasonable, reference category: good/excellent self-reported health.

Discussion

In a general population sample of adults aged 20-72 years from the Netherlands, we found that COPD-prevalence varied depending on the used definition (2.9-10.9%). The overlap between COPD-prevalence based on the four different operational definitions was low. Self-reported or EMR-based COPD identified less than 30% of all subjects with spirometry-based COPD, but specificity was high. Despite the variation in prevalence estimates, low overlap and low sensitivity, the direction of associations between potential risk factors and all four operational definitions of COPD were more or less similar, although the magnitude and statistical significance of the associations varied between the definitions. The combination of a relatively low prevalence and high specificity of self-reported and EMR-based COPD compared to both LLN and GOLD as a reference explains the minor changes in the associations between risk factors with the different COPD-definitions[28]. A high specificity causes a relatively low number of 'false positive' COPD cases in the 'true positive' COPD group. COPD-prevalence was substantially higher based on pre- instead of post-BD measurements. We found similar associations with risk factors when using pre- or post-BD spirometry, but the associations with risk factors were stronger and more often significant when COPD was based on post-BD measurements.

The highest COPD-prevalence was found based on the GOLD-definition (10.9%), followed by spirometry LLN-definition (5.9%), self-report (4.6%) and EMR (2.9%). Prevalence estimates were comparable with spirometry-based prevalence estimates in the larger general population studies. The PLATINO-study (n=5,571, age ≥40 years) in Latin America found a prevalence varying between 7.8%-19.7% based on post-BD GOLD-definition[26]. The BOLD-study (n=9,425, 52-60 years) estimated world-wide COPD-prevalence to be 10.1% (GOLD-2 or higher) based on post-BD measurements[25]. The number of studies in the general population comparing prevalence estimates based on different data sources is limited. Celli *et al.* found a twice as high COPD-prevalence based on pre-BD spirometry compared with self-reported COPD (self-report: 7.7%, LLN: 14.2%, GOLD: 16.8%) in the United States population (n=9,838, mean±SD 48.3 ± 13.6 years) (6). Despite the use of pre-BD spirometry, a Swedish study conducted in a population-based sample (n=3,892, mean±SD 51.7 ± 10.6 years), found a lower COPD-prevalence compared with our results, (LLN: 4.2%, GOLD: 9.4%, self-report: 0.8%)[5]. This is possibly explained due to exclusion of subjects with physician-diagnosed asthma in the Swedish study. Mohangoo *et al.* (8)(n=12,699, mean±SD 39±23 years) found almost twice as high self-reported "asthma or COPD" prevalence compared to the prevalence based on GP data. Our study also found higher COPD-prevalence based on self-reported data compared to GP data.

Other studies also confirmed underestimation of COPD in the general population when using self-reported or EMR-data[2,3,5,7,10]. Pulmonary specialists may argue that COPD only based on spirometry is an overestimation since for a clinical COPD diagnosis also other indicators are needed like respiratory symptoms, family history of COPD, or history of exposure to risk factors[1]. We want to emphasize that this study aims to assess COPD for epidemiological usage and not for clinical case finding. Therefore COPD based on only lung function criteria is justifiable. Therefore COPD based on only lung function criteria is justifiable. On the other hand there are also arguments for underestimation of COPD prevalence based on spirometry since the likelihood of producing a reproducible spirometric measurement decreases with disease severity. We excluded non-reproducible tests and therefore it is likely to selectively exclude a higher proportion of subjects with airflow obstruction[2]. Furthermore, COPD is a slowly progressive disease and symptoms slowly worsen over time[1]. People adapt to these slowly developing respiratory problems and might be unaware of their condition and may not visit a GP. This could explain the low sensitivity of self-reported or EMR–based COPD. Furthermore, self-reported COPD or a diagnosis of COPD in EMR will also depend on the severity of the disease which is highly associated with care seeking behavior[29]. The CCQ-score and self-reported health – indicators of the severity of the disease - were both more strongly associated with self-reported- and EMR-based COPD compared to spirometry-based COPD. Decline in lung function occurs faster in earlier stages of the disease. Therefore, early diagnosis may slow disease progression by physical activity and prevention of exposure to smoke and other noxious agents. In addition, pharmacological intervention may control symptoms and improve quality of life[30,31].

A follow-up study by de Marco *et al.*[11] in young adults (ECRHS-II study, n=4,636, 20-44 years old at the time of inclusion) studied risk factors of new-onset COPD and compared associations between risk factors and several pre-BD spirometric COPD-definitions. The association with LLN-based COPD incidence and gender, age, and being underweight lost their statistical significance compared to GOLD-based COPD incidence. We found similar associations with age, gender and underweight and these associations were also stronger with pre-BD GOLD-based COPD compared with LLN. However, being underweight was stronger associated with LLN-based COPD than GOLD when using post-BD measurements.

In our study, most associations between risk factors and different COPD-definitions had a similar magnitude and overlapping confidence intervals, except for the associations with allergy indicators. We found strong positive associations between self-reported COPD and indicators for allergy. Allergy is associated with asthma, and the association between COPD and current asthma was more prominent for self-reported COPD than for the other COPD-definitions. The associations between self-reported COPD and allergy indicators became weaker when subjects with current asthma were excluded, this indicates that some misclassification in self-reported COPD may be present due to overlap with asthma. Except for allergy indicators, this study overall indicates that for epidemiological studies with the aim to evaluate risk factors for COPD, the influence of the used definition seems to be limited. However, we focused only on risk factors that are known to be associated with COPD, and we can only speculate that the influence of different COPD-definitions on associations with unknown risk factors is limited.

Our population-based study is unique in the simultaneous use of three different data sources to assess COPD: post-BD spirometry, GP registrations, and ECRHS questionnaire items. We applied stringent quality standards to both spirometry and EMR data. In most population-based epidemiological studies based on spirometry, only pre-BD lung function measurements are available. It is not unsurprising that the prevalence estimates were higher when COPD was based on pre-BD spirometry. By using post-BD spirometry we studied fixed airway obstruction, which will reduce the number of false-positives due to overlap with asthma[32,33]. Nevertheless, the influence of using pre-BD instead of post-BD definitions on associations with potential risk factors, including current asthma, was limited. As expected, associations were somewhat stronger and more often significant when COPD was based on post-measurements, since a reduction in the number of false-positives will reduce measurement error and consequently will strengthen risk factor associations. To the best of our knowledge, this was not studied before in a population–based study. Another strength of our study was the extensive non-response analysis from the source population up to the current study population. We previously compared characteristics of non-responders and responders of the questionnaire survey (source-population)[16]. This study continued the non-response analysis by comparing characteristics of responders and non-responders in different stages of the data collection. Participants of the medical examination were older, more often female and more often former smokers compared to subjects who were invited but did not participate. Both in the previous analysis[16] and in the present study we were able to demonstrate that selection bias did not affect the associations under study, e.g. the association between self-reported COPD and potential risk factors (see Table S3.1).

The three different data sources were not collected at the same time, which is a limitation of our study. Questionnaire data on COPD were collected in November 2012, EMR from 2010-2012 were used, and spirometry was conducted between March 2014 and February 2015. However, it is unlikely that the lack of overlap is to a large degree explained by COPD development during the relatively short data collection period. Previous studies that did collect self-reported data and spirometry data synchronically, also found a large degree of non-overlap[5,6].
General population studies are often conducted in urban populations. Our study population lived in a rural area outside the larger cities and farmers were excluded. The prevalence of GP-diagnosed COPD in the study area did not differ from the prevalence in other Dutch rural areas without livestock farming (42.6 vs. 47.1 prevalence per 1,000 for patients aged >40 years, average over 2007-2013)[34], and we have no reason to expect that agreement between different COPD-definitions would be different in other areas.

Conclusions

The operational definition used for COPD greatly influences prevalence estimates. Self-reported or EMR-based COPD identified less than 30% of all subjects with COPD based on persistent airflow limitation, which implies that a substantial number of subjects with COPD cannot be identified by questionnaires or medical records. However, the effect of the different COPD-definitions on associations with potential risk factors was limited, except for indicators of allergy, which were more strongly associated with self-reported COPD compared to the other definitions. In addition, the use of pre-BD spirometry instead of post-BD spirometry resulted in higher prevalence estimates, but had a minimal effect on associations with potential risk factors. Researchers using these operational definitions to group individuals according to COPD status, need to be aware of the impact of such choices. Results of this study may be informative for population-based epidemiological studies with the aim to evaluate potential risk factors for COPD.

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Supplementary tables

Table S3.1 Association between self-reported COPD and several characteristics compared between different population subsets to study potential selection bias.

Characteristics	Self-reported COPD			
	Respondents of screening questionnaire who are eligible for follow-up study	**Subjects who agreed to be contacted for a follow-up study**	**Invited subjects for medical examination**	**Participants of medical examination**
Subjects n	14,163	8,714	7,180	2,494
Age (per 10 years)	**1.53 (1.42-1.66)**	**1.55 (1.41-1.72)**	**1.55 (1.39-1.73)**	**1.74 (1.39-2.16)**
Female gender	1.04 (0.88-1.22)	0.92 (0.75-1.13)	0.87 (0.69-1.09)	0.87 (0.61-1.25)
Ever smoker	**2.02 (1.68-2.43)**	**1.72 (1.37-2.16)**	**1.51 (1.18-1.94)**	**1.49 (1.01-2.20)**
Current asthma	**25.58 (20.77-31.50)**	**24.14 (18.74-31.09)**	**25.85 (19.44-34.39)**	**24.80 (15.29-40.23)**

OR and 95% CI were adjusted for age, gender, and ever smoking. Bold type indicates statistical significance ($p<0.05$). Self-reported COPD was defined as a positive answer to the question: 'Have you ever been told by a doctor that you had chronic obstructive pulmonary disease or emphysema?'. The sub-populations are represented in Figure 1 (main article).

Table S3.2 Associations between risk factors and severity measures with four different definitions of COPD, only subjects older than 39 years of age are included.

	Subjects aged 40 years and older (n=1,626)			
	Self-report	**EMR**	**Spirometry LLN**	**Spirometry GOLD**
N (%)	81 (5.0%)	52 (3.2%)	103 (6.3%)	196 (12.1%)
Age (per 10 years),mean (SD)	**1.70 (1.23-2.34)**	**2.26 (1.44-3.55)**	1.02 (0.77-1.34)	**1.64 (1.31-2.06)**
Female gender	0.71 (0.44-1.14)	1.20 (0.67-2.14)	0.75 (0.49-1.15)	**0.49 (0.35-0.68)**
Ever smoker	1.34 (0.81-2.21)	**4.13 (1.83-9.34)**	**4.46 (2.48-8.02)**	**3.47 (2.33-5.17)**
Pack years (per 10 years). Mean* (SD))	**1.19 (1.06-1.33)**	**1.20 (1.06-1.35)**	**1.30 (1.18-1.43)**	**1.23 (1.13-1.34)**
Occupational exposure to vapors, gases, dust or fumes	1.17 (0.70-1.94)	1.49 (0.79-2.82)	1.09 (0.69-1.73)	1.17 (0.82-1.67)
BMI < 20 (ref = BMI 20-25)	**7.08 (2.27-22.08)**	**9.47 (2.50-35.80)**	**5.28 (1.86-15.01)**	**2.79 (1.00-7.77)**
BMI > 25 (ref = BMI 20-25)	0.83 (0.49-1.39)	0.77 (0.41-1.46)	**0.58 (0.37-0.91)**	**0.57 (0.40-0.82)**
High education level (ref = low/ medium)	0.66 (0.37-1.19)	**0.26 (0.09-0.73)**	**0.47 (0.26-0.83)**	**0.63 (0.42-0.94)**
Current asthma	**31.2 (16.7-58.4)**	**7.16 (3.23-15.88)**	**2.62 (1.24-5.56)**	**2.55(1.34-4.84)**
Self-reported ever allergy	**3.17 (1.94-5.17)**	**1.86 (1.03-3.38)**	1.22 (0.78-1.89)	1.28 (0.91-1.80)
Atopy	**2.34 (1.45-3.76)**	1.83 (0.99-3.36)	1.34 (0.85-2.12)	1.05 (0.73-1.52)
> 1 positive for specific IgE	**2.59 (1.55-4.33)**	1.76 (0.87-3.56)	1.50 (0.89-2.52)	1.30 (0.86-1.98)
Total IgE >= 100 IU/ml	**2.87 (1.74-4.73)**	**2.56 (1.36-4.81)**	**1.90 (1.17-3.08)**	1.37 (0.91-2.06)
GOLD-1 † (ref = $FEV_1/FVC > 0.7$)	**2.69 (1.28-5.63)**	**4.33 (1.62-11.56)**	NA	NA
GOLD 2-4 † (ref = $FEV_1/FVC > 0.7$)	**28.5 (14.5-56.1)**	**52.5 (23.9-115.7)**	NA	NA
CCQ-score‡, mean (SD)	**3.95 (2.92-5.35)**	**4.02 (2.81-5.76)**	**3.10 (2.34-4.12)**	**2.17 (1.70-2.78)**
Less than good self-reported health §	**6.03 (3.68-9.88)**	**6.67 (3.58-12.43)**	**2.40 (1.55-3.72)**	**1.58 (1.10-2.26)**

Data are presented as mean ±SD or %, unless otherwise stated. OR and 95% CI were adjusted for age, gender, ever smoking and pack years (number of pack years was mean-centered for ex- and current smokers). Bold type indicates statistical significance ($p<0.05$). Self-reported: self-reported data based on the ECRHSIII screening questionnaire, EMR: Electronic Medical Records, spirometry: post-bronchodilator lung function measurement. Used definitions for COPD based on different databases are presented in Table 3.2. *Mean packyears are calculated for ex-smokers and current smokers. † GOLD 1: FEV1/FVC<0.70 and FEV1≥ 80% predicted, GOLD 2-4: FEV1/FVC<0.7 and FEV1<80% predicted. ‡ Clinical COPD Questionnaire (CCQ)-score (van der Molen et al. 'Development, validity and responsiveness of the Clinical COPD. Questionnaire.' Health Qual Life Outcomes 2003;1:13.) NA: Not available, as very few (LLN) or no (GOLD) subjects with spirometry-based COPD had FEV1/FVC>0.7. §Less than good self-reported health: bad/moderate/reasonable, reference category: good/excellent self-reported health.

Table S3.3 Associations between risk factors and severity measures with four different definitions of COPD, subjects with current asthma are excluded.

	Subjects without current asthma (n=1,716)			
	Self-report	**EMR**	**Spirometry LLN**	**Spirometry GOLD**
N (%)	52 (3.0%)	42 (2.5%)	95 (5.5%)	180 (10.5%)
Age (per 10 years),mean (SD)	**2.28 (1.51-3.45)**	**2.76 (1.66-4.59)**	1.06 (0.83-1.35)	**1.78 (1.44-2.21)**
Female gender	0.82 (0.46-1.48)	1.08 (0.56-2.06)	0.78 (0.50-1.22)	**0.51 (0.36-0.72)**
Ever smoker	1.58 (0.83-3.01)	**3.86 (1.59-9.36)**	**4.37 (2.41-7.92)**	**3.59 (2.37-5.44)**
Pack years (per 10 years).Mean* (SD))	**1.19 (1.04-1.35)**	**1.18 (1.03-1.34)**	**1.32 (1.19-1.46)**	**1.22 (1.12-1.34)**
Occupational exposure to vapors, gases, dust or fumes	1.11 (0.59-2.10)	1.19 (0.59-2.40)	0.98 (0.61-1.59)	1.10 (0.76-1.59)
BMI<20 (ref=BMI 20-25)	**9.81 (2.61-36.91)**	**7.94 (1.78-35.39)**	**5.73 (2.05-16.03)**	2.45 (0.83-7.25)
BMI>25 (ref=BMI 20-25)	0.63 (0.34-1.18)	0.64 (0.32-1.27)	**0.53 (0.33-0.84)**	**0.57 (0.40-0.82)**
High education level (ref=low/ medium)	0.56 (0.26-1.23)	**0.24 (0.07-0.78)**	**0.49 (0.27-0.88)**	**0.60 (0.39-0.91)**
Self-reported ever allergy	**2.60 (1.42-4.77)**	1.75 (0.90-3.40)	1.20 (0.76-1.90)	1.19 (0.83-1.71)
Atopy	1.66 (0.90-3.06)	1.39 (0.69-2.81)	1.14 (0.70-1.85)	0.89 (0.60-1.32)
>1 positive for specific IgE	1.28 (0.60-2.73)	1.12 (0.46-2.74)	1.09 (0.61-1.95)	0.98 (0.61-1.57)
Total IgE≥100 IU/ml	**2.48 (1.31-4.70)**	**2.07 (1.00-4.29)**	**1.74 (1.04-2.92)**	1.20 (0.77-1.86)
GOLD-1 † (ref=FEV_1/FVC>0.7)	2.06 (0.72-5.86)	**6.70 (2.31-19.42)**	NA	NA
GOLD 2-4 † (ref=FEV_1/FVC>0.7)	**44.8 (19.8-101.1)**	**76.2 (30.4-190.5)**	NA	NA
CCQ-score‡, mean (SD)	**3.99 (2.77-5.75)**	**3.77 (2.55-5.59)**	**2.80 (2.07-3.78)**	**2.07 (1.58-2.70)**
Less than good self-reported health §	**7.15 (3.82-13.36)**	**6.09 (3.09-11.98)**	**2.12 (1.34-3.36)**	**1.47 (1.01-2.15)**

Data are presented as mean ±SD or %, unless otherwise stated. OR and 95% CI were adjusted for age, gender, ever smoking and pack years (number of pack years was mean-centered for ex- and current smokers). Bold type indicates statistical significance ($p<0.05$). Self-reported: self-reported data based on the ECRHSIII screening questionnaire, EMR: Electronic Medical Records, spirometry: post-bronchodilator lung function measurement. Used definitions for COPD based on different databases are presented in Table 3.2. *Mean packyears are calculated for ex-smokers and current smokers. † GOLD 1: FEV_1/FVC<0.70 and FEV_1≥80% predicted, GOLD 2-4: FEV_1/FVC<0.7 and FEV_1<80% predicted. ‡ Clinical COPD Questionnaire (CCQ)-score (van der Molen et al. 'Development, validity and responsiveness of the Clinical COPD Questionnaire.' Health Qual Life Outcomes 2003;1:13.) NA: Not available, as very few (LLN) or no (GOLD) subjects with spirometry-based COPD had FEV_1/FVC>0.7. §Less than good self-reported health: bad/moderate/reasonable, reference category: good/excellent self-reported health.

Chapter 4

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).

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].

Materials and methods

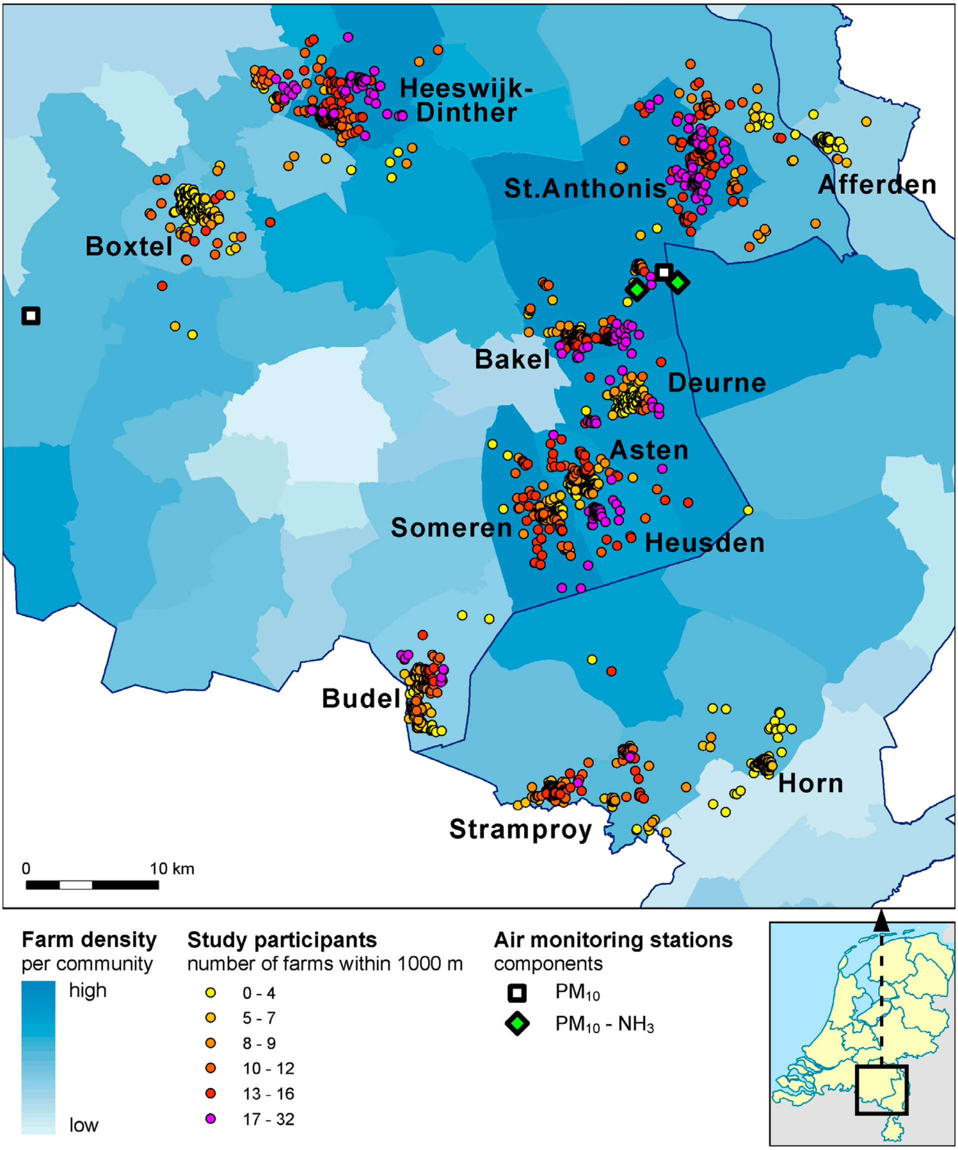
Study population and design

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 4.1). 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].

Figure 4.1 Map of the study area.



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 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 4.1). 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.

Table 4.1 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
Never smoker	1122 (45.0%)
Ever smoker	1112 (44.6%)
Current smoker	252 (10.1%)
Pack years†	18.1 ± 17.9
Education level, low	607 (24.3%)
Education level, medium	1112 (44.6%)
Education level, high	746 (29.9%)
Farm childhood	837 (33.6%)
Grown up in study area	1871 (75.0%)
Lung function characteristics	
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 ǁ	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 ever smoked. 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. ǁ 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).

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 4.2 and more details in 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 (Supplementary Table S4.3).

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 Supplementary Table S4.1). 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 4.2). 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 500m and 1,000m. Spirometry results expressed as z-scores showed minimal differences compared to results presented as % predicted values (see supplementary Figure S4.1). 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 4.2). 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 Supplementary Figure S4.2).

Table 4.2 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)

Table 4.2 (continued)

	Agreed to be contacted for follow-up study			Participated in medical examination		
	Yes	No	Adjusted OR (95% CI)	Yes	No	Adjusted OR (95% CI)
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 $g*year\text{-}1*m^{-2}$)	1.17	1.18	1.00 (0.99-1.02)	1.83	1.33	**1.08 (1.06-1.10)**

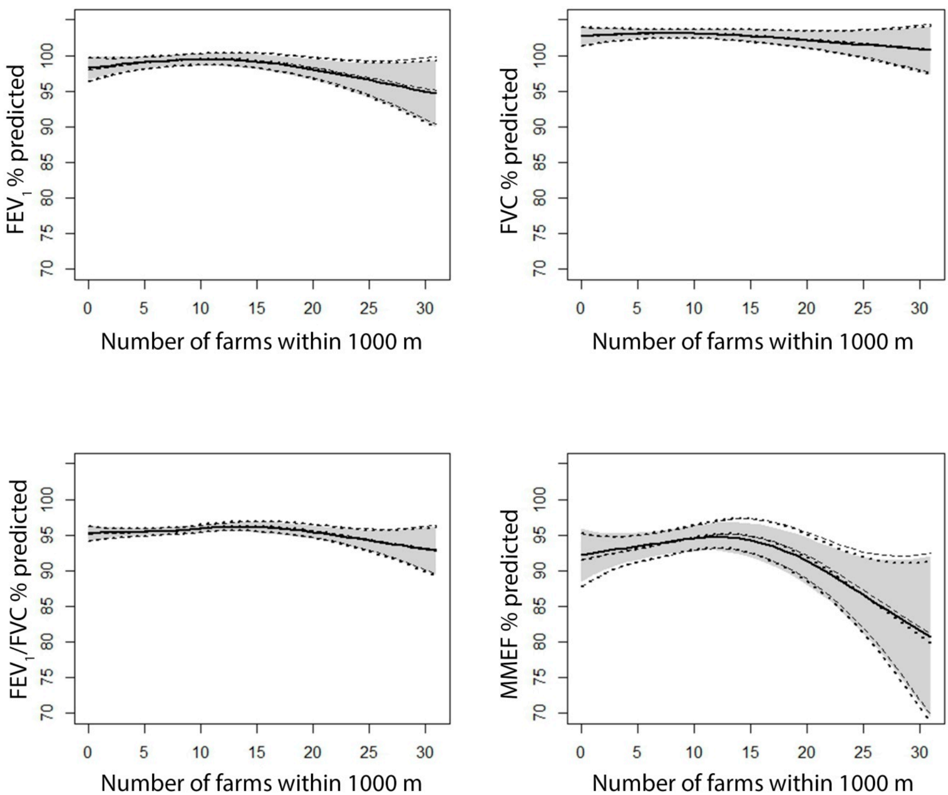
The likelihood of agreeing to follow-up / being a participant is modeled for different characteristics with logistic regression. OR (95% CI) were adjusted for age, gender and smoking habits. Bold type indicates statistical significance ($p < 0.05$). In total 14,163 subjects responded to the short questionnaire[20], 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.

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 4.3) 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 Supplementary Figure S4.3A). Adjustment for farm density around the home address did not change the association between lung function and NH_3 (see Figure 4.4). Spirometry results expressed as z-scores showed minimal differences compared to results presented as % predicted values (see Supplementary Figure S4.4). Smoothed plots showed similar negative linear associations between all lung function variables and week-average PM_{10} (See Supplementary Figure S4.5). However, in a two-pollutant model, only NH_3 remained associated with lung function (see Figure 4.4 and Supplementary Figure S4.5 and S4.6).

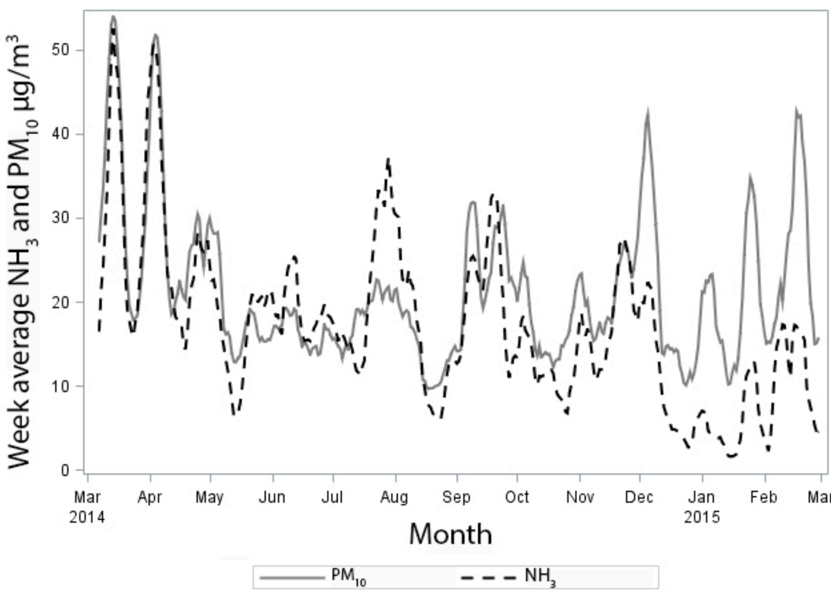
Figure 4.2 Association between the number of livestock farms within 1,000 m of the home address and lung function in 2,308 residents.



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: FEV_1:0.116; FVC:0.347; FEV_1/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 NH_3 (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: 0.142; FVC:0.355; FEV_1/FVC:0.106; MMEF:0.051. The dotted lines show the models with further adjustment for week-average ambient PM_{10} (μg/m^3) levels prior to the lung function test. P-values of the smooth terms after adjustment for week-average PM_{10} levels are: FEV_1: 0.101; FVC:0.361; FEV_1/FVC:0.081; MMEF:0.030.

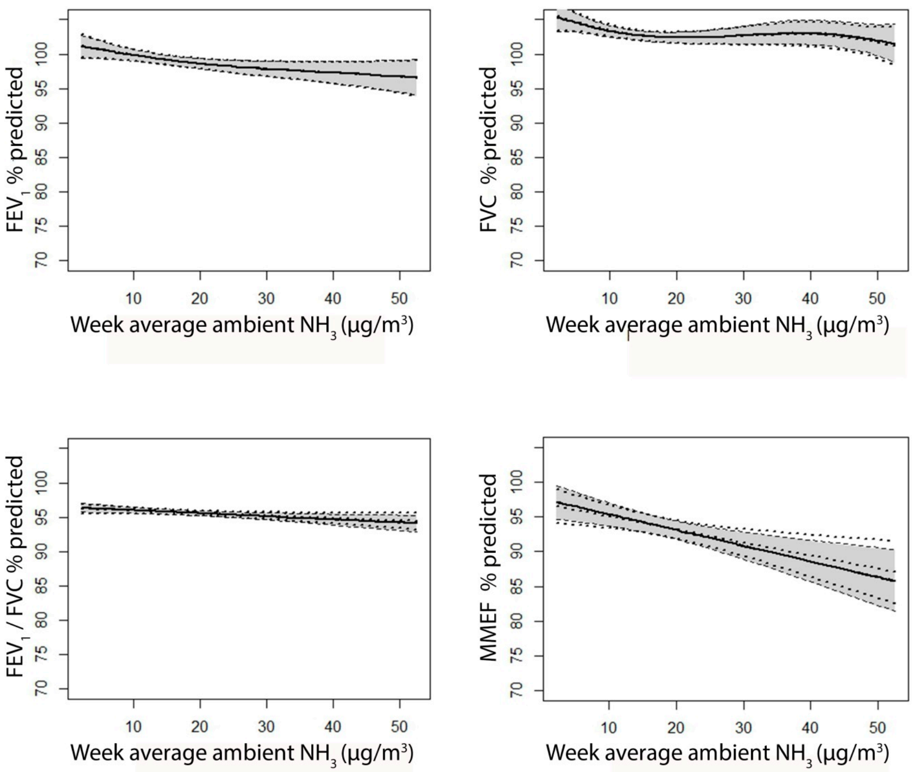
Linear regression analyses showed that a change in week-average NH_3 levels between the P10 and P90 (25.1 μg/m^3) was associated with a difference in FEV_1 of -2.22 (95%CI -3.69 to -0.74), FVC: -1.07 (95%CI -2.33 to 0.20), FEV_1/FVC: -1.12 (95%CI -1.96 to -0.28) and MMEF: -5.67 (95%CI -8.80 to -2.55). 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 supplementary Figure E6). 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.

Figure 4.3 Week-average ambient NH_3 and PM_{10} ($\mu g/m^3$) levels in the study area.



Week-average ambient NH_3 and PM_{10} 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 NH_3 and PM_{10} levels was r=0.64.

Figure 4.4 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 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:0.098; FEV_1/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: FEV_1: 0.015; FVC:0.107; FEV_1/FVC:0.008; MMEF:<0.001. The dotted lines show the models for further adjustment for week-average ambient PM_{10} (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: 0.101; FVC:0.133; FEV_1/FVC:0.196; MMEF:0.070.

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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Supplementary methods, tables and figures

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].

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 S4.1 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 and PM_{10} 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} $r\geq0.88$). 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 2.5 µm ($PM_{2.5}$) 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 s4.2.

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 supplementary Figure E3A and B). 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 4.2 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 (Supplementary Table S4.3). 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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Table S4.1 Overview of the number of farms and farm animals in the study area.

	N farms	Mean nr of animals per farm	Number of animals in quartiles			
			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.

Table S4.2 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 S4.3 Associations between different farm exposure estimates and indicators of respiratory health compared between different population subsets to study potential selection bias.

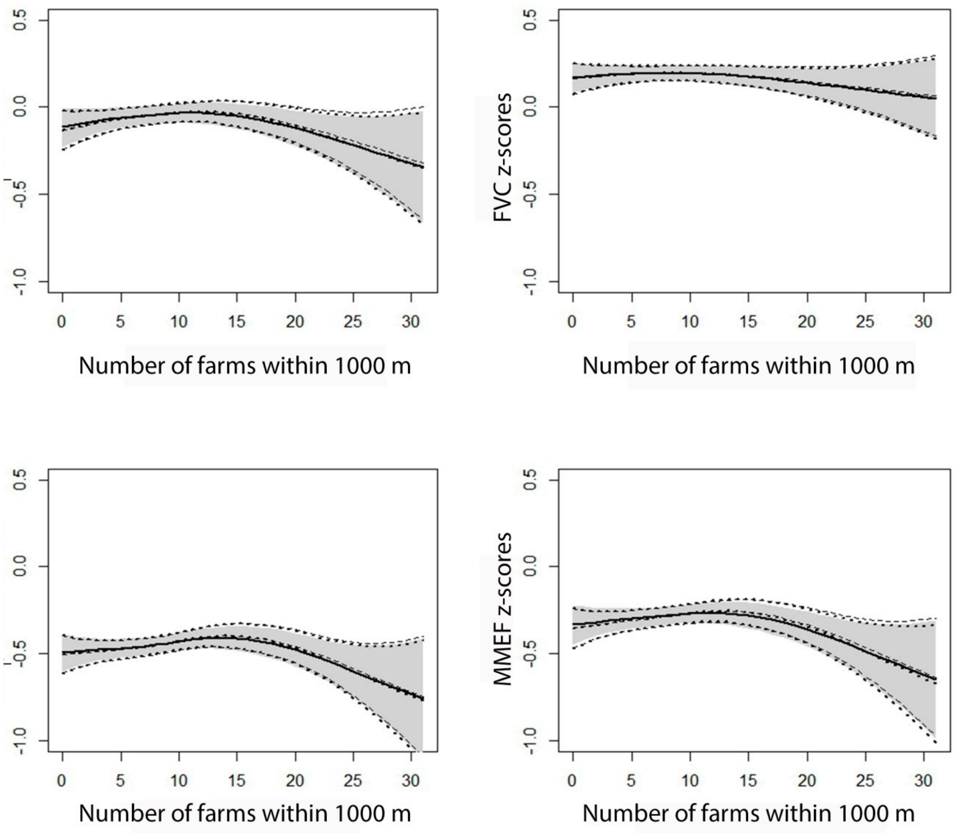
		Source population (n=14,163)	Subjects who agreed to follow-up (n=8,714)	Subjects invited for medical examination (n=7,180)	Subjects who participated in medical examination (n=2,494)
		OR (95% CI) adjusted	**OR (95% CI) adjusted**	**OR (95% CI) adjusted**	**OR (95% CI) adjusted**
Current asthma	**Presence 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)**

Table S4.3 (continued)

		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)
	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)

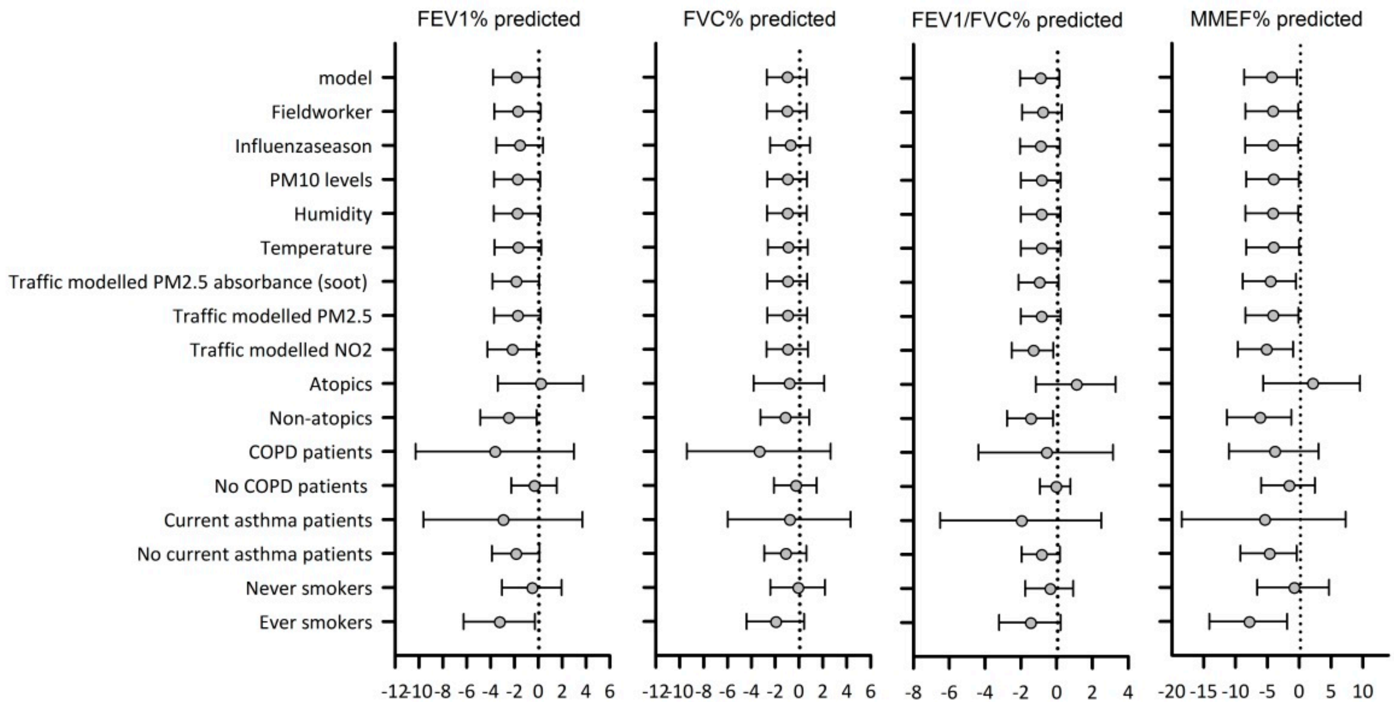
OR and 95% CI were adjusted for age, gender and smoking habits. Bold type indicates statistical significance ($p<0.05$). Information on self-reported respiratory health, age, gender and smoking habits was assessed with the first questionnaire collected in November 2012 as described previously[1]. The definitions used for self-reported respiratory morbidity are presented in Table S4.2.

Figure S4.1 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.



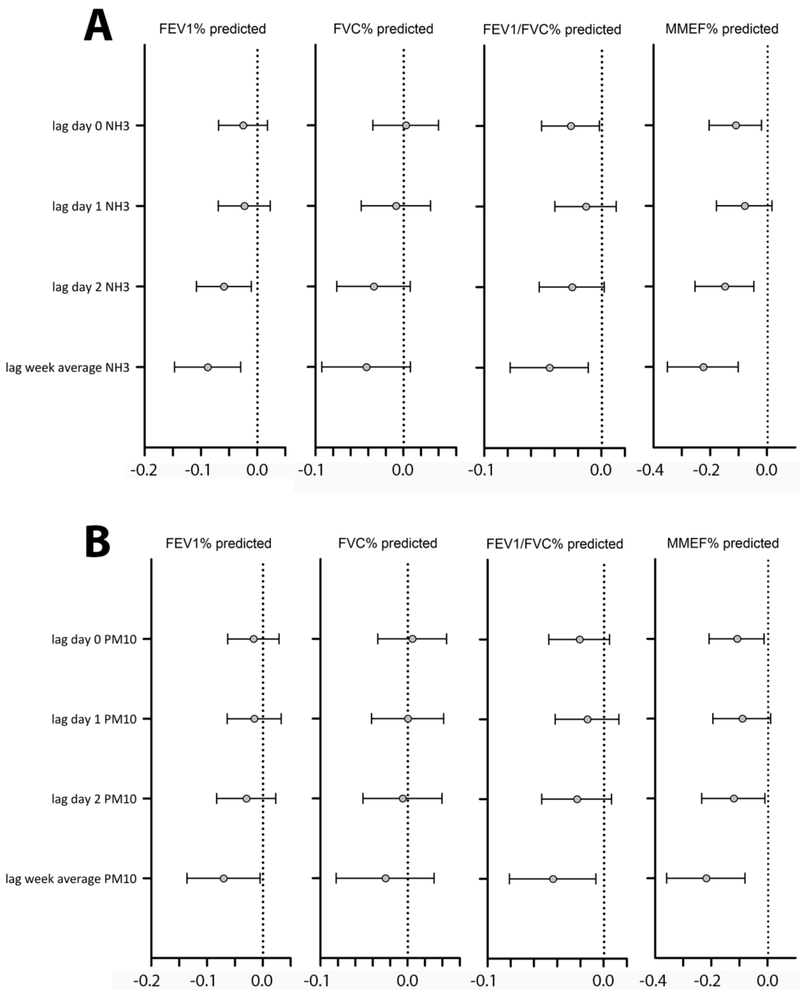
Smoothed plots show the association between the number of livestock farms within 1,000 m of the home address and lung function. P-values of the smooth terms are: FEV_1:0.108; FVC:0.402; FEV_1/FVC:0.140; MMEF: 0.073. Adjustment for age, gender and height was made by calculating % predicted spirometry 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_3 ($\mu g/m^3$) 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_{10} ($\mu g/m^3$) levels 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 S4.2 Sensitivity analyses on ‘Hotspot model’, i.e. ≥17 farms versus <17 farms within 1,000 m.



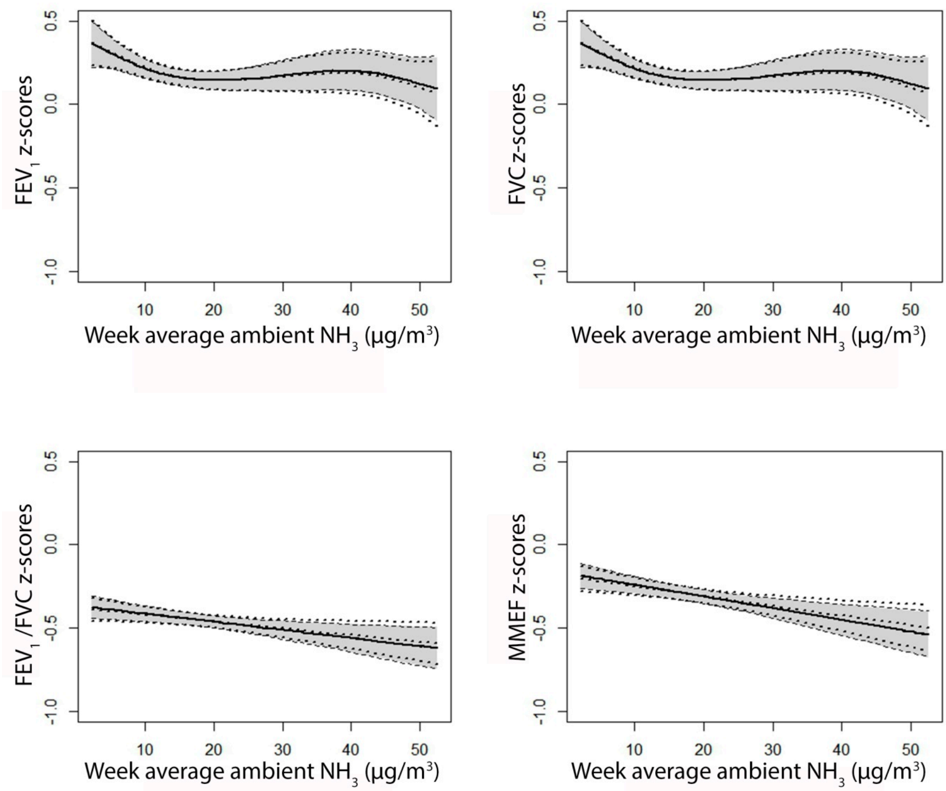
The association between living in a ‘hotspot’ and lung function variables, and additional sensitivity analyses (additional adjustment and analyses in subgroups) were analyzed with linear regression analyses. Adjustment for age, gender and height was made by calculating % predicted spirometry variables 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 FEV1 of -1.86 (95%CI -3.80 to 0.09), FVC:-1.03 (95%CI -2.70 to 0.64), FEV1/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 FEV1/FVC (p=0.03) and MMEF (p=0.02).

Figure S4.3 Association between lung function variables and ambient NH_3 and PM_{10} levels on multiple lag days.



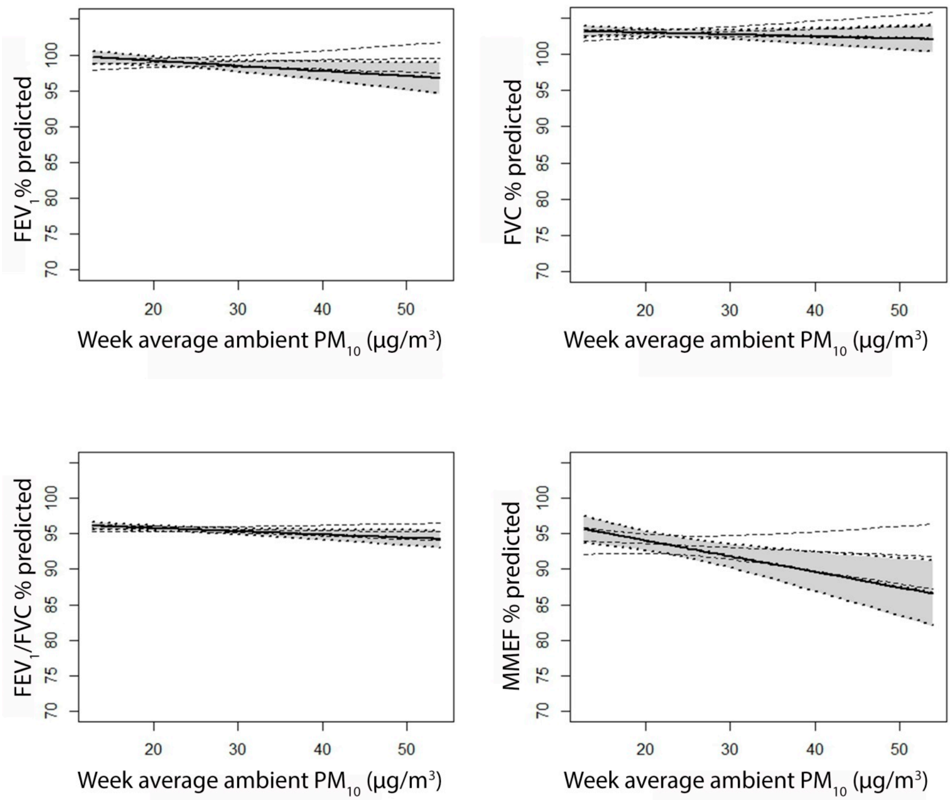
Associations are presented for a 1 µg/m^3 increase of NH_3 (A) or PM_{10} (B).

Figure S4.4 Associations between ambient NH_3 ($\mu g/m^3$) in the week before the lung function test and lung function expressed as z-scores in 2308 residents.



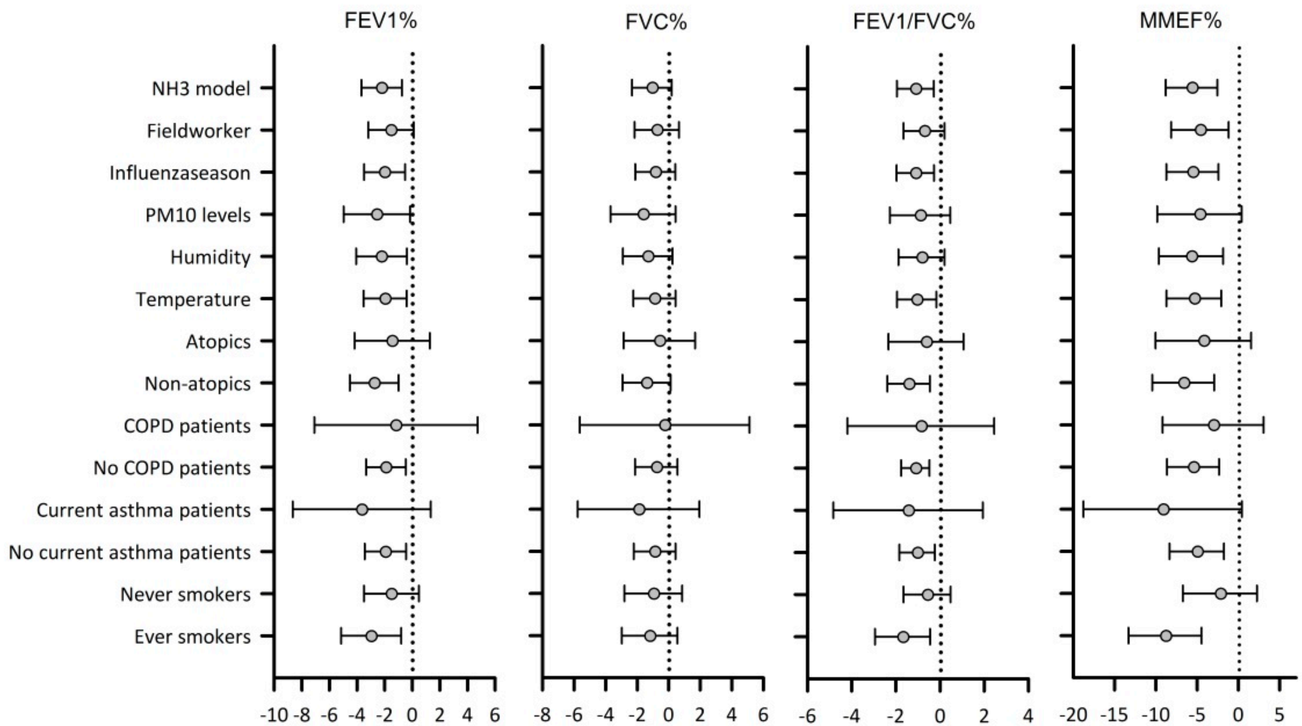
Smoothed plots show the association between the week-average ambient NH_3 ($\mu g/m^3$) levels prior to the lung function test and lung function. P-values of the smooth terms are: FEV_1: 0.012; FVC:0.062; FEV_1/FVC:0.007; MMEF:<0.001. Adjustment for age, gender and height was made by calculating % predicted 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_{10} ($\mu g/m^3$) 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 S4.5 Associations between ambient PM_{10} ($\mu g/m^3$) 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_{10} ($\mu g/m^3$) 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_3 ($\mu g/m^3$) 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 S4.6 Sensitivity analyses on NH3 model.



The association between week-average ambient NH3 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 NH3 model are expressed per P90-P10 increase in week-average NH3 (P90-P10 NH3 = 25.1 µg/m3). NH3 ('NH3 model') was associated with a change in FEV1 of -2.22 (95%CI -3.69 to -0.74), FVC: -1.07 (95%CI -2.33 to 0.20), FEV1/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 NH3 levels were observed.

Chapter 5

Residential proximity to livestock farms is associated with a lower prevalence of atopy

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Abstract

Objectives: Exposure to farm environments during childhood and adult life seems to reduce the risk of atopic sensitization. Most studies have been conducted among farmers, but people living in rural areas may have similar protective effects for atopy. This study aims to investigate the association between residential proximity to livestock farms and atopy among non-farming adults living in a rural area in the Netherlands.

Methods: We conducted a cross-sectional study among 2,443 adults (20-72 years). Atopy was defined as specific IgE to common allergens and/or total IgE ≥100 IU/ml. Residential proximity to livestock farms was assessed as 1) distance to the nearest pig, poultry, cattle or any farm, 2) number of farms within 500m and 1000m, and 3) modelled annual average fine dust emissions from farms within 500m and 1000m. Data were analysed with multiple logistic regression and generalized additive models.

Results: The prevalence of atopy was 29.8%. Subjects living at short distances from farms (<327 m, first tertile) had a lower odds for atopy compared to subjects living further away (>527 m, third tertile) (OR 0.79, 95% CI 0.63-0.98). Significant associations in the same direction were found with distance to the nearest pig or cattle farm. The associations between atopy and livestock farm exposure were somewhat stronger in subjects who grew up on a farm.

Conclusions: Living in close proximity to livestock farms seems to protect against atopy. This study provides evidence that protective effects of early-life and adult farm exposures may extend beyond farming populations.

Introduction

It is now well established that children growing up on farms are less likely to develop allergic disease than children living in the same area but with non-farming parents[1,2]. This protective effect seems to be retained in adulthood, since adults with early-life exposure to a farm environment still have a lower prevalence of atopy[3-8]. A few epidemiological studies indicate that not only exposure during early life is protecting but occupational farm exposures during adulthood may also prevent from atopic sensitization[9-12]. Farming families are exposed to higher loads of microbial agents and to greater microbial diversity[13-15]. Environmental exposure to endotoxin – a cell-wall component of gram-negative bacteria - was inversely associated with atopy among children in rural environments[13]. There is some evidence that exposure to greater microbial diversity during early life, but possibly also during adult life, prevents the development of allergic diseases[14,16,17].

Although the beneficial effect of farm exposure has mainly been shown in farming families, it may extend to inhabitants of rural areas since livestock farm emissions include particles containing microorganisms[18]. Previous studies have indeed shown that higher levels of microbial exposure were found in close proximity to farms and in the neighborhoods of farming areas[19-21]. The association between atopy and farm proximity is poorly studied in the general and non-farming populations. A Danish study found an urban-rural gradient of allergic sensitization in adults depending on their residence during childhood[22]. Moreover, a German study found a similar urban-rural effect on atopic sensitization by comparing atopy prevalence in farmers, rural, suburban and urban residents. Both studies suggest that living in a rural environment might be protective[23]. Two cross-sectional studies in a rural area in the Netherlands found inverse associations between indicators of livestock farm emissions and allergic rhinitis among subjects of a general population[24,25]. However, both studies lacked information on history of livestock farm exposure, and allergic rhinitis was based on self-reported data[24] and Electronic Medical Records[25].

The current study aims to investigate the association between farm proximity and atopy among 2,443 non-farming adults living in a rural area with a high farm density in the Netherlands. To our knowledge this is the first study that studied the association between residential proximity to livestock farms, while taking the contribution of a farm childhood into account. Furthermore, our analysis is based on objective markers of atopy - including total IgE and specific IgE to common allergens – which lowers the risk of misclassification.

Methods

Study population and study design

This study is part of the VGO study (Dutch acronym for Livestock Farming and Neighboring Residents' Health), a cross-sectional study conducted in the eastern part of the province of Noord-Brabant and the northern part of Limburg, a rural area in the South of the Netherlands characterized by a high farm density. The study population originates from participants of a questionnaire survey (n=14,163) conducted in November – December 2012 which is previously described by Borlée *et al.*[24]. Questionnaire respondents 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). Between March 2014 and February 2015, in total 7,180 participants were invited for medical examination which resulted in 2,494 participants (response 34.7%). From 2,443 individuals a serum sample could be obtained (98.0%). The medical examination included collection of serum and an extended questionnaire and height and weight measurement, more details are previous described[26,27].

The study protocol (13/533) was approved by the Medical Ethical Committee of the University Medical Centre Utrecht. All 2,494 subjects signed an informed consent form.

Atopy: IgE Serology

In our main analyses, atopy was defined as specific serum IgE antibodies ≥0.35 IU/ml 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[28]. All samples were tested in the same laboratory.

Livestock farm exposure

Livestock farm exposure proxies were computed for each subject. We used data from the Provincial databases with 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. Livestock farm proximity to the home address for each participant 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) distance (m) to the nearest pig, poultry, cattle, any livestock farm; 2) total number of farms within 500 and 1,000 m (pig, poultry, cattle farms and any farm (independent of animal species)); 3) inverse-distance weighted fine dust emissions from all farms within 500m and 1,000 m as described previously[25].

Questionnaire

The questionnaire, collected during the medical examination, comprised amongst others items on symptoms and diseases, smoking habits, education, profession, current animal contact, place of birth and history of living on a farm during childhood.

Data analysis

Associations between proxies of livestock farm exposures and atopy were assessed by multiple logistic regression analysis. The distance to the nearest farm (pig, poultry, cattle and any livestock farm) and weighted fine dust emission from farms within 500 and 1000m was categorized into tertiles based on an equal number of atopy cases in each category, which provides a similar variance for odds ratios across categories. The shape of the relationship between atopy and livestock farm exposure variables was further 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. To test whether the goodness-of-fit of the models that contain splines was significantly better than linear models, we used Chi-Square tests. Associations between atopy and potential confounders were analyzed with univariate logistic regression and confounders with a P-value <0.2 were selected beforehand. Associations between atopy and the potential confounders were studied with multiple logistic regression modelling following a forward stepwise procedure based on improvement of Akaike’s Information Criterion (AIC). As a result, all models were adjusted for gender, age, smoking habits (ever smoking and pack years), education (high versus middle / low education), being born in the study area, and history of living on a farm during childhood. The presence of a specific farm animal farm was also adjusted for the presence of other types of farm animal species. To evaluate potential heterogeneity of effects due to a history of living on a farm, we stratified for farm childhood and moreover we tested for interaction (farm childhood * farm proximity). Data were analysed using SAS 9.4 (SAS Institute Inc. Cary, NC, USA) and R version 3.02 (www.r-project.org).

Several sensitivity analyses were conducted to investigate the robustness of our findings. First, we repeated data analyses with two alternative definitions of atopy: a positive test to at least one specific allergen (yes/no), or total IgE>100 IU/ml (yes/no). Second, we studied the potential effect of variables associated with current contact with livestock farm animals, namely contact with farm animals at home or during a farm visit, and contact with animals during study or work. Further, we assumed that prolonged exposure might have a stronger protective effect, therefore we conducted sensitivity analyses with subjects who lived at least 5 years in their current home. We also stratified analyses by 'allergic symptoms' to assess the effect of exposure on atopy in combination with symptoms and without symptoms. 'Allergic symptoms' were defined as self-reported allergy against house dust mite, animals, or plants and pollen including one of the following symptoms: sneezing or running nose, shortness of breath, itchy skin or erythema, itchy or tearing eyes. We also tested for interaction between farm proximity and allergic symptoms. Finally, to evaluate potential migration of atopic subjects from rural areas to more urbanized areas, we compared associations with the number of years subjects lived in their current home and farm proximity (minimal distance to the nearest farm and farm density within 1000m) stratified by atopy. Atopic subjects living in close proximity to farms, might migrate more often and therefore live a shorter period in their home. If selective migration due to atopic sensitization occurred, we would expect a different relationship between the number of years they have lived in their current home and farm proximity among atopic and non-atopic individuals. These analyses were adjusted for gender, age, smoking habits, education, being born in the study area, and history of living on a farm during childhood as well.

Results

Our study population consisted for 54.5% of females and the average age was 56.4 years (Table 5.1). The prevalence of atopy was 29.8% in the total population. IgE to grass (11.8%) and house dust mite (11.7%) were more prevalent than IgE against cat (5.2%) and dog (3.9%). In total 33.5% had a history of living on a farm during childhood, those were mostly raised on mixed farms with multiple animal species and crop farming (data not shown). Subjects who grew up on a farm were less often atopic compared to subjects who did not have a farm childhood history (21.6% *versus* 33.9% see Table 5.2).

Table 5.1 Characteristics of the study population.

Characteristics	All (n=2,443)
Age, years	56.4 ± 11.0
Female gender	1,331 (54.5)
BMI*	27.0 ± 4.2
Ever smoker	1,403 (57.4)
Pack years†	17.9 ± 17.7
Born in study area	1,831 (75.0)
High education≠	738 (30.2)
History of living on a farm during childhood	818 (33.5)
Contact at home or during farm visit with farm animals∞	1014 (41.5)
During work/study contact with animals	148 (6.1)
Atopic sensitisation	
Atopy	727 (29.8)
Total IgE ≥ 100 Ku/L	495 (20.3)
Specific IgE to ≥1 common allergen	444 (18.2)
House dust mite IgE	285 (11.7)
Grass IgE	287 (11.8)
Cat IgE	127 (5.2)
Dog IgE	95 (3.9)
Distance to the nearest farm (meters)	
Any farm	439 ± 263
Pig farm	692 ± 343
Poultry farm	873 ± 408
Cattle farm	503 ± 271
Mean number of livestock farms within 500m	
Any farm	1.8 ± 2.1
Pig farm	0.4 ± 0.9
Poultry farm	0.2 ± 0.5
Cattle farm	0.9 ± 1.2
Mean number of livestock farms within 1000m	
Any farm	9.3 ± 5.9
Pig farm	2.3 ± 2.6
Poultry farm	1.1 ± 1.4
Cattle farm	4.0 ± 2.9
Modelled fine dust emission	
Weighted fine dust emission from farms within 500m, median ± SD ($g*year^{-1} * m^{-2}$)	0.07 ± 63.12
Weighted fine dust emission from farms within 1000m, median ± SD ($g*year^{-1} * m^{-2}$)	1.83 ± 12.76

Data are presented as mean ± SD or n (%), unless indicated otherwise. *BMI: body mass index=mass (kg)/(height (m))$^{2.}$ † Mean pack years for subjects who ever smoked. Number of pack-years = (packs smoked per day) × (years as a smoker). ≠ High educational level: upper vocational education or university. ∞Farm animals were horses, pigs, poultry, cows, goats and sheep. Contact was defined as touching the animal and/or touching the droppings of the animal.

Table 5.2 Association between atopy and livestock farm exposures in the total population (n=2,443), and stratified by a history of living on a farm during childhood.

	All (n=2,443) OR (95%CI) Unadjusted	**All (n=2,443) (Atopy = 29.8%) OR (95%CI) Adjusted**	**Farm Childhood (n=818) (Atopy = 21.6%) OR (95%CI) Adjusted**	**Non-Farm Childhood (Atopy = 33.9%) (n=1,601) OR (95%CI) Adjusted**	**Interaction P-value#**
Minimal distance to the nearest farm (tertiles)*					
>527 m	1	1	1	1	0.122
327 - 527 m	1.06 (0.86-1.32)	1.06 (0.85-1.33)	0.82 (0.53-1.28)	1.16 (0.89-1.50)	
<327 m	**0.76 (0.61-0.93)**	**0.79 (0.63-0.98)**	**0.61 (0.40-0.92)**	0.86 (0.66-1.11)	
Test for trend	**0.007**	**0.029**	**0.016**	0.245	
Minimal distance to the nearest pig farm (tertiles)*					
>835 m	1	1	1	1	0.876
558 - 835 m	0.89 (0.72-1.10)	0.89 (0.70-1.13)	0.66 (0.40-1.08)	0.97 (0.74-1.29)	
<558	**0.74 (0.60-0.91)**	**0.73 (0.57-0.93)**	0.78 (0.49-1.24)	**0.69 (0.52-0.93)**	
Test for trend	**0.005**	**0.009**	0.446	**0.010**	
Minimal distance to the nearest poultry farm (tertiles)*					
>1035	1	1	1	1	0.093
684 -1035 m	0.93 (0.76-1.16)	0.97 (0.77-1.22)	0.72 (0.46-1.14)	1.07 (0.81-1.40)	
<684	0.91 (0.74-1.13)	0.95 (0.75-1.20)	0.73 (0.47-1.14)	1.05 (0.80-1.38)	
Test for trend	0.395	0.670	0.204	0.757	
Minimal distance to the nearest cattle farm (tertiles)*					
>624	1	1	1	1	**0.035**
390 - 624 m	0.83 (0.67-1.03)	0.86 (0.69-1.08)	0.80 (0.51-1.25)	0.88 (0.68-1.14)	
<390 m	**0.72 (0.58-0.89)**	**0.76 (0.60-0.96)**	**0.56 (0.36-0.89)**	0.85 (0.65-1.11)	
Test for trend	**0.002**	**0.020**	**0.012**	0.232	
Number of farms within 500 m (per farm increase)					
Any farm	**0.94 (0.91-0.99)**	0.96 (0.91-1.00)	**0.90 (0.83-0.98)**	0.98 (0.93-1.04)	0.073
Pig farm	**0.85 (0.76-0.95)**	0.88 (0.79-1.00)	0.84 (0.68-1.03)	0.92 (0.78-1.07)	0.306
Poultry farm	0.99 (0.84-1.17)	1.09 (0.91-1.30)	1.02 (0.76-1.36)	1.13 (0.90-1.43)	0.534
Cattle farm	0.97 (0.90-1.04)	0.99 (0.92-1.08)	0.93 (0.81-1.08)	1.02 (0.93-1.12)	0.277
Number of farms within 1,000 m (per farm increase)					
Any farm	0.99 (0.98-1.01)	1.00 (0.98-1.01)	0.98 (0.95-1.01)	1.00 (0.98-1.02)	0.105
Pig farm	0.97 (0.94-1.01)	0.98 (0.94-1.02)	0.95 (0.89-1.01)	1.00 (0.95-1.04)	0.117
Poultry farm	0.99 (0.93-1.06)	1.01 (0.95-1.08)	1.03 (0.92-1.15)	1.00 (0.92-1.09)	0.935
Cattle farm	1.00 (0.97-1.03)	1.01 (0.98-1.05)	1.01 (0.95-1.07)	1.01 (0.97-1.05)	0.401
Weighted fine dust emission from farms within 500m ($g*year^{-1} * m^{-2}$) ∞					
$<4*10^{-4}$	1	1	1	1	0.470
$4*10^{-4}$ – 0.29	1.03 (0.83-1.27)	1.04 (0.83-1.29)	0.78 (0.50-1.23)	1.12 (0.87-1.45)	
>0.29	0.84 (0.68-1.04)	0.88 (0.71-1.10)	0.80 (0.54-1.19)	0.91 (0.70-1.18)	
Test for trend	0.115	0.285	0.286	0.545	
Weighted fine dust emission from farms within 1000m ($g*year^{-1} * m^{-2}$) ∞					
<0.69	1	1	1	1	0.595
0.69 – 3.71	0.98 (0.75-1.14)	0.94 (0.75-1.17)	0.82 (0.52-1.29)	0.98 (0.77-1.26)	
>3.71	0.82 (0.67-1.02)	0.87 (0.69-1.09)	0.80 (0.52-1.22)	0.88 (0.67-1.15)	
Test for trend	0.075	0.215	0.327	0.369	

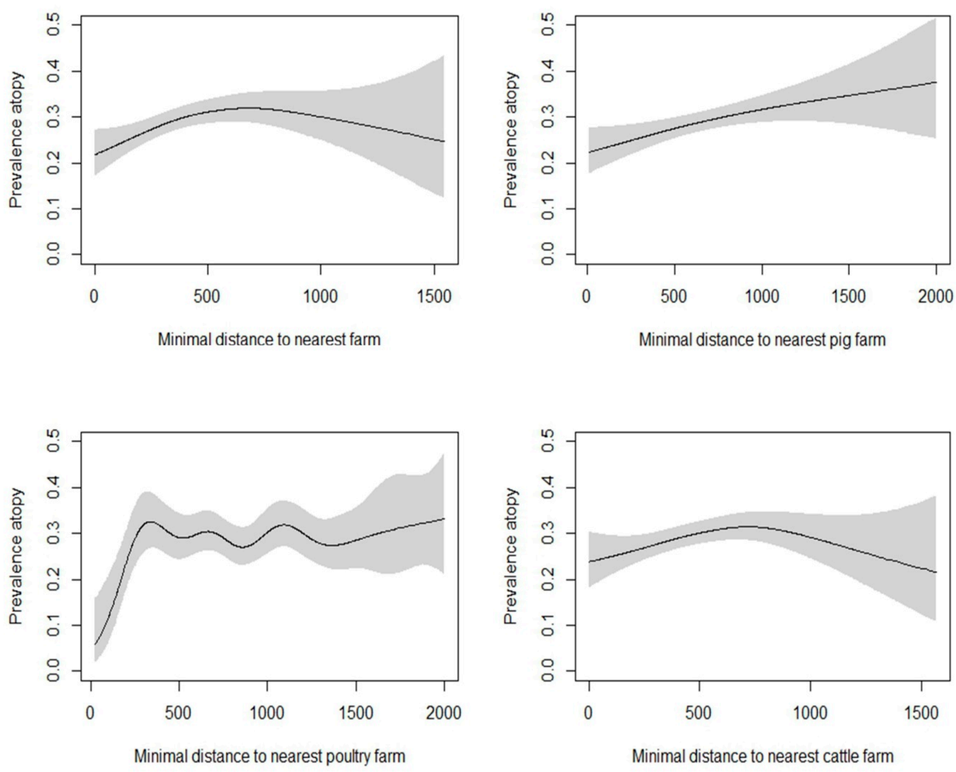
The association between environmental livestock farm exposure and atopy was modeled with logistic regression. Analyses were adjusted for gender, age, smoking habits, education, being born in the study area, and having grown up on a farm. The presence of specific animal farm was also adjusted for the presence of other types of farm animal species. ∞ The distance to the nearest farm (pig, poultry, cattle and any farm) and weighted fine dust emission from farms within 500 and 1000m was categorized into tertiles based on an equal number of atopy cases in each category (dummy variables). # P-value of interaction between farm childhood * farm proximity.

Association between livestock farm exposures and atopy

Associations between atopy and proxies of livestock farms are shown in Table 5.2. Subjects living at short distances from a farm (<327 m, first tertile) had a lower odds for atopy compared to subjects living further away (reference category: >527 m, third tertile) (OR 0.79, 95% CI 0.63-0.98). A statistically significantly test-for-trend was found for distance to the nearest farm and atopy. The same associations and trends were observed when analysing the distance to the nearest pig or cattle farm (first *versus* third tertile, pig farm: OR 0.73, 95%CI 0.57-0.93, cattle farm: OR 0.76, 95% CI 0.60-0.96). Proxies for farm density (number of farms in a radius around the home address) were also associated with atopy. The number of farms and pig farms within 500m was associated with a lower prevalence of atopy (per increase of one farm OR: 0.96, 95% CI 0.91-1.00, per increase of one pig farm OR 0.88, 95% CI 0.79-1.00). No associations were observed between atopy and farm density within 1000 meter or modeled fine dust. In Figure 5.1 the shape of each relationship between the distance to the nearest pig, poultry, cattle and any livestock farm and atopy are shown. The spline for atopy with distance to the nearest pig farm did not have a better fit than the linear relationship. The other splines (cow, poultry and any farm) fitted significantly ($p<0.05$) better than linear models. Figure 5.2 shows the shape of the relationships between atopy and the number of farms and weighted fine dust emission within a 500m and 1000m radius from the home. All four spline models did not fit significantly ($p>0.05$) better than linear models.

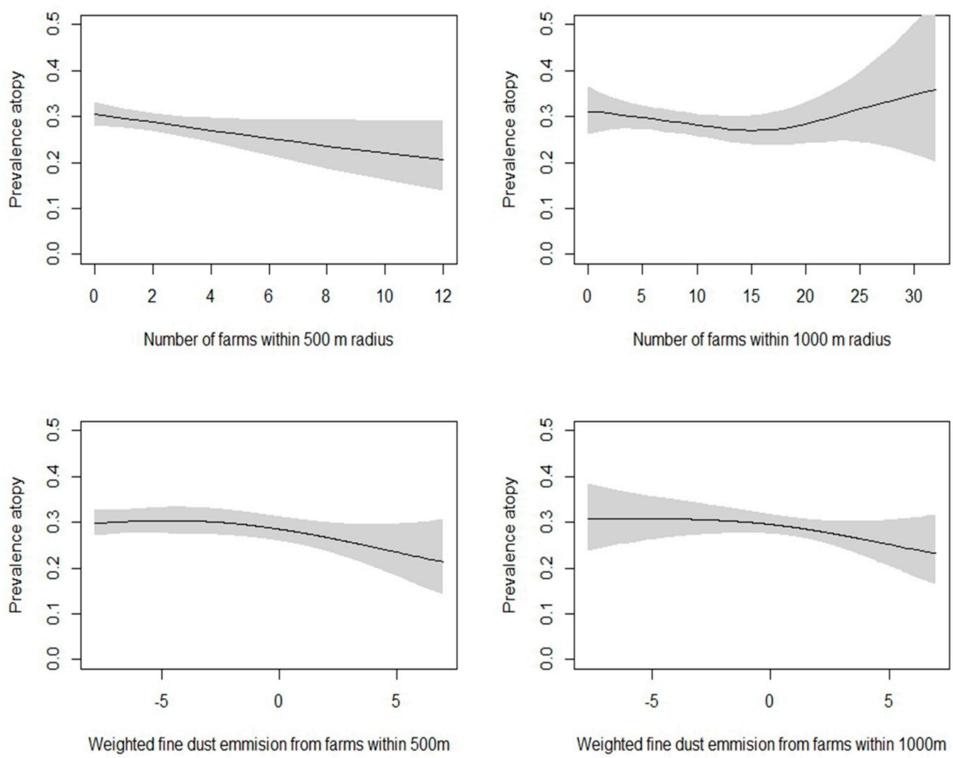
Aassociations between atopy and livestock farm exposures were somewhat stronger when we only considered subjects with a history of living on a farm during childhood (see Table 5.2). Subjects with a farm childhood living at short distances from a farm or a cattle farm had a lower odds for atopy compared to subjects living further away (first *versus* third tertile, any farm: OR 0.61, 95% CI 0.40-0.92, cattle farm: OR 0.56, 95% CI 0.36-0.89). A significant interaction was observed between farm childhood and distance to the nearest cattle farm (p-value 0.035), and a borderline significant interaction was found with the number of farms within 500m (p-value 0.070). Spline analysis indicate a linear relationship between atopy and distance to the nearest farm for subjects who were grown up on a farm: atopy prevalence increases in a monotonous manner with increasing distance to the nearest farm (results not shown). Among subjects not grown up on a farm, the relationship between atopy and distance to the nearest farm fitted significantly ($p<0.05$) better than a linear model. The spline for atopy and distance to the nearest cattle farm did have a significantly (p-value <0.05) better fit than the linear model for subjects who were grown up on a farm, but had not a better fit than the linear model for subjects who were not grown up on a farm (results not shown). Among subjects without a farm childhood, the distance to the nearest pig farm was negatively associated with atopy (first versus third tertile, pig farm: OR 0.69, 95% CI 0.52-0.93). No other significant associations were observed among subjects without a history of living on a farm during childhood.

Figure 5.1 Associations between the distance to the nearest pig, poultry, cattle and any livestock farm and atopy in 2,443 residents.



Smoothed plots show the associations between the distance to the nearest pig, poultry, cattle and any farm and atopy. Associations are adjusted for gender, age, smoking habits, education, being born in the study area, and having grown up on a farm. Models on distance to specific animal farms, were also adjusted for the presence of other types of farm animal species within 1000m. The p-values of the smooth terms are: any farm: 0.025, pig farm: 0.027, poultry farm: 0.195, cattle farm: 0.0918. The association between distance to the nearest pig farm and atopy did not fit better with a spline, indicating a linear relationship. The other spline models (cow, poultry and any farm) fitted significantly ($p < 0.05$) better than the linear models.

Figure 5.2 Associations between the number of farms and weighted fine dust emission from farms within a 500m and 1000m radius from the home and atopy in 2,443 residents.



Smoothed plots show the associations between the number of farms and weighted fine dust emission within a 500m and 1000m radius from the home and atopy. Associations are adjusted for gender, age, smoking habits, education, being born in the study area, and having grown up on a farm. The p-values of the smooth terms are: number of farms within 500m: 0.049, number of farms within 1000m: 0.414, weighted fine dust emission within 500m: 0.174, weighted fine dust emission within 1000m: 0.312. All four models with spline were not significantly ($p>0.05$) better than the linear models.

Sensitivity analyses

Overall, associations using specific serum IgE for atopy (prevalence: 18.2%) or IgE>100 IU/ml for atopy (prevalence: 20.3%) were statistically less strongly significant but showed similar directions and had overlapping confidence intervals (see Table S5.1). No clear differences in the results were observed between the two atopy definitions.

Sensitivity analyses with adjustment for current farm animal contact or farm visits did not change the associations between livestock farm exposures (see Table S5.2). Sensitivity analyses with subjects that lived at least 5 years in their current home (n=2,227) showed slightly stronger effects; confidence intervals became narrower (see Table S5.2), indicating that a prolonged exposure to livestock farms might have a stronger effect. Sensitivity analyses stratified for allergic symptoms showed a similar protective effect among asymptomatic subjects (n=1,799) as in the total population (see Table S5.3). In symptomatic subjects (n=644) weaker associations were observed and the test for trend was not statistically significant. However, no significant interaction was observed between indicators of farm proximity and allergic symptoms.

Atopic subjects and non-atopic subjects showed a similar negative relationship between the distance to the nearest farm and the number of years they have lived in their current home (p-value interaction term: 0.439) (see Figure S5.1), suggesting that selective migration does not explain the observed associations between atopy and farming. However, a significant interaction (p-value 0.027) was observed between atopy and the number of farms within 1000m. This indicates that non-atopic subjects living in an area with a high farm density might migrate less frequently compared to atopic subjects (see Figure S5.2).

Discussion

This large population-based study among non-farming subjects shows that current exposure to a livestock farm environment, assessed as residential proximity to livestock farms, seems to protect against atopy in adults. Associations were found between atopy and distance to a livestock farm, in particular to the nearest pig or cattle farm. Proxies for farm density – such as the number of farms within 500 m - were also clearly associated with a lower atopy prevalence.

The study was conducted in The Netherlands which is a small country with a high population density in combination with a high livestock farm density. Farms located in the study area are a mix of small farms with relatively few animals to large farms with thousands of animals. The study area was chosen because it is characterised by a high farm density. The present study is the first to investigate this relationship with atopy based on objective markers. Results of this study confirms the results of two previous studies among non-farming populations which found inverse associations between indicators of livestock farm proximity and allergic rhinitis based on self-reported data[24] and Electronic Medical Records[25]. Results of two other studies also indicate that living in a rural environment might be protective[22,23]. However, in these latest studies livestock farm exposure was not assessed at the individual level. As expected, we found that a farm childhood history was associated with a lower prevalence of atopy. Associations between atopy and livestock farm exposures were somewhat stronger among subjects who grew up on a farm. Among subjects who grew up on a farm, those living in closer proximity to livestock farms had a lower atopy prevalence than those living further away, suggesting that prolonged farm exposures may be especially effective to prevent development of atopy. Previous studies among farmers confirm our results, showing that continued involvement in farming exposure might be required to maintain optimal protection among farmers[3,7,29,30].

Several studies have shown that exposure to greater microbial diversity may prevent the development of allergic diseases[14,16,17]. Overall understanding how microbial diversity can protect against allergic diseases is incomplete. The microbiome – the complete microbial community that exists in the human host and is influenced by environmental exposure - seems to play an important role in the immune system in many ways[31]. Regulatory T-cells (Tregs) for example, are able to inhibit the development of allergic Th2 responses[32]. The microbiome influences the generation and maintenance of Tregs, amongst others by microbial products and microbe-microbe interactions which contribute to Treg formation and function. The microbiome also influences regulatory B-cells (Bregs), though these mechanisms are less well understood. Allergy-promoting Th2 and Th17 responses can also be driven by the microbiome. Several microorganisms have been identified that either inhibit or promote Th2 or Th17 responses[31]. We assume that farm proximity is associated with a higher diversity of environmental microbial exposure. Although we did not measure microbial diversity directly in this study, previous studies show associations with residential farm proximity and other microbial agents. A study conducted by de Rooij *et al.*[21] showed that endotoxin concentrations in ambient air in a livestock dense area, was associated with spatial livestock-related characteristics of the surroundings. Moreover, previous studies measured elevated levels of endotoxin and other microbial proxies emitted from stables 30-250m downwind of livestock farms[19,33,34]. High endotoxin levels are associated with higher microbial richness[17]. A Finnish study found that living in an environment with high environmental biodiversity - mainly more forest and agricultural land - was associated with a higher diversity of commensal microbiota of the skin[35].

Moreover, atopic sensitization was inversely associated with environmental biodiversity and diversity of commensal microbiota of the skin. Although these studies support our assumption that farm proximity is associated with higher exposure to microbial diversity, further microbiological characterization of the subjects' residential environment would help to understand the present findings.

One could argue that our exposure variables weighted fine dust emission within 500m and 1000m are most reliable since these variables contain information on modeled emission of farms and it takes into account the weighted distances of those farms to the home. However, no association with atopy was observed. An explanation could be that the (microbial) composition of fine dust plays an important role. Our results showed differences between specific type of farms; we observed associations with pig and cattle farms, but no association with poultry farms. A study of Illi *et al.* among 7,682 children from rural areas showed protective effects on atopic sensitization with cattle, but no effects with pig or poultry[36]. This could indicate that the composition of emissions from farms are different between specific types of farms and may have different effects on atopy.

Another explanation for the protective effect of living near livestock farms could be migration of atopic subjects from rural areas to more urbanized areas. We showed protective effects on atopy among non-symptomatic individuals, where one would not expect health-related migration to occur. Furthermore, if selective migration due to atopic sensitization occurred, we would expect a different relationship between the number of years they have lived in their current home and farm proximity among atopic and non-atopic individuals. Atopic subjects and non-atopic subjects showed a similar negative relationship between the distance to the nearest farm and the number of years they have lived in their current home. The significant interaction between atopy and the number of farms within 1000m suggests that non-atopic subjects living in an area with a high farm density migrate less often than atopic subjects. However, overall, these sensitivity analyses do not support the hypothesis that selective migration fully explains the protective effect of farm proximity on atopy.

Detailed non-response analyses were previously conducted and we demonstrated that selection bias did not affect associations between farm exposures and respiratory health (amongst others nasal allergies)[24,26,27]. Data on farm exposure and Electronic Medical Records (EMR) of the general practitioner were available of the total source population (source population: n=27,869[24]). This enabled us to compare characteristics of non-responders and responders in different stadia of the data collection.

A limitation of our study is the cross-sectional design, which limits interpretation of the possible impact of selective migration, causality, and timing of a protective effect. Longitudinal studies are needed to assess the role of past and current exposure on atopic sensitization and causality of associations.

In conclusion, living in close proximity to livestock farms seems to protect against atopy. Our population-based study provides evidence that protective effects of early-life and adult farm exposures may extend beyond farming populations.

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Supplementary tables and figures

Table S5.1 Association between atopy based on two alternative definitions and livestock farm exposures.

	Specific IgE to ≥1 common allergen		Total IgE ≥100 Ku/L	
	OR (95%CI) Unadjusted	**OR (95%CI) Adjusted**	**OR (95%CI) Unadjusted**	**OR (95%CI) Adjusted**
Minimal distance to the nearest farm (tertiles)*				
>527 m	1	1	1	1
327 - 527 m	0.98 (0.77-1.26)	0.98 (0.76-1.27)	1.23 (0.95-1.58)	1.24 (0.96-1.61)
<327 m	**0.78 (0.61-0.99)**	0.80 (0.62-1.03)	0.84 (0.66-1.09)	0.89 (0.69-1.16)
Test for trend	**0.034**	0.076	0.158	0.343
Minimal distance to the nearest pig farm (tertiles)*				
>835 m	1	1	1	1
558 - 835 m	0.93 (0.73-1.19)	0.94 (0.71-1.24)	0.91 (0.70-1.17)	0.93 (0.70-1.23)
<558	0.79 (0.62-1.01)	**0.75 (0.56-0.99)**	**0.73 (0.67-0.94)**	0.77 (0.58-1.03)
Test for trend	0.053	**0.036**	**0.013**	0.067
Minimal distance to the nearest poultry farm (tertiles)∞				
>1035	1	1	1	1
684 -1035 m	0.99 (0.78-1.27)	1.02 (0.78-1.33)	0.91 (0.71-1.18)	0.99 (0.75-1.30)
<684	0.93 (0.73-1.19)	0.95 (0.73-1.25)	0.97 (0.76-1.25)	1.06 (0.81-1.38)
Test for trend	0.564	0.715	0.838	0.678
Minimal distance to the nearest cattle farm (tertiles)∞				
>624	1	1	1	1
390 - 624 m	0.89 (0.69-1.13)	0.89 (0.69-1.16)	0.83 (0.64-1.07)	0.87 (0.67-1.13)
<390 m	0.82 (0.64-1.04)	0.84 (0.64-1.10)	**0.77 (0.60-0.99)**	0.84 (0.64-1.10)
Test for trend	0.106	0.203	**0.045**	0.220
Number of farms within 500m (per farm increase)				
Any farm	0.96 (0.92-1.01)	0.93 (0.81-1.07)	**0.94 (0.90-0.99)**	0.96 (0.91-1.01)
Pig farm	0.89 (0.78-1.01)	1.07 (0.87-1.32)	**0.82 (0.70-0.94)**	**0.83 (0.72-0.98)**
Poultry farm	0.96 (0.79-1.17)	1.01 (0.92-1.11)	1.01 (0.82-1.23)	1.06 (0.86-1.31)
Cattle farm	0.99 (0.91-1.08)	0.99 (0.91-1.09)	0.96 (0.88-1.05)	1.00 (0.91-1.10)
Number of farms within 1000m (per farm increase)				
Any farm	1.00 (0.98-1.01)	0.99 (0.95-1.04)	0.99 (0.97-1.01)	1.00 (0.98-1.02)
Pig farm	0.99 (0.95-1.03)	1.00 (0.95-1.04)	0.97 (0.93-1.01)	0.98 (0.94-1.03)
Poultry farm	0.99 (0.92-1.06)	1.00 (0.92-1.08)	1.01 (0.94-1.09)	1.04 (0.96-1.12)
Cattle farm	1.01 (0.97-1.04)	1.01 (0.98-1.05)	0.99 (0.96-1.03)	1.01 (0.97-1.05)
Weighted fine dust emission from farms within 500m (g*year-1 * m^{-2}) ∞				
<4*10^{-4}	1	1	1	1
4*10^{-4} – 0.29	0.97 (0.76-1.23)	0.96 (0.74-1.23)	1.22 (0.95-1.56)	1.25 (0.97-1.61)
>0.29	0.85 (0.67-1.07)	0.88 (0.68-1.13)	0.93 (0.72-1.19)	0.97 (0.75-1.25)
Test for trend	0.174	0.308	0.600	0.898
Weighted fine dust emission from farms within 1000m (g*year^{-1} * m^{-2}) ∞				
<0.69	1	1	1	1
0.69 – 3.71	1.01 (0.80-1.28)	1.01 (0.78-1.29)	0.96 (0.75-1.24)	1.00 (0.78-1.29)
>3.71	0.84 (0.66-1.07)	0.87 (0.67-1.13)	0.87 (0.68-1.12)	0.93 (0.72-1.22)
Test for trend	0.164	0.312	0.288	0.617

The association between environmental livestock farm exposure and atopy based on two different definitions was modeled with logistic regression. All analyses were adjusted for gender, age, smoking habits, education, being born in the study area and having grown up on a farm. The presence of a specific animal farm was also adjusted for the presence of other types of farm animal species. The distance to the nearest farm (pig, poultry, cattle and any farm) and weighted fine dust emission from farms within 500 and 1000m was categorized into tertiles based on an equal number of atopy (at least one of both definitions) cases in each category (dummy variables).

Table S5.2 Association between atopy and livestock farm exposures adjusted for current farm contact and living at least 5 years in current home.

	Total population (n=2,443) OR (95%CI) Adjusted	Total population adjusted for animal contact at home or during farm visit# OR (95%CI) Adjusted	Total population adjusted for animal contact during work or study OR (95%CI) Adjusted	Only subjects living 5 years or more in current home (n=2,227) OR (95%CI) Adjusted
Minimal distance to the nearest farm (tertiles)∞				
>527 m	1	1	1	1
327 - 527 m	1.06 (0.85-1.33)	1.06 (0.85-1.33)	1.07 (0.85-1.34)	1.07 (0.84-1.35)
<327 m	**0.79 (0.63-0.98)**	**0.78 (0.63-0.98)**	**0.79 (0.64-0.99)**	**0.79 (0.62-0.99)**
Test for trend	**0.029**	**0.028**	**0.033**	**0.033**
Minimal distance to the nearest pig farm (tertiles) ∞				
>835 m	1	1	1	1
558 - 835 m	0.89 (0.70-1.13)	0.89 (0.70-1.13)	0.89 (0.70-1.14)	0.83 (0.64-1.07)
<558	**0.73 (0.57-0.93)**	**0.73 (0.57-0.93)**	**0.73 (0.57-0.94)**	**0.69 (0.53-0.89)**
Test for trend	**0.009**	**0.009**	**0.011**	**0.004**
Minimal distance to the nearest poultry farm (tertiles) ∞				
>1035	1	1	1	1
684 -1035 m	0.97 (0.77-1.22)	0.97 (0.77-1.22)	0.97 (0.77-1.22)	0.99 (0.77-1.26)
<684	0.95 (0.75-1.20)	0.95 (0.75-1.20)	0.95 (0.76-1.20)	0.98 (0.77-1.25)
Test for trend	0.670	0.670	0.696	0.875
Minimal distance to the nearest cattle farm (tertiles) ∞				
>624	1	1	1	1
390 - 624 m	0.86 (0.69-1.08)	0.86 (0.68-1.08)	0.86 (0.69-1.08)	0.80 (0.51-1.25)
<390 m	**0.76 (0.60-0.96)**	**0.76 (0.60-0.96)**	**0.76 (0.61-0.96)**	**0.56 (0.36-0.89)**
Test for trend	**0.020**	**0.019**	**0.023**	**0.039**
Number of farms within 500m (per farm increase)				
Any farm	**0.96 (0.91-1.00)**	**0.95 (0.90-0.99)**	**0.95 (0.91-0.99)**	**0.96 (0.91-1.00)**
Pig farm	**0.88 (0.79-1.00)**	**0.86 (0.76-0.98)**	**0.87 (0.77-0.98)**	**0.86 (0.76-0.98)**
Poultry farm	1.08 (0.90-1.30)	1.05 (0.88-1.25)	1.06 (0.88-1.26)	1.11 (0.92-1.34)
Cattle farm	1.00 (0.92-1.08)	0.99 (0.92-1.07)	0.99 (0.92-1.07)	1.01 (0.93-1.10)
Number of farms within 1000m (per farm increase)				
Any farm	1.00 (0.98-1.01)	0.99 (0.98-1.01)	0.99 (0.98-1.01)	0.99 (0.98-1.01)
Pig farm	0.98 (0.94-1.02)	0.97 (0.94-1.01)	0.97 (0.94-1.01)	0.97 (0.93-1.01)
Poultry farm	1.01 (0.95-1.08)	0.99 (0.93-1.06)	0.99 (0.93-1.06)	1.01 (0.94-1.08)
Cattle farm	1.01 (0.98-1.05)	1.01 (0.97-1.04)	1.01 (0.97-1.04)	1.01 (0.97-1.04)
Weighted fine dust emission from farms within 500m (g*year^{-1} * m^{-2}) ∞				
<4*10^{-4}	1	1	1	1
4*10^{-4} – 0.29	1.04 (0.83-1.29)	1.04 (0.83-1.29)	1.04 (0.83-1.30)	1.06 (0.84-1.33)
>0.29	0.88 (0.71-1.10)	0.88 (0.71-1.10)	0.89 (0.72-1.11)	0.89 (0.71-1.12)
Test for trend	0.285	0.285	0.324	0.346
Weighted fine dust emission from farms within 1000m (g*year^{-1} * m^{-2}) ∞				
<0.69	1	1	1	1
0.69 – 3.71	0.94 (0.75-1.17)	0.94 (0.75-1.17)	0.94 (0.75-1.17)	0.94 (0.75-1.18)
> 3.71	0.87 (0.69-1.09)	0.87 (0.69-1.09)	0.87 (0.70-1.09)	0.85 (0.67-1.08)
Test for trend	0.215	0.215	0.230	0.180

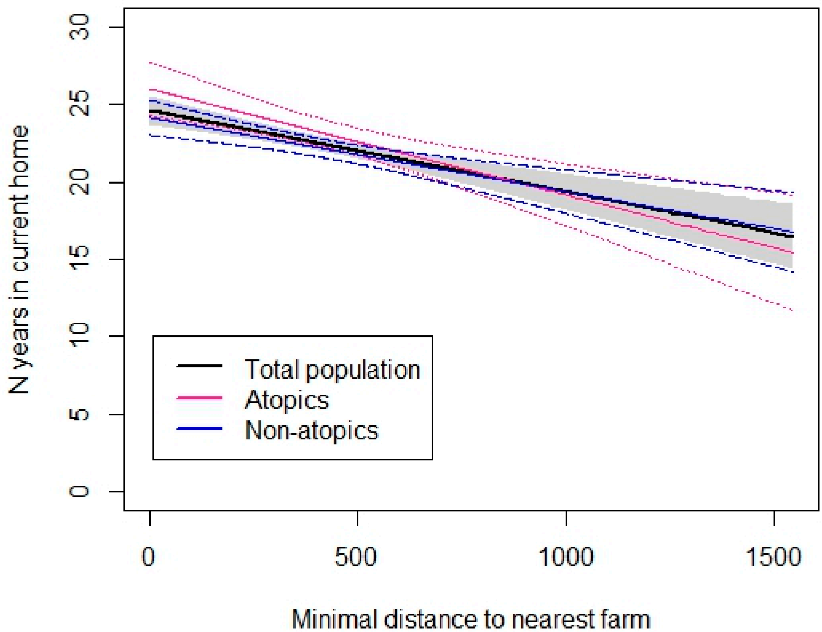
The association between environmental livestock farm exposure and atopy was modeled with logistic regression. All analyses were adjusted for gender, age, smoking habits, education, being born in the study area. The presence of a specific animal farm was also adjusted for the presence of other types of farm animal species. Horses, pigs, poultry, cows, goats and sheep. Contact was defined as touching the animal and/or touching the droppings of the animal. ∞ The distance to the nearest farm (pig, poultry, cattle and any farm) and weighted fine dust emission from farms within 500 and 1000m was categorized into tertiles based on an equal number of atopy (at least one of both definitions) cases in each category (dummy variables).

Table S5.3 Association between atopy and livestock farm exposures in subjects stratified by a history of living on a farm during childhood, and stratified by symptom reporting. Symptoms are defined as self-reported allergy and symptoms#.

	Symptomatic (n=644) (atopy = 57.6%) OR (95%CI) Adjusted	Non-symptomatic (n=1,799) (atopy = 19.8%) OR (95%CI) Adjusted	Interaction P-value
Minimal distance to the nearest farm (tertiles)∞			
>527 m	1	1	0.071
327 - 527 m	1.27 (0.83-1.95)	0.94 (0.70-1.27)	
<327 m	1.08 (0.71-1.65)	**0.70 (0.52-0.94)**	
Test for trend	0.722	**0.015**	
Mean distance to the nearest pig farm (tertiles) ∞			
>835 m	1	1	0.370
558 - 835 m	0.86 (0.53-1.40)	0.79 (0.57-1.08)	
<558	0.73 (0.45-1.20)	**0.66 (0.48-0.92)**	
Test for trend	0.203	**0.013**	
Mean distance to the nearest poultry farm (tertiles) ∞			
>1035	1	1	0.516
684 -1035 m	0.68 (0.43-1.07)	1.05 (0.77-1.43)	
<684	0.68 (0.43-1.09)	1.04 (0.76-1.41)	
Test for trend	0.124	0.821	
Mean distance to the nearest cattle farm (tertiles) ∞			
>624	1	1	0.185
390 - 624 m	1.02 (0.66-1.57)	0.79 (0.58-1.06)	
<390 m	0.93 (0.59-1.45)	**0.69 (0.51-0.93)**	
Test for trend	0.738	**0.018**	
Number of farms within 500m (per farm increase)			
Any farm	0.95 (0.88-1.03)	**0.94 (0.88-1.00)**	0.561
Pig farm	0.89 (0.72-1.10)	**0.84 (0.71-1.00)**	0.584
Poultry farm	1.01 (0.72-1.42)	1.09 (0.86-1.38)	0.642
Cattle farm	0.95 (0.82-1.10)	1.00 (0.90-1.11)	0.840
Number of farms within 1000m (per farm increase)			
Any farm	0.99 (0.97-1.02)	0.99 (0.97-1.01)	0.394
Pig farm	0.99 (0.92-1.06)	**0.95 (0.90-1.00)**	0.132
Poultry farm	1.01 (0.88-1.15)	1.01 (0.93-1.10)	0.806
Cattle farm	0.99 (0.93-1.05)	1.01 (0.97-1.06)	0.744
Weighted fine dust emission from farms within 500m (g*year-1 * m-2) ∞			
<4*10-4	1	1	0.210
4*10-4 – 0.29	1.13 (0.83-1.96)	0.95 (0.71-1.27)	
>0.29	1.11 (0.74-1.68)	0.80 (0.59-1.07)	
Test for trend	0.556	0.130	
Weighted fine dust emission from farms within 1000m ($g*year^{-1} * m^{-2}$) ∞			
<0.69	1	1	0.196
0.69 – 3.71	1.32 (0.87-2.01)	0.74 (0.55-0.99)	
>3.71	0.91 (0.59-1.40)	0.83 (0.62-1.12)	
Test for trend	0.718	0.215	

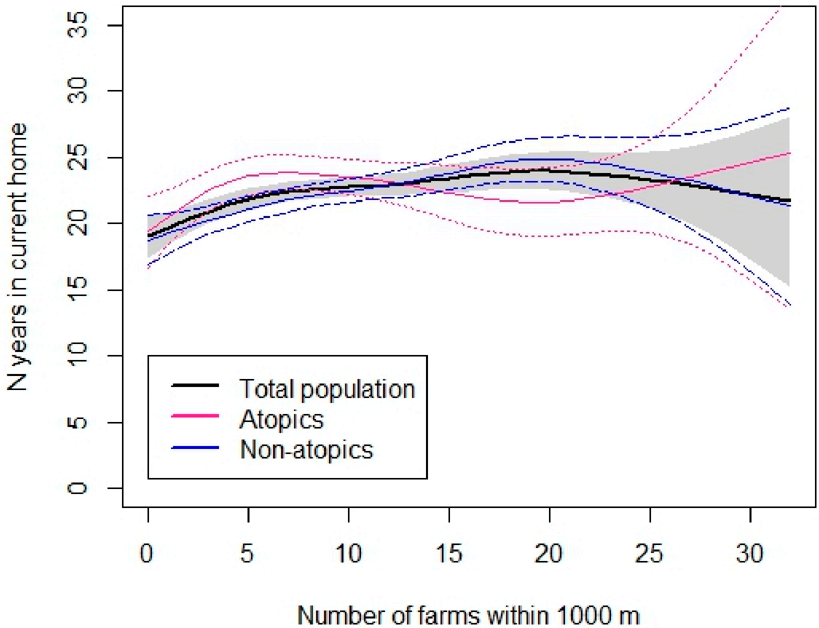
The association between environmental livestock farm exposure and atopy was modeled with logistic regression. All analyses were adjusted for gender, age, smoking habits, education, being born in the study area and having grown up on a farm. The presence of a specific animal farm was also adjusted for the presence of other types of farm animal species. # Symptomatic was defined as self-reported allergy against house dust mite, animals and plant and/or pollen and reporting at least one of the followin symptoms: sneezing or running nose, shortness of breath, itchy or red colored skin, itchy or tearing eyes. ∞ The distance to the nearest farm (pig, poultry, cattle and any farm) and weighted fine dust emission from farms within 500 and 1000m was categorized into tertiles based on an equal number of atopy (at least one of both definitions) cases in each category (dummy variables).

Figure S5.1 Association between the number of years subjects have lived in their current home and the distance to the nearest farm, stratified for atopy.



The smoothed plots shows the association between the distance to the nearest farm and the number of years in the current home (solid line and grey area). Associations are adjusted for age, gender, atopy and having grown up on a farm. Models were stratified by atopy (atopics: pink lines, non-atopics: blue lines). P-values of the smooth terms are: total population: < 0.001, atopics: 0.001, non-atopics: < 0.001. No significant interaction term was observed between minimal distance to the nearest farm and atopy (p-value: 0.439).

Figure S5.2 Association between the number of years subjects have lived in their current home and the number of farms within 1000m, stratified for atopy.



The smoothed plots shows the association between the number of farms within 1000m and the number of years in the current home (solid line and grey area). Associations are adjusted for age, gender, atopy and having grown up on a farm. Models were stratified by atopy (atopics: pink lines, non-atopics: blue lines). P-values of the smooth terms are: total population: <0.001, atopics: 0.106, non-atopics: <0.001. The interaction term between the number of farms within 1000m and atopy was: p-value: 0.027.

Chapter 6

Attitude towards livestock farming in residential areas: does it influence epidemiological associations?

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Abstract

Background: Attitudes towards environmental risks may be a source of bias in environmental health studies since concerns about environmental hazards may influence self-reported outcomes. We aimed to identify determinants associated with attitude towards livestock farming. The second aim was to assess whether the earlier observed association between proximity to goat farms and self-reported pneumonia was biased by participants' attitude.

Methods: We developed an attitude-score for 2,457 VGO participants by factor analysis of 13 questionnaire items related to attitude towards livestock farming. Linear regression analysis was used to assess associations between attitude and potential determinants.

Results: In general, the study population had a positive attitude towards farming. Older participants, females, ex-smokers, and individuals with a higher education had a more negative attitude. Self-reported symptoms were associated with a more negative attitude. Awareness bias might have played a role here. The attitude-score was associated with exposure to livestock farms and could therefore potentially confound exposure response relations. However, no indication was found that the association between proximity to goat farms and pneumonia was confounded or modified by attitude.

Conclusions: When relying on self-reported data in environmental health studies, we recommend to measure attitude towards a potential hazard to study the potential impact of awareness bias.

Introduction

There is an ongoing debate about livestock farming and potential health risks for surrounding populations[1-5]. The Netherlands is a small country with one of the highest population densities in the world in combination with one of the highest livestock densities[6]. A small survey (n=1,090) on the public's view on intensive livestock farming showed disagreement among the Dutch general population about large-scale intensive farming[7]. Most arguments against intensive livestock farming were focused on animal welfare, and potential risks for public health.

Potential environmental risks – such as air pollution from livestock farms - may be a source of concern about health effects in exposed individuals. In environmental health studies, information on health outcomes is often self-reported and attitudes towards environmental risks may be a source of bias since concerns about environmental hazards may influence self-reported outcomes. Moffatt[8] describes such 'awareness bias' as the propensity to report more illness and symptoms as a result of proximity to a potential hazard, in the absence of a biological effect. Perception of exposure, causal beliefs and concerns, and media coverage play an important role in symptom reporting[8-13], and may act as a confounder or even effect modifier.

Actual or perceived exposure to a hazard, and cultural and social factors may influence someone's risk perception, which results in a variation of attitudes towards a potential environmental risk among individuals[14]. A non-systematic review of Marcon and co-workers found that determinants of environmental risk perception mainly comprise demographic, socio-economic and exposure indicators[15]. They studied environmental risk perception among a population of Italian parents and found female gender, age of parents, young age of children, a higher level of education, and exposure indicators to be associated with a higher risk perception. However, the authors did not investigate whether risk perception affected associations between environmental pollution and self-reported health outcomes[15].

The VGO study (Dutch acronym for Livestock Farming and Neighbouring Residents' Health) aims to investigate respiratory health of residents living in close proximity of livestock farms in the Netherlands. One of the main findings was a higher risk of pneumonia for residents living in close proximity to goat farms[16]. Pneumonia was defined as self-reported pneumonia or based on a diagnosis of pneumonia by the general practitioner, recorded in the Electronic Medical Record. Since the studied health outcome was (partly) self-reported and therefore not objectively assessed, potential awareness bias may have resulted in confounding or effect modification by attitude towards farming.

In the current study we aim to explore determinants that are associated with attitude towards livestock farming in the residential environment. To achieve this, we developed an attitude-score using factor analysis of 15 questionnaire items. The second aim was to assess whether the earlier observed association[16] between proximity to goat farms and self-reported pneumonia was biased by participants' attitude.

Methods

Study design and population

The study population originates from participants of a questionnaire survey (n=14,163)[17]. Respondents who were willing to participate in a follow-up study, and who were not working or living on a farm were eligible for a medical examination (n=8,714). Between March 2014 and February 2015, 7,180 persons were invited for medical examination and 2,494 participated (response 34.7%). The medical examination consisted amongst others of a second and more extended questionnaire and spirometry. More details about the recruitment of the study population and the medical examination are described previously by Borlée *et al.*[18,19]. 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.

Medical examination

The questionnaire comprised amongst others items on education, profession, residential history, smoking habits, non-specific symptoms[20] and diseases. Moreover, the questionnaire contained also 15 statements on attitude towards farming in their residential environment. Statements were mostly adopted from a survey among the general Dutch population which was focused on the public's view on intensive livestock farming[7]. Pre- and post-BD spirometry was conducted[19].

Construction of a score for attitude towards livestock farming in the residential environment

Response options of the 15 statements were coded based on a five-point Likert scale (Table 6.1). Principal factor analysis was used to identify one or more latent factors which can be interpreted as an 'attitude towards farming'. Standardized factor scores (z-scores, hereafter named 'attitude-score') were computed as linear combinations of scoring coefficients and standardized questionnaire responses for each participant, where a higher score indicates a more positive attitude towards farming.

Livestock farm exposure variables

The following livestock farm exposure proxies were studied for each subject: 1) number of farms within 500 and 1,000 m and 2) presence of a farm (pig, poultry, cattle, goat, sheep, horse) within 1,000 m (Y/N) (21).

Data analysis

Linear regression analysis was used to assess the association between attitude and potential determinants. The potential determinants of attitude studied were: 1) personal characteristics; 2) health status, 3) exposure to livestock farms. Analyses on non-specific symptoms were conducted on each symptom separately, as well as for clusters which were previously studied by Yzermans *et al.*[20]. Two adjusted models were assessed: model A: adjusted for age and gender, and model B: adjusted for: age, gender, born in study area, childhood on a farm, BMI≥30, visited a farm last 12 months, high education. The effect of attitude towards farming on the relationship between the earlier observed association[16] between self-reported pneumonia and goat farm proximity was analysed by adding the attitude-score as a confounder. The interaction between goat farm exposure and the attitude-score was also tested. Sensitivity analyses were conducted after excluding subjects who attributed their symptoms to presence of livestock farms in their environment. Data were analysed using SAS 9.4 (SAS Institute Inc. Cary, NC, USA).

More details on the study methodology are provided in the supplement.

Table 6.1 Statements regarding attitude towards farming in the residential environment and the distribution of 2,457 participants' responses to the 15 statements.

Question	Reverse-scored?	Included in final factor?	Factor loading	More negative attitude	Neutral attitude	More positive attitude	Missing
S1. Livestock farms are a heavy burden for my living environment	Yes	Yes	0.70	15.0%	28.4%	56.2%	0.3%
S2. Farmers do their best to prevent heavy disturbances in my living environment	No	Yes	0.54	11.4%	43.0%	45.0%	0.6%
S3. Livestock farms are important for the Dutch economy	No	Yes	0.60	5.4%	20.9%	73.3%	0.4%
S4. I am happy with the livestock farmers in my neighbourhood	No	Yes	0.71	21.1%	50.2%	28.6%	0.1%
S5. There is too much discussion about the disadvantages of livestock farming	No	Yes	0.60	23.4%	38.2%	38.0%	0.5%
S6. The odour of manure disturbs me every time	Yes	Yes	0.58	30.1%	26.3%	43.4%	0.2%
S7. Livestock farming is a threat for my health	Yes	Yes	0.80	15.5%	41.6%	42.6%	0.2%
S8. I think the threat for my health due to livestock farming increased in the last decade	Yes	Yes	0.74	25.2%	32.4%	42.2%	0.2%
S9. If farmers monitor the health of their animals well, livestock farming is not a threat for my own health	No	Yes	0.49	18.2%	35.1%	46.6%	0.2%
S10. I am concerned about the impact of antibiotic use in livestock farming for my own health	Yes	No	-	65.8%	23.4%	10.7%	0.2%
S11. I am concerned about new diseases that can be transmitted from animals to humans	Yes	No	-	63.4%	24.7%	11.9%	0.1%
S12. I have health problems that are caused by livestock farms in my living environment	Yes	Yes	0.56	4.3%	31.6%	63.5%	0.6%
S13. A livestock farmer loves his animals and takes good care of them	No	Yes	0.57	7.2%	31.9%	60.6%	0.3%
S14. If there is no disturbance for me or my family, livestock farming may increase	No	Yes	0.65	33.0%	29.9%	36.8%	0.2%
S15. Construction of bigger stables disturbs the landscape	Yes	Yes	0.53	50.5%	27.4%	22.0%	0.1%

For comparability purposes, responses of negatively-keyed statements were reverse-scored. Response options were coded based on a five-point Likert scale ('Strongly disagree", "Disagree", "Neutral", "Agree", "Strongly agree") but are represented in the table as a three-point scale. The answers "Strongly disagree" and "Disagree" were merged and translated as "More negative attitude", the answers "Agree" and "Strongly agree" were also merged and translated to a "More positive attitude".

Results

Study population

The study population was on average 56.4 ± 11.1 years old and 54.6% of the study population consisted of females (Table 6.2). In total 76.1% was born in the study area and one third (33.8%) had grown up on a farm. The number of missing answers to the 15 statements was low for all items (<0.6%) (Table 6.1). The majority of participants answered neutral or positive to all statements, with the exception of three statements regarding antibiotic usage in livestock farming, zoonotic diseases, and disturbance of the landscape due to construction of bigger sheds.

Construction of 'attitude-score '

After first exploratory factor analyses, statement 10 and 11 were removed since their residual correlation coefficients were >0.1. The final factor analysis was performed on the remaining 13 statements and one latent factor was identified (Eigenvalue =5.14) and explained 97.6% of the total variance. Cronbach's alpha was 0.89, suggesting a good internal consistency. Factor loadings, i.e. the correlations of the individual questionnaire items with the factor, ranged from 0.49 to 0.80 (Table 6.1).

Determinants of attitude

Older participants, females, ex-smokers (*versus* never smokers), and individuals with a higher education (*versus* low and middle education) had a more negative attitude towards farming (Table 6.2). As expected, determinants related to familiarity with a farming environment – such as childhood on a farm, born in the study area, or a recent farm visit - were associated with a more positive attitude towards farming.

Aside from 'headache', all separately reported non-specific symptoms were significantly associated with a more negative attitude towards farming (Table 6.3). Also, the total number of symptoms and the number of symptoms within clusters were negatively associated with attitude, with statistically significant tests for trend ($p<0.0001$) (Table S6.1). As expected, subjects who attributed their health complaints to livestock farming had a more negative attitude towards farming. Except for self-reported COPD, all self-reported health outcomes were associated with a lower attitude-score, while lung function was not associated with attitude (Table 6.3).

Table 6.2 Characteristics of the study population of 2,457 adults from a general, non-farming population, and association between potential determinants and the attitude-score.

Personal characteristics	**Mean (SD) or %**	**Unadjusted β (95%CI)**	**Model A* Adjusted β (95%CI)**	**Model B* Adjusted β (95%CI)**
Age (per 10 years), mean (SD)	56.4 (11.1)	**-0.17 (-0.21--0.14)**	**-0.18 (-0.21--0.14)**	**-0.21 (-0.24--0.17)**
Female (%)	54.6	-0.02 (-0.10-0.05)	**-0.08 (-0.16--0.01)**	**-0.09 (-0.17--0.02)**
Born in the study area (%)	75.6	**0.29 (0.20-0.38)**	**0.22 (0.13-0.31)**	**0.23 (0.14-0.31)**
Childhood on a farm (%)	33.8	**0.22 (0.15-0.30)**	**0.30 (0.22-0.38)**	**0.11 (0.02-0.20)**
Ex-smoker (%)	44.6	**-0.18 (-0.26--0.11)**	**-0.08 (-0.16--0.01)**	**-0.09 (-0.17--0.01)**
Current smoker (%)	10.2	**0.18 (0.05-0.30)**	**0.13 (0.01-0.26)**	0.09 (-0.03-0.21)
BMI ≥ 30 ∞(%)	20.6	**0.22 (0.13-0.31)**	**0.25 (0.16-0.34)**	**0.24 (0.15-0.33)**
Higher education (%)	30.2	**-0.19 (-0.27--0.11)**	**-0.29 (-0.37--0.21)**	**-0.24 (-0.32--0.16)**
Paid work (%)	57.5	**0.20 (0.12-0.28)**	-0.05 (-0.15-0.04)	-0.08 (-0.17-0.02)
Retired (%)	28.3	**-0.25 (-0.33--0.17)**	0.02 (-0.09-0.12)	0.08 (-0.02-0.19)
Having pets, last 5 years (%)	52.4	**0.17 (0.09-0.25)**	0.07 (-0.01-0.15)	0.04 (-0.03-0.12)
Having farm animals as a hobby, last 5 years (%)	18.2	**0.12 (0.02-0.21)**	0.09 (0.00-0.19)	0.02 (-0.08-0.11)
During current work/study contact with animals (%)	6.1	**0.18 (0.03-0.34)**	0.21 (0.05-0.36)	0.10 (-0.05-0.26)
Visited a farm last 12 months (%)	62.6	**0.22 (0.14-0.29)**	0.16 (0.09-0.24)	0.12 (0.05-0.20)

Potential determinants of the 'attitude-score ' (z-score obtained from factor analysis) were analyzed with linear regression analysis. Bold type indicates statistical significance ($p < 0.05$). Regression coefficients display a change in the attitude-score for a difference in determinants as indicated in the table (e.g. for 10 years increase in age, or for being female *versus* male). A negative association means that the determinant is associated with a more negative attitude towards farming and a positive association means that the determinant is associated with a more positive attitude towards farming. * Model A was adjusted for age and gender, model B was adjusted for: age, gender, born in study area, childhood on a farm, BMI≥30, visited a farm last 12 months, high education. ∞BMI: body mass index = mass (kg)/ height $(m)^2$.

The following proxy measures of livestock farm exposure were statistically significantly associated with a more negative attitude: larger number of farms within 500 m and 1,000 m of the home and presence of pig or a goat farm within 1,000m (Table 6.4).

Table 6.3 Associations between the attitude-score and self-reported- and objective measured health determinants.

Health status	%	Unadjusted β (95%CI)	Model A* Adjusted β (95%CI)	Model B* Adjusted β (95%CI)
Psychological/neurovegetative symptoms				
Feeling down/ depressed	8.7	**-0.18 (-0.31--0.05)**	**-0.22 (-0.35--0.08)**	**-0.20 (-0.34--0.06)**
Acute (intense) stress or crisis	5.7	-0.14 (-0.30-0.02)	**-0.18 (-0.34--0.02)**	**-0.17 (-0.33--0.01)**
Feeling anxious/nervous/ tense	13.0	**-0.24 (-0.35--0.13)**	**-0.29 (-0.40--0.18)**	**-0.25 (-0.36--0.14)**
Feeling irritable/ angry	15.0	**-0.22 (-0.32--0.11)**	**-0.28 (-0.38--0.17)**	**-0.26 (-0.37--0.16)**
Sleep problems	25.0	**-0.24 (-0.33--0.16)**	**-0.21 (-0.29--0.12)**	**-0.21 (-0.30--0.13)**
Fatigue/ tiredness	37.9	**-0.15 (-0.22--0.07)**	**-0.24 (-0.32--0.16)**	**-0.23 (-0.31--0.15)**
Musculoskeletal symptoms				
Arm/ elbow/ hand/ wrist symptoms	24.5	**-0.12 (-0.20--0.03)**	**-0.09 (-0.17-0.00)**	**-0.09 (-0.18-0.00)**
Back problems	32.2	**-0.08 (-0.16-0.00)**	**-0.09 (-0.17--0.01)**	**-0.08 (-0.16-0.00)**
Neck- or shoulder symptoms	36.8	**-0.11 (-0.18--0.03)**	**-0.11 (-0.18--0.03)**	**-0.11 (-0.19--0.04)**
Leg/ hip/ knee/ foot symptoms	30.7	**-0.15 (-0.23--0.07)**	**-0.11 (-0.19--0.03)**	**-0.13 (-0.22--0.05)**
Pain in muscles	23.1	**-0.14 (-0.23--0.05)**	**-0.15 (-0.24--0.07)**	**-0.13 (-0.22--0.04)**
Gastrointestinal symptoms				
Abdominal/ stomach pain	20.6	**-0.22 (-0.31--0.12)**	**-0.24 (-0.34--0.15)**	**-0.23 (-0.32--0.13)**
Nausea	9.1	**-0.13 (-0.26-0.00)**	**-0.20 (-0.33--0.07)**	**-0.16 (-0.29--0.03)**
Diarrhea or constipation	21.5	**-0.13 (-0.23--0.04)**	**-0.16 (-0.25--0.07)**	**-0.11 (-0.21--0.02)**
Dizziness or feeling light-headed	19.5	**-0.23 (-0.33--0.14)**	**-0.26 (-0.35--0.17)**	**-0.26 (-0.36--0.16)**
Headache	27.2	-0.01 (-0.09-0.08)	**-0.10 (-0.18--0.01)**	-0.08 (-0.17-0.01)
Cardiac symptoms				
Pain or pressure in chest	8.9	**-0.23 (-0.36--0.10)**	**-0.24 (-0.37--0.11)**	**-0.25 (-0.38--0.12)**
Heart palpitations/ awareness	11.6	**-0.30 (-0.42--0.18)**	**-0.29 (-0.41--0.18)**	**-0.34 (-0.46--0.22)**
Shortness of breath or wheezing	7.6	**-0.27 (-0.42--0.13)**	**-0.31 (-0.45--0.17)**	**-0.34 (-0.48--0.19)**
Pulmonary symptoms				
Cough	27.0	**-0.24 (-0.33--0.16)**	**-0.23 (-0.31--0.15)**	**-0.21 (-0.29--0.12)**
Nasal symptoms	28.3	**-0.27 (-0.35--0.18)**	**-0.27 (-0.35--0.19)**	**-0.25 (-0.33--0.16)**
Symptoms from several other organs				
Ear symptoms	13.2	**-0.21 (-0.33--0.10)**	**-0.20 (-0.31--0.09)**	**-0.19 (-0.30--0.07)**
Eye irritation	19.1	**-0.30 (-0.40--0.21)**	**-0.29 (-0.38--0.19)**	**-0.26 (-0.36--0.17)**
Skin problems	21.0	**-0.21 (-0.30--0.12)**	**-0.21 (-0.30--0.12)**	**-0.21 (-0.30--0.11)**

Table 6.3 (continued)

Health status	%	Unadjusted β (95%CI)	Model A* Adjusted β (95%CI)	Model B* Adjusted β (95%CI)
Self-reported (respiratory) health				
Self-reported ever asthma	6.3	**-0.16 (-0.31-0.00)**	**-0.22 (-0.37--0.07)**	**-0.19 (-0.35--0.04)**
Self-reported current asthma	4.9	**-0.18 (-0.36--0.01)**	**-0.25 (-0.42--0.08)**	**-0.23 (-0.40--0.06)**
Self-reported COPD	5.1	**-0.25 (-0.43--0.08)**	-0.16 (-0.33-0.01)	-0.15 (-0.32-0.02)
Self-reported pneumonia confirmed by GP or specialist	5.3	**-0.21 (-0.38--0.04)**	**-0.18 (-0.34--0.01)**	**-0.24 (-0.40--0.07)**
Attribution health complaints by livestock farming	7.8	**-1.25 (-1.38--1.11)**	**-1.20 (-1.33--1.08)**	**-1.19 (-1.32--1.06)**
Objectively measured respiratory health mean (SD) (lung function parameters expressed as IQR increase)¥				
COPD based on lung function (%)§	9.0	-0.09 (-0.22-0.04)	0.00 (-0.13-0.14)	0.00 (-0.13-0.13)
Lung function parameters (mean (SD)), per IQR □				
FEV1 % predicted	99.4 (15.0)	**-0.06 (-0.10--0.01)**	-0.05 (-0.09-0.00)	-0.02 (-0.07-0.03)
FVC % predicted	103.1 (12.8)	**-0.10 (-0.15--0.05)**	**-0.10 (-0.15--0.05)**	-0.04 (-0.09-0.01)
FEV1/FVC % predicted	95.8 (8.5)	0.03 (-0.01-0.07)	**0.04 (0.00-0.09)**	0.01 (-0.03-0.05)
MMEF % predicted	94.0 (32.2)	0.00 (-0.05-0.04)	0.01 (-0.04-0.06)	0.01 (-0.04-0.06)

Associations between the 'attitude-score ' (z-score obtained from factor analysis) and self-reported health and objective measured health were analyzed with linear regression analysis. Bold type indicates statistical significance ($p < 0.05$). Regression coefficients display a change in the attitude-score for a difference in health determinants as indicated in the table. A negative association means that the determinant is associated with a more negative attitude towards farming and a positive association means that the determinant is associated with a more positive attitude towards farming. * Model A was adjusted for age and gender, model B was adjusted for: age, gender, born in study area, childhood on a farm, BMI ≥ 30 (BMI: body mass index = mass (kg)/ height $(m)^2$), visited a farm last 12 months, high education. ¥ In total 2059 subjects had lung function measurements of good quality (C or better)(19). COPD based on lung function: a post bronchodilator (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)[18,19].
□ Adjusted models (A + B) with lung function parameters were also adjusted for current smoking.

Excluding subjects who attributed their health symptoms to livestock farms in their environment (n=191, 7.8%), did not change associations between attitude and personal characteristics (Table S6.2) and associations with farm exposures (Table S3). However, associations between the attitude-score and self-reported health symptoms were attenuated in the sensitivity analyses. In particular, associations with musculoskeletal symptoms, gastrointestinal symptoms and self-reported respiratory health symptoms showed weaker associations, mostly with p-values >0.05 (Table S6.4).

Attitude towards farming as a confounder or effect modifier

Residents living within 1,000 m of a goat farm had a higher risk of self-reported pneumonia (OR 1.78 (95%CI 1.07-2.95)). Adding the attitude-score as a confounder hardly changed the association (OR 1.72 (95%CI 1.04-2.86)). In addition, adding the attitude-score and the confounders used in model B showed similar results (OR

1.73 (95%CI 1.03-2.93). No significant interaction-term was observed between attitude and living within 1,000 m of a goat farm (model A: p-value for interaction 0.631 and model B: p-value 0.627), suggesting that the association between goat farms and pneumonia was not modulated by attitude. Excluding subjects who attributed their health symptoms to livestock farms in their environment, did not change the association between self-reported pneumonia and living within 1,000 m of a goat farm (OR 1.75 (95%CI 1.02-3.01).

Table 6.4 Associations between the attitude-score and determinants of livestock farm exposure.

	Mean (SD) or n (%)	Unadjusted β (95%CI)	Model A* Adjusted β (95%CI)	Model B* Adjusted β (95%CI)
Number of livestock farms, mean (SD)				
Nr of farms within 500 m	1.8 (2.1)	-0.01 (-0.02-0.01)	-0.01 (-0.03-0.01)	**-0.02 (-0.04-0.00)**
Nr of farms within 1,000 m	9.3 (5.9)	-0.01 (-0.01-0.00)	**-0.01 (-0.01-0.00)**	**-0.01 (-0.02--0.01)**
Presence of farms within 1,000 m per animal category, n (%)				
Any farm	2357 (95.9)	0.09 (-0.10-0.28)	0.07 (-0.11-0.26)	0.02 (-0.17-0.21)
Pig farm	1949 (79.3)	-0.08 (-0.18-0.01)	**-0.09 (-0.18-0.00)**	**-0.13 (-0.22--0.04)**
Poultry farm	1356 (55.2)	0.07 (0.00-0.15)	0.06 (-0.02-0.13)	0.01 (-0.07-0.08)
Cattle farm	2314 (94.2)	0.05 (-0.11-0.21)	0.04 (-0.12-0.20)	0.01 (-0.15-0.17)
Goat farm	266 (10.8)	**-0.12 (-0.25-0.00)**	**-0.16 (-0.28--0.04)**	**-0.19 (-0.31--0.08)**
Sheep farm	771 (31.4)	-0.01 (-0.09-0.07)	-0.02 (-0.10-0.06)	-0.04 (-0.12-0.04)
Horse farm	1763 (71.8)	0.04 (-0.05-0.12)	0.04 (-0.04-0.12)	0.02 (-0.06-0.10)

Associations between the 'attitude-score ' (z-score obtained from factor analysis) and determinants of livestock farm exposure were analyzed with linear regression analysis. Bold type indicates statistical significance ($p<0.05$). Regression coefficients display a change in the attitude-score for a difference in determinants as indicated in the table. A negative association means that the determinant is associated with a more negative attitude towards farming and a positive association means that the determinant is associated with a more positive attitude towards farming. * Model A was adjusted for age and gender, model B was adjusted for: age, gender, born in study area, childhood on a farm, BMI≥30 (BMI: body mass index = mass (kg)/ height (m)^2), visited a farm last 12 months, high education.

Discussion

In general, the study population had a relatively positive attitude towards farming. Most questions were answered with a neutral to positive tendency. Familiarity with farming could possibly explain the predominantly positive attitude. One third of the study population had grown up on a farm. The study area, in which 75.6% of the study population was born, is characterised by the highest farm density of the Netherlands. Previous studies on risk perception show that common risks are judged more acceptable than uncommon and unknown risks[22]. Agricultural activities are familiar and common among the majority of the study population and therefore probably more acceptable. Attitude was indeed positively associated with determinants related to familiarity with a farming environment – such as childhood on a farm, being born in the study area, or a recent farm visit.

In 2011, a survey on the public's view of the Dutch population on intensive livestock farming was conducted[7]. This survey consisted of two parts: 1) a qualitative part that explored arguments that play a role in the discussion on intensive livestock farming in the Netherlands, 2) the second part consisted of an online survey among 1,090 subjects from the Dutch general population. The 15 statements in our questionnaire were adopted from or inspired by this survey. Results of the online survey showed a lot of similarities with the answers to the statements given by our study population, even though our study population is living in a rural area with high livestock farm density. This might explain why our study population considers the benefits for the local (and Dutch) economy more important than the general Dutch population from the previous survey (73.3% *versus* 52%). In the online survey one of the most important arguments against intensive livestock farming was focused on potential risks for public health, and especially on antibiotic resistant bacteria and zoonotic diseases[7]. The majority of our study population mentioned to be concerned about antibiotic usage in livestock farming and zoonotic diseases. The use of antibiotics in livestock production can lead to increased occurrence of antimicrobial resistance in bacteria which may transmit to humans[23]. Previous studies show increased risks of livestock-related antimicrobial resistance among farmers with direct animal contact[24,25]. This may have contributed to concerns about antimicrobial resistance in the study population, despite the large reduction of antimicrobials use of more than 60% in livestock farming since 2009 in the Netherlands[26]. In the current VGO-study, no increased risk was observed between farm proximity and carriage of extended-spectrum β-lactamase- (ESBL) and pAmpC-producing Enterobacteriaceae[27]. However, a slightly increased risk was observed between living near farms and carriage of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA), although the prevalence was low, and there is a high likelihood of a chance finding[28]. A Q-fever outbreak in the study area between 2007 and 2011, which most likely originated from infected goat farms, may have contributed to our study population's concerns on emerging zoonotic infections[29]. More than 3,500 acute Q-fever patients were officially registered, and it was estimated that 74 patients died. A study focused on regional differences in public perceptions regarding Q-fever found that this epidemic caused increased perceived anxiety and preventive behaviour – e.g. taking preventive measures – among subjects living in regions with high Q-fever incidence[30].

In general, self-reported health symptoms were associated with a more negative attitude. Subjects who reported to attribute their health complaints to livestock farming had a much lower average attitude-score than other participants. This is in line with previous studies that showed positive associations between concern and reporting factors related to illness[8,11]. Awareness bias[8] might have played a role since we only observed an association between attitude and self-reported respiratory health and not with objectively measured respiratory health. Several indicators of livestock farm exposure were associated with a more negative attitude. Subjects who live in areas with a high number of livestock farms, especially in close proximity of pig and goat farms, had a more negative attitude towards farming than subjects living in areas with less livestock farms. The association with goat farms might be explained by the Q-fever outbreak in the study area[29]. The observed association with pig farms could possibly explained by odour annoyance. Pig farms emit more offensive odour in comparison with cattle and poultry farms[31]. Odour annoyance is common in populations living in the proximity of livestock farms and is a main source of annoyance[32,33]. A Dutch study showed that the number of pigs, but also the number of poultry and cattle, around homes of residents was associated with odour annoyance[34].

Our second aim was to assess whether the earlier observed association between goat farm exposure and self-reported pneumonia was biased by participants' attitude, i.e. by confounding or effect modification. In case of confounding the effect of the confounder is mixed with the effect of interest. As a result there is a biased estimate of risk. For a variable to be a confounder it must have three characteristics[35]: 1) it must be a risk factor for the disease, 2) it must be associated with the exposure under study in the population from which the cases derive and 3) a confounding variable must not be an intermediate step in the causal path between the exposure and the disease. Attitude towards farming meets the first two conditions: a more negative attitude was associated with self-reported pneumonia (first condition), and a more negative attitude was associated with living with one or more goat farms within 1,000 m(second condition). The third condition is more difficult to answer. Is attitude an intermediate factor on the pathway between living near goat farms and self-reported pneumonia? One could argue that attitude is an intermediate step in the causal path since people living in close proximity of goat farms might have a more negative attitude towards farming due to the Q-fever outbreak, and therefore report more often pneumonia. However, previous studies also found associations between goat farm exposure and pneumonia recorded in Electronic Medical Records (EMR) of the general practitioner[2,16]. EMR-based pneumonia is not biased by attitude of the patient, since this information is based on the examination by a physician. Therefore, attitude is unlikely to be an intermediate in the causal path and thus meets all three conditions of a potential confounder. Controlling for confounding can be achieved by multiple regression analysis. In general, if a potential confounder changes the estimates of the risk by 10% or more, than it is considered to be a confounder. Adding the attitude-score as a confounder to the model resulted in minor changes of the association (<10%). We also checked whether attitude acted as an effect modifier. There is effect modification when the selected effect measure for the risk factor under study varies across levels of another factor[35], which was not observed in our study. In conclusion, we did not find any indication that the earlier observed association between goat farm exposure and self-reported pneumonia[16] was confounded or modified by attitude towards farming.

Strengths of our study are our large, population-based sample and the low amount of missing data on the attitude statements. Both self-reported-and objectively assessed data on respiratory health was available; this enabled us to compare associations with attitude and to explore awareness bias. Nevertheless, a number of limitations should be considered. First, the cross-sectional design makes it difficult to infer causality. Second, attitude towards farming may have contributed to the decision whether or not to participate to the medical examination and to the questionnaire survey where the study population originates from. Our previous studies showed that participants of the medical examination[19] and responders to the questionnaire survey[36] lived in closer proximity to farms compared to subjects who did not participate and to non-responders respectively. We have no information on attitude towards farming from the source population, therefore it was not possible to analyze the effect of participation bias on the average reported attitude.

In conclusion, we developed an attitude-score to measure attitude towards farming in the residential environment. In general, the study population had a positive attitude towards farming, in particular if participants were more familiar with farming. Older participants, females, ex-smokers, and individuals with a higher education had a more negative attitude. Self-reported symptoms were also associated with a more negative attitude. Awareness bias might have played a role since we only observed associations between attitude and self-reported respiratory health symptoms and not with objectively measured respiratory health. The attitude-score was associated with exposure to livestock farms and could therefore be a potential confounder. However, we did not find any indication that the association between proximity to goat farms and self-reported pneumonia was confounded or modified by attitude. Overall, results of the current study indicate that awareness bias might play a role when using self-reported data in environmental health studies. When relying on self-reported data, we recommend to estimate attitude towards a potential hazard to assess the potential influence of awareness bias on epidemiological associations.

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Supplementary methods and tables

Methods (more detailed)

Study design and population

The VGO 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 (n=14,163) conducted in December 2012 and previously described by Borlée *et al.*[1]. Questionnaire respondents who were willing to participate in a follow-up study, and who were not working or living on a farm were eligible for a medical examination (n=8,714). Between March 2014 and February 2015, 7,180 persons were invited for medical examination and 2,494 participated (response 34.7%). The medical examination consisted amongst others of a second and more extended questionnaire and spirometry. More details about the recruitment of the study population and the medical examination are described previously by Borlée *et al.*[2,3].

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.

Data collection

Questionnaire

The questionnaire completed by the participants during the medical examination comprised amongst others items on education, profession, residential history, smoking habits, non-specific symptoms and diseases. The Symptoms and Perceptions (SaP) questionnaire was used to measure non-specific symptoms[4]. These symptoms - such as headache, fatigue, sleep difficulties – are very common in the general population, refer to multiple organ systems and can be caused by a variety of factors. Moreover, the questionnaire contained also items on attitudes towards farming in their residential environment. Statements were mostly adopted from a survey among the general Dutch population which was focused on the public's view on intensive livestock farming[5].

Construction of score for attitude towards livestock farming in the residential environment

The questionnaire included 15 statements related to attitudes towards livestock farming in the residential environment (see table 1). Response options were coded based on a five-point Likert scale: “Strongly disagree“, “Disagree”, “Neutral” (neither agree nor disagree), “Agree”, and “Strongly agree”. Responses to negatively-keyed statements were reverse-scored (see Table 6.1). Correlation between the statements was checked with a Pearson correlation matrix. Principal factor analysis was used to identify one or more latent factors which can be interpreted as an ‘attitude towards farming’. The number of factors determined was based on the Kaiser-Guttman criterion (retain factors having an eigenvalue greater than 1), and by observing the point where the scree plot bends. Final factor analysis was done after excluding items with residual correlations or partial correlations <0.1. Standardized factor scores (z-scores, hereafter named ‘attitude score’) were computed as linear combinations of scoring coefficients and standardized questionnaire responses for each participant, where a higher score indicates a more positive attitude towards farming. In total, data from 37 subjects were excluded from the analyses since they had more than 3 missing answers among the 15 statements (n=36), or answered all 15 statements with ”Strongly disagree” before recoding (n=1). Missing answers of all 2,457 remaining subjects were assigned to ”Neutral”.

Spirometry

Pre- and post-BD spirometry was conducted according to European Respiratory Society (ERS) guidelines and the European Community Respiratory Health Survey III (ECRHS-III)[6], and is described in more detail by Borlée *et al*.[3].

Livestock farm exposure variables

Livestock farm exposure proxies were computed for each subject as described previously[7]. In short, livestock farm proximity to the home address for each participant 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) number of farms within 500 and 1,000 m and 2) presence of a farm (pig, poultry, cattle, goat, sheep, horse) within 1,000 m (Y/N).

Data analysis

Linear regression analysis was used to assess the association between attitude and potential determinants. The potential determinants of attitude studied were:

1) general characteristics: age, gender, born in study area, childhood on a farm, smoking habits, BMI, education, employment status, direct contact with (farm) animals; 2) health status: non-specific symptoms, self-reported respiratory health, lung function (spirometry), 3) exposure to livestock farms. SaP questionnaire analyses were conducted on each symptom separately, as well as for clusters which were previously studied by Yzermans *et al.*[4]: psychological/neurovegetative, musculoskeletal, gastrointestinal, cardiac, pulmonary, and symptoms from several other organ systems. The number of reported symptoms in each cluster was categorized into tertiles plus a reference category (no reported symptoms in the cluster). Symptom clusters that consisted only out of two symptoms were categorized into 'no symptoms', '1 symptom' or '2 symptoms'. Cardiac symptoms were dichotomized into 'no symptoms' and '1 or 2 symptoms'. Potential confounders (general characteristics) with a p-value <0.2 were selected beforehand by linear regression of attitude on potential confounders following a forward stepwise procedure based on improvement of Akaike's Information Criterion (AIC). As a result, two adjusted models were run: the basic model A (only adjusted for age and gender) and the full Model B (age, gender, born in study area, childhood on a farm, BMI≥30, visited a farm last 12 months, high education). Sensitivity analyses were conducted after excluding subjects who attributed their symptoms to presence of livestock farms in their environment.

Freidl *et al.*[8] found in the same study population (2,494 participants of the medical examination) a higher risk of pneumonia for residents living in close proximity to goat farms. Pneumonia was defined as self-reported physician-diagnosed pneumonia over the past three years, or pneumonia recorded in the Electronic Medical Records (EMR). In the current study, pneumonia was defined as only self-reported physician-diagnosed pneumonia over the past three years reported in the questionnaire. The association between pneumonia and goat farm proximity was analysed using multiple logistic regression. The effect of attitude towards farming on the relationship between self-reported pneumonia and goat farm proximity was analysed by adding the attitude score as a confounder. We also tested interaction between goat farm exposure and the attitude score. A sensitivity analysis was conducted after excluding subjects who attributed their symptoms to presence of livestock farms in their environment.
Data were analysed using SAS 9.4 (SAS Institute Inc. Cary, NC, USA).

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Table S6.1 Association between the attitude-score and (clusters of) total number of self-reported non-specific symptoms.

Reported number of symptoms	**Median (IQR)**	**Unadjusted β (95%CI)**	**Model A* Adjusted β (95%CI)**	**Model B* Adjusted β (95%CI)**
Total number of symptoms	4 (5)			
0 symptoms	13.7 %	1	1	1
1 - 3 symptoms	32.7 %	-0.04 (-0.17-0.08)	-0.06 (-0.18-0.06)	-0.06 (-0.19-0.06)
4 to 6 symptoms	25.0 %	**-0.25 (-0.38--0.13)**	**-0.29 (-0.41--0.16)**	**-0.28 (-0.41--0.15)**
>6 symptoms	28.6 %	**-0.40 (-0.52--0.27)**	**-0.45 (-0.57--0.32)**	**-0.44 (-0.56--0.31)**
Test for trend		**<.0001**	**<.0001**	**<.0001**
Psychological/neurovegetative symptoms	1 (2)			
0 symptoms	47.9 %	1	1	1
1 symptom	25.3%	-0.04 (-0.13-0.05)	**-0.09 (-0.18-0.00)**	-0.09 (-0.18-0.00)
2 symptoms	12.5 %	**-0.25 (-0.37--0.13)**	**-0.31 (-0.43--0.20)**	**-0.28 (-0.40--0.17)**
>2 symptoms	14.4%	**-0.32 (-0.43--0.21)**	**-0.39 (-0.50--0.28)**	**-0.38 (-0.50--0.27)**
Test for trend		**<.0001**	**<.0001**	**<.0001**
Musculoskeletal symptoms	1 (2)			
0 symptom	33.5 %	1	1	1
1 symptom	24.8 %	-0.04 (-0.14-0.06)	-0.04 (-0.14-0.06)	-0.04 (-0.14-0.06)
2-3 symptoms	29.8 %	**-0.18 (-0.28--0.09)**	**-0.18 (-0.28--0.09)**	**-0.20 (-0.29--0.10)**
>3 symptoms	11.8%	**-0.21 (-0.33--0.08)**	**-0.18 (-0.31--0.06)**	**-0.18 (-0.31--0.05)**
Test for trend		**<.0001**	**<.0001**	**<.0001**
Gastrointestinal symptoms	1 (2)			
0 symptoms	49.8 %	1	1	1
1 symptom	23.9 %	-0.05 (-0.14-0.04)	-0.09 (-0.18-0.00)	-0.05 (-0.14-0.05)
2 symptoms	13.2 %	**-0.21 (-0.32--0.09)**	**-0.25 (-0.37--0.14)**	**-0.20 (-0.31--0.08)**
>2 symptoms	13.2%	**-0.25 (-0.36--0.13)**	**-0.34 (-0.46--0.22)**	**-0.31 (-0.43--0.19)**
Test for trend		**<.0001**	**<.0001**	**<.0001**
Cardiac symptoms	0 (0)			
1 or 2 cardiac symptoms	20.8%	**-0.28 (-0.37--0.19)**	**-0.29 (-0.38--0.20)**	**-0.33 (-0.42--0.23)**
Pulmonary symptoms	0 (1)			
0 symptoms	59.2 %	1	1	1
1 symptom	26.3%	**-0.25 (-0.34--0.16)**	**-0.23 (-0.32--0.15)**	**-0.21 (-0.30--0.12)**
>1 symptoms	14.5%	**-0.35 (-0.46--0.24)**	**-0.35 (-0.46--0.24)**	**-0.32 (-0.43--0.21)**
Test for trend		**<.0001**	**<.0001**	**<.0001**
Symptoms from several other organs	0 (1)			
0 symptoms	88.2 %	1	1	1
1 symptom	9.4 %	**-0.25 (-0.33--0.16)**	**-0.25 (-0.33--0.16)**	**-0.24 (-0.32--0.15)**
>1 symptoms	2.4 %	**-0.36 (-0.48--0.24)**	**-0.34 (-0.45--0.22)**	**-0.31 (-0.43--0.19)**
Test for trend		**<.0001**	**<.0001**	**<.0001**

Associations between the 'attitude-score ' (z-score obtained from factor analysis) and (clusters of) total number of non-specific symptoms were analyzed with linear regression analysis. Bold type indicates statistical significance ($p<0.05$). Symptoms are divided in clusters previously proposed by Yzermans *et al.*[20]. A negative association means that the determinant is associated with a more negative attitude towards farming and a positive association means that the determinant is associated with a more positive attitude towards farming. * Model A was adjusted for age and gender, model B was adjusted for: age, gender, born in study area, childhood on a farm, BMI≥30 (BMI: body mass index = mass (kg)/ height $(m)^2$), visited a farm last 12 months, high education.

Sensitivity analyses

Table S6.2 Characteristics of the study population (n=2,266) without subject who attributed their health complaints by exposure to livestock farming (n=191) and associations between potential determinants with the attitude score.

General characteristics	Unadjusted β (95%CI)	Model A* Adjusted β (95%CI)	Model B* Adjusted β (95%CI)
Age (per 10 years), mean (SD)	**-0.16 (-0.19--0.12)**	**-0.16 (-0.19--0.13)**	**-0.19 (-0.22--0.15)**
Female (%)	-0.02 (-0.09-0.06)	-0.07 (-0.14-0.00)	**-0.08 (-0.16--0.01)**
Born in the study area (%)	**0.28 (0.19-0.36)**	**0.22 (0.13-0.30)**	**0.11 (0.02-0.20)**
Childhood on a farm (%)	**0.22 (0.14-0.30)**	**0.29 (0.22-0.37)**	**0.23 (0.15-0.31)**
Ex-smoker (%)	**-0.16 (-0.23--0.08)**	-0.06 (-0.14-0.01)	-0.07 (-0.15-0.00)
Current smoker (%)	0.11 (-0.01-0.23)	0.07 (-0.05-0.19)	0.03 (-0.09-0.15)
BMI ≥30 ∞(%)	**0.22 (0.13-0.31)**	**0.25 (0.16-0.34)**	**0.24 (0.15-0.32)**
Higher education (%)	**-0.17 (-0.25--0.09)**	**-0.27 (-0.35--0.19)**	**-0.22 (-0.30--0.14)**
Paid work (%)	**0.17 (0.10-0.24)**	-0.07 (-0.16-0.02)	-**0.09 (-0.19-0.00)**
Retired (%)	**-0.23 (-0.32--0.15)**	0.00 (-0.10-0.10)	0.07 (-0.04-0.17)
Having pets, last 5 years (%)	**0.16 (0.09-0.23)**	0.07 (-0.01-0.14)	0.04 (-0.03-0.12)
Having farm animals as a hobby, last 5 years (%)	**0.11 (0.02-0.21)**	**0.09 (0.00-0.19)**	0.03 (-0.07-0.12)
During current work/study contact with animals (%)	**0.25 (0.10-0.41)**	**0.23 (0.08-0.38)**	**0.16 (0.01-0.31)**
Visited a farm last 12 months (%)	**0.19 (0.12-0.27)**	**0.15 (0.07-0.22)**	**0.11 (0.03-0.18)**

Potential determinants of the 'attitude score' (z-score obtained from factor analysis) were analyzed with linear regression analysis. Bold type indicates statistical significance ($p<0.05$). Regression coefficients display a change in the attitude score for a difference in determinants as indicated in the table (e.g. for 10 years increase in age, or for being female *versus* male). A negative association means that the determinant is associated with a more negative attitude towards farming and a positive association means that the determinant is associated with a more positive attitude towards farming. * Model A was adjusted for age and gender, model B was adjusted for: age, gender, born in study area, childhood on a farm, BMI≥30, visited a farm last 12 months, high education. ∞BMI: body mass index = mass (kg)/ height (m)^2.

Table S6.3 Associations between the attitude score and determinants of livestock farm exposure. Subjects who attributed their health complaints by exposure to livestock farming (n=191) were excluded in this sensitivity analysis.

	Unadjusted β (95%CI)	Model A* Adjusted β (95%CI)	Model B* Adjusted β (95%CI)
Number of livestock farms, mean (SD)			
Nr of farms within 500 m	0.00 (-0.01-0.02)	0.00 (-0.02-0.02)	-0.01 (-0.03-0.01)
Nr of farms within 1,000 m	0.00 (-0.01-0.00)	0.00 (-0.01-0.00)	**-0.01 (-0.02-0.00)**
Presence of farms within 1,000 m per animal category, n (%)			
Any farm	0.12 (-0.07-0.31)	0.11 (-0.08-0.29)	0.04 (-0.14-0.23)
Pig farm	-0.04 (-0.13-0.05)	-0.05 (-0.14-0.04)	**-0.09 (-0.18--0.01)**
Poultry farm	0.05 (-0.02-0.12)	0.04 (-0.03-0.11)	0.00 (-0.08-0.07)
Cattle farm	0.06 (-0.09-0.22)	0.06 (-0.10-0.21)	0.01 (-0.14-0.17)
Goat farm	-0.11 (-0.23-0.00)	**-0.14 (-0.26--0.03)**	**-0.17 (-0.29--0.06)**
Sheep farm	0.03 (-0.05-0.11)	0.02 (-0.06-0.10)	0.00 (-0.08-0.08)
Horses farm	0.05 (-0.04-0.13)	0.05 (-0.03-0.13)	0.03 (-0.05-0.11)

Associations between the 'attitude score' (z-score obtained from factor analysis) and determinants of livestock farm exposure were analyzed with linear regression analysis. Bold type indicates statistical significance ($p<0.05$). Regression coefficients display a change in the attitude score for a difference in determinants as indicated in the table. A negative association means that the determinant is associated with a more negative attitude towards farming and a positive association means that the determinant is associated with a more positive attitude towards farming. * Model A was adjusted for age and gender, model B was adjusted for: age, gender, born in study area, childhood on a farm, BMI≥30 (BMI: body mass index = mass (kg)/ height (m)^2), visited a farm last 12 months, high education.

Table S6.4 Associations between the attitude score and self-reported- and objective measured health determinants. Subjects who attributed their health complaints by exposure to livestock farming (n=191) were excluded in this sensitivity analysis.

Health status	Unadjusted β (95%CI)	Model A* Adjusted β (95%CI)	Model B* Adjusted β (95%CI)
Psychological/neurovegetative symptoms			
Feeling down/ depressed	-0.11 (-0.25-0.02)	-0.15 (-0.28--0.02)	-0.13 (-0.27-0.00)
Acute (intense) stress or crisis	-0.07 (-0.23-0.09)	-0.12 (-0.28-0.04)	-0.11 (-0.28-0.05)
Feeling anxious/nervous/ tense	**-0.21 (-0.33--0.10)**	**-0.25 (-0.36--0.15)**	**-0.23 (-0.34--0.11)**
Feeling irritable/ angry	**-0.15 (-0.26--0.05)**	**-0.21 (-0.31--0.11)**	**-0.19 (-0.30--0.09)**
Sleep problems	**-0.19 (-0.28--0.10)**	**-0.15 (-0.24--0.07)**	**-0.16 (-0.25--0.07)**
Fatigue/ tiredness	**-0.09 (-0.16--0.01)**	**-0.18 (-0.25--0.10)**	**-0.16 (-0.24--0.08)**
Musculoskeletal symptoms			
Arm/ elbow/ hand/ wrist symptoms	-0.05 (-0.14-0.04)	-0.03 (-0.12-0.05)	-0.03 (-0.12-0.05)
Back problems	-0.04 (-0.12-0.04)	-0.05 (-0.12-0.03)	-0.04 (-0.12-0.04)
Neck- or shoulder symptoms	-0.06 (-0.14-0.02)	-0.06 (-0.14-0.01)	-0.07 (-0.15-0.00)
Leg/ hip/ knee/ foot symptoms	**-0.12 (-0.20--0.04)**	-0.08 (-0.16-0.00)	**-0.11 (-0.19--0.03)**
Pain in muscles	-0.07 (-0.16-0.02)	-0.09 (-0.17-0.00)	-0.08 (-0.17-0.01)
Gastrointestinal symptoms			
Abdominal/ stomach pain	**-0.17 (-0.27--0.08)**	**-0.20 (-0.29--0.11)**	**-0.19 (-0.28--0.10)**
Nausea	-0.06 (-0.19-0.07)	-0.13 (-0.26-0.00)	-0.10 (-0.23-0.03)
Diarrhea or constipation	-0.07 (-0.16-0.02)	-0.10 (-0.18--0.01)	-0.07 (-0.16-0.03)
Dizziness or feeling light-headed	**-0.15 (-0.25--0.06)**	**-0.18 (-0.27--0.08)**	**-0.17 (-0.27--0.07)**
Headache	0.07 (-0.01-0.15)	-0.01 (-0.09-0.08)	0.01 (-0.07-0.10)
Cardiac symptoms			
Pain or pressure in chest	**-0.14 (-0.28--0.01)**	**-0.15 (-0.28--0.01)**	**-0.17 (-0.30--0.03)**
Heart palpitations/ awareness	**-0.22 (-0.34--0.10)**	**-0.21 (-0.33--0.10)**	**-0.26 (-0.38--0.14)**
Shortness of breath or wheezing	-0.13 (-0.28-0.02)	**-0.17 (-0.32--0.03)**	**-0.21 (-0.36--0.05)**
Pulmonary symptoms			
Cough	**-0.11 (-0.20--0.03)**	**-0.10 (-0.19--0.02)**	-0.07 (-0.16-0.02)
Nasal symptoms	**-0.10 (-0.19--0.02)**	**-0.11 (-0.19--0.03)**	-0.08 (-0.16-0.00)
Symptoms from several other organs			
Ear symptoms	**-0.15 (-0.26--0.04)**	**-0.13 (-0.24--0.02)**	**-0.13 (-0.24--0.02)**
Eye irritation	**-0.17 (-0.27--0.07)**	**-0.16 (-0.25--0.06)**	**-0.12 (-0.22--0.03)**
Skin problems	**-0.15 (-0.24--0.06)**	**-0.14 (-0.23--0.05)**	**-0.14 (-0.23--0.05)**
Self-reported (respiratory) health			
Self-reported ever asthma	-0.09 (-0.25-0.07)	-0.16 (-0.32-0.00)	-0.13 (-0.28-0.03)
Self-reported current asthma	-0.09 (-0.27-0.09)	-0.16 (-0.34-0.01)	-0.13 (-0.31-0.05)
Self-reported COPD	**-0.21 (-0.39--0.04)**	-0.13 (-0.30-0.05)	-0.10 (-0.28-0.07)
Self-reported pneumonia confirmed by GP or specialist	-0.08 (-0.25-0.09)	-0.05 (-0.22-0.11)	-0.11 (-0.28-0.06)
Objectively measured respiratory health mean (SD) (lung function parameters expressed as IQR increase)¥			
COPD based on lung function (%)§	-0.07 (-0.19-0.06)	0.02 (-0.11-0.15)	0.02 (-0.11-0.15)
Lung function parameters (mean (SD)), per IQR □			
FEV1 % predicted	**-0.06 (-0.11--0.02)**	**-0.06 (-0.10--0.01)**	-0.03 (-0.08-0.01)
FVC % predicted	**-0.10 (-0.15--0.05)**	**-0.10 (-0.14--0.05)**	-0.04 (-0.09-0.00)
FEV1/FVC % predicted	0.02 (-0.02-0.06)	0.03 (-0.01-0.07)	0.00 (-0.04-0.04)
MMEF % predicted	-0.02 (-0.07-0.03)	0.00 (-0.02-0.01)	-0.01 (-0.06-0.04)

Associations between the 'attitude score' (z-score obtained from factor analysis) and self-reported health and objective measured health were analyzed with linear regression analysis. Bold type indicates statistical significance ($p < 0.05$). Regression coefficients display a change in the attitude score for a difference in health determinants as indicated in the table. A negative association means that the determinant is associated with a more negative attitude towards farming and a positive association means that the determinant is associated with a more positive attitude towards farming. * Model A was adjusted for age and gender, model B was adjusted for: age, gender, born in study area, childhood on a farm, BM≥30 (BMI: body mass index = mass (kg)/ height $(m)^2$), visited a farm last 12 months, high education. ¥ In total 2059 subjects had lung function measurements of good quality (C or better)[3]. § COPD based on lung function: a post bronchodilator (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)[2,3]. □ Adjusted models (A + B) with lung function parameters were also adjusted for current smoking.

Chapter 7

General discussion

Main findings

The aim of this thesis was to explore associations between air pollution from livestock farms and respiratory health of non-farming residents living in close proximity to farms in a rural area in the Netherlands. This was achieved through the VGO study. Prior to the VGO-study, the IVG study[1] was conducted. In this thesis, data of the VGO study were analysed which consisted of two parts (see Figure 1.2 in the Introduction). The first part consisted of a questionnaire survey among 14,882 subjects living in a rural area in the Netherlands. Of these respondents, 2,494 subjects participated in the second part. This part included a health examination survey consisting of measuring other parameters, a second and more extended questionnaire, spirometric lung function measurements and collection of serum for assessing sensitization to a panel of common allergens.

Chapter 2 describes results of the questionnaire survey among 14,883 subjects. Analyses were conducted on 12,117 responders, after excluding farmers (subject who reported to be living or working on a farm) and subjects who were living at least one year at their current home address. Inverse associations were found between living in the proximity of livestock farms and self-reported respiratory health of residents. Prevalence of self-reported asthma, COPD and nasal allergies was lower among residents living at a short distance from a farm compared to residents living further away (Q1 (<290m), compared to Q4 (>640m), asthma: OR 0.83, 95% CI 0.71–0.98, COPD: OR 0.71, 95% CI 0.56–0.91 and nasal allergies: 0.87, 95% CI 0.77–0.98). On the other hand, results also showed more respiratory symptoms among COPD patients who were living in close proximity to livestock farms. Wheezing and use of inhaled corticosteroids among COPD patients was positively associated with several indicators of livestock farm exposures. This may indicate an increased risk of exacerbations among COPD patients who have a high exposure to livestock farm emissions.

In the VGO study, respiratory health of residents was assessed based on three data sources namely: self-reported data, general practitioner (GP) diagnosis (based on Electronical Medical Records (EMR)), and spirometry results. In **Chapter 3**, we compared four different definitions for determining COPD prevalence to get more insight into the possible effects of using various COPD-definitions on prevalence estimates and their associations with potential risk factors. The operational definition used for COPD greatly influences prevalence estimates which varied from 2.9% to 10.9%. Self-reported or GP-diagnosed COPD identified less than 30% of all

COPD cases based on persistent airflow limitations, which implies that a substantial number of subjects with COPD cannot be identified by questionnaires or medical records. However, the effect of the different COPD-definitions on associations with potential risk factors was limited, except for indicators of allergy, which were more strongly associated with self-reported COPD compared to the other definitions. In addition, the use of pre-bronchodilator instead of post-bronchodilator spirometry results in the COPD definition led to higher prevalence estimates, but had minimal effect on associations with potential risk factors.

Chapter 4 shows that air pollution from livestock farms is associated with a reduced lung function level in neighbouring residents. Respiratory health of the VGO study population was assessed with a pre- and post-bronchodilator pulmonary function test. Associations were found between lung function and both spatial and temporal livestock farm exposure estimates. A spatial association was found between the number of livestock farms within a 1,000 m buffer from the home address and lung function, which was statistically significant for the maximum mid-expiratory flow (MMEF). Subjects living in a 'hotspot'(>17 farms within 1,000m) had a 4.5% lower MMEF (95% CI, -8.64 to -0.36). In addition, associations with temporal livestock farm exposure estimates were observed. Lung function variables (Forced Expiratory Volume in 1 second (FEV_1), FEV_1/Forced Vital Capacity (FVC) and MMEF) were lower when measured after weeks with higher ammonia (NH_3) levels and PM_{10} levels. In a two-pollutant model, only ammonia remained statistically significantly associated. A 25 $\mu g/m^3$ increase in ammonia 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). Livestock production is the major contributor to ambient ammonia levels. Our results indicate that the spatial association was especially apparent in non-atopic subjects and in patients with COPD, while the temporal association with ammonia was observed in the whole population.

Chapter 5 shows that current exposure to a livestock farm environment, assessed as residential proximity to livestock farms, seems to protect against atopy in adults. Atopy was defined as specific serum IgE antibodies ≥0.35 IU/ml to one or more common allergens and/or a total IgE higher than 100 IU/ml. The prevalence of atopy was 29.8%. Subjects living at short distances from farms (<327 m, 1st tertile) had a lower odds for atopy compared to subjects living further away (>527 m, 3rd tertile) (OR 0.79, 95% CI 0.63-0.98). Significant associations in the same direction were found with distance to the nearest pig or cattle farm. The negative associations between atopy and livestock farm exposure were somewhat stronger in subjects who grew up on a farm.

Awareness bias is described as the propensity to report more illness and symptoms as a result of proximity to a potential hazard, in the absence of a biological effect[2]. Attitude towards environmental risks – such as air pollution emitted by farms - may be a source of bias since concerns about environmental hazards may influence self-reported health outcomes. One of the findings of the VGO study was a higher risk of self-reported pneumonia for residents living in close proximity to goat farms[3]. Since the studied health outcome was self-reported and therefore not objectively assessed, potential awareness bias may have resulted in confounding or effect modification by attitude towards farming. In **Chapter 6** a score was developed to measure the attitude towards livestock farming in a residential environment. In general, the VGO study population had a positive attitude towards farming, in particular if participants were more familiar with farming. Awareness bias might have played a role since we observed associations between attitude and self-reported respiratory health, while objectively measured respiratory health was not associated with attitude. The attitude score was also associated with exposure to livestock farms and could therefore be a potential confounder. However, we did not find any indication that the earlier observed associations between proximity to goat farms and self-reported pneumonia was confounded or modified by attitude.

What do these studies add to the scientific literature?

Spatial and temporal associations between lung function and livestock farm exposure

Prior to 2012, when this research project started, there were only three articles published in which respiratory health of neighbouring residents of livestock farms was measured objectively using spirometry. Most studies used self-reported health outcomes or diagnostic data from Electronic Medical Records (see Chapter 1, Table 1.1). Spirometry results are objective measurements and are not influenced by symptom-perception, recall-bias, misdiagnosis and access to health care[4]. One of the three articles reported on a panel study conducted in the United States in which FEV_1 was measured in 101 adults. A temporal effect of farm-related pollutants was observed[5]. The other two articles arose from a cross-sectional study in Lower Saxony in Germany. In this study a decrease in FEV_1 was observed in 1,030 adults living with more than 12 stables within 500 m of the home address[6]. Farm density was also a predictor for self-reported wheezing. Moreover, subjects who were exposed to higher annual ammonia levels showed a significantly lower FEV_1 (-8%) compared to a control reference group (total population n=457) (7). More recently, in 2015, two articles were published about a longitudinal study among 58 asthmatic children from Washington State (USA). Among those susceptible subjects, week-average ammonia levels were associated with a decrease of FEV_1 (-3.8% for an IQR increase ammonia)[8]. Also, a small but statistically significant decrease of FEV_1 (-0.9%) was associated with an IQR increase of day-average $PM_{2.5}$ concentrations[9].

In our study (Chapter 4), we were able to confirm and expand some of these earlier findings. A spatial association was found between farm density (the number of farms within 1,000m) and MMEF. This spatial association was especially apparent in subjects without atopy and in patients with COPD. Another study within VGO found the number of farms as a predictor for self-reported wheezing[10]. An association between farm density and FEV_1 was previously observed in the German study[6]. We also found temporal associations between airway obstruction and week-average ambient ammonia and PM_{10} levels. In a model with mutual adjustment for ammonia and PM_{10}, the association with ammonia remained, but the association with PM_{10} was no longer observed. Such a temporal association with ammonia levels was also observed in the panel study among asthmatic children[8], but never before in a large population-based study.

Livestock production is the major contributor to ambient ammonia levels. Considering the ambient ammonia levels during the study period, and the toxicological effects described at these levels, it is unlikely that ammonia alone caused a direct effect on respiratory health of residents. It is more plausible that ambient ammonia levels serve as a marker for airborne emissions from livestock farms and that associated air pollutants affect respiratory health. H_2S, particulate matter (PM_{10}, $PM_{2.5}$ and $PM_{2.5-10}$) and endotoxin - cell-wall components of gram-negative bacteria - are co-pollutants that may affect respiratory health[5,11]. Another explanation is that ambient ammonia forms secondary inorganic aerosols (SIA), which contribute highly to atmospheric $PM_{2.5}$ concentrations[12,13]. Ammonia 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. Secondary particle formation takes time, and $PM_{2.5}$ can be transported over long distances[13,14]. Without further studies of the local atmospheric chemistry we cannot support the possibility of these transformations happening locally. However, a recent study from Barcelona suggested fast formation of secondary ammonium sulphate within the urban area[15].

Another possibility is that peak emissions of ammonia (or co-pollutants) may affect respiratory health. Previous epidemiological studies have demonstrated that fluctuations of air pollution are associated with acute respiratory symptoms, especially among susceptible subjects[16,17]. Our cross-sectional study lacks the ability to investigate the association of acute respiratory responses with ammonia fluctuations. A longitudinal panel study design is more suitable to detect effects of air pollutant fluctuations. Regardless of whether the decreased lung function reported here was caused by exposure to ammonia or to other co-pollutants, our findings add to the existing body of evidence for a relationship between livestock farm proximity and respiratory health. Further epidemiological studies are needed to replicate our findings and findings of other studies before we can infer causality. Moreover, more detailed characterization of livestock associated environmental exposures, including bioaerosol analysis and SIA formation, is needed.

More symptoms among COPD patients

Results of the questionnaire survey (Chapter 2) showed more respiratory symptoms among COPD patients who are living in close proximity to livestock farms. Wheezing and use of inhaled corticosteroids among COPD patients was positively associated with several indicators of livestock farm's exposure. This may indicate an increased risk of exacerbations among COPD patients who have a high exposure to livestock farm emissions. Moreover, results of our health examination survey (Chapter 4) suggest that the negative association between the number of livestock farms and lung function was restricted to patients with COPD.

Another study within VGO, using EMR data, confirmed our results and found a higher exacerbation rate among COPD patients in the VGO study area compared with patients living in a rural control area, whereas the incidence rate of asthma exacerbations did not differ between the two regions. COPD exacerbations also appeared to be increased among patients living in close proximity of poultry farms[18]. A study in Pennsylvania (USA) describes associations between farm proximity and asthma exacerbations[19]. It should be noted that patients with COPD are also at high risk for developing pneumonia[20]. There is evidence that chronic use of inhaled corticosteroids is associated with a higher prevalence of pneumonia[21]. Three previous studies – all conducted in the Netherlands - have indicated that living close to goat and poultry farms constitute an increased risk for pneumonia[3,22,23]. For poultry farms, it was hypothesized that endotoxin and other farm-related air pollutants may predispose susceptible individuals to respiratory infections[22]. The increased pneumonia incidence near goat farms is more difficult to explain, since these farms do not emit large quantities of dust and endotoxin. So far, there is no evidence of zoonotic pathogens playing a role, except during outbreak situations[24]. Expansion of these epidemiological studies and identifying causal agents is therefore a topic of ongoing research. Pneumonia diagnosis was based on EMR-data[22,23] and on a combination of EMR-data and self-reports[3]. One of these studies was conducted among the VGO study population[3]. None of the three studies found significant associations between exposure to other farm animals than goat or poultry and pneumonia. Observed effects among COPD patients in our studies (Chapter 2 and 4) were not associated with a specific farm animal type but were associated with more general farm's exposure estimates (number of farms within 1000 m, and distance to nearest farm).

Results of our study confirm previous findings that livestock farm emissions affect in particular susceptible patients with a pre-existing lung disease[25,26]. However, we only observed effects among COPD patients and not among asthma patients or subjects with nasal allergies. Due to practical reasons, we selected a population-based sample of patients aged between 18 and 70 years at the start of the study. We did not select our population for risk groups for specific diseases. For example, COPD and pneumonia are more common, and often more severe, among older age groups[27]. Asthma is the most common respiratory disease that occurs in childhood[27]. Further research into the impact of emissions from livestock farms on respiratory health of susceptible subgroups (e.g., children, elderly, and patients with respiratory disease) is warranted.

Beneficial health effects

Our study also found results that are indicative of potentially beneficial health effects of living in close proximity to farms. Based on data of the questionnaire survey, prevalence of self-reported asthma, COPD and nasal allergies was lower among residents living at a short distance from a farm (Chapter 2). In addition, results of the health examination survey indicated that current exposure to a livestock farm environment, assessed as residential proximity to livestock farms, seems to protect against atopy in adults (Chapter 5). The negative associations between atopy and livestock farm exposure were somewhat stronger in subjects who grew up on a farm.

The beneficial effect of farm exposure and atopic diseases has mainly been shown in farming families. It is now well established that children growing up on farms are less likely to develop allergic disease than children living in the same area but with non-farming parents[28,29]. A few epidemiological studies indicate that occupational farm exposures during adulthood may also prevent from atopic sensitization, also in farmers without a farm childhood[30-32]. The association between atopy and farm proximity is poorly studied in the general and non-farming populations. A German found an urban-rural effect on atopic sensitization by comparing atopy prevalence in farmers, rural, suburban and urban residents[33]. Another Danish study also found an urban-rural gradients depending on childhood exposure[34]. The explorative study which was conducted in the Netherlands (part of IVG study[1], see Introduction), found inverse associations between indicators of air pollution from livestock farms and asthma, allergic rhinitis and COPD based on a GP-diagnosis (EMR-data) of 92,548 patients[35], but in that study, information on farm childhood and other potential confounders was lacking. Our study provides evidence that protective effects of early-life and adult farm exposures may extend beyond farming populations.

A protective effect on IgE-mediated allergies and asthma has been attributed to higher and more diverse environmental exposures to microbial components[36-39]. However, the observed negative association between farm density and COPD (Chapter 2), which could be interpreted as a protective effect, is not easily explained since IgE does not play a role in COPD. In occupational settings, an increased risk of COPD (defined by the 5% lower limit of normal (LLN) pre-bronchodilator FEV1/FVC ratio) was reported for livestock farmers compared with crop farmers[40]. One explanation could be that self-reported COPD is associated with non-allergic asthma or with the asthma-COPD overlap syndrome (ACOS)[41]. A study among Norwegian farmers (n=2,169) found that exposure to endotoxins and fungal spores appeared to have a protective effect on atopic asthma but may induce non-atopic asthma[42]. In Chapter 3 we showed that self-reported COPD identified 21% and 30% of COPD-cases based on respectively spirometry-GOLD and LLN definition. We found strong positive associations between self-reported COPD and indicators for allergy. These associations became weaker when subjects with current asthma were excluded. This indicates that some misclassification in self-reported COPD may be present due to overlap with asthma and might explain the observed protective effect on COPD in Chapter 2.

We assume that farm proximity is associated with a higher diversity of environmental microbial exposure which was put forward as an explanation of the protective effects on atopic diseases[39,43]. We did not measure microbial diversity directly in this study. However, previous studies have shown associations with residential farm proximity and (proxies of) microbial agents. Emission of bio-aerosols containing (parts of) microbial agents is measured by air-measurements up- and downwind from farms. Culturable bacteria measured downwind from swine facilities were higher than upwind[44]. Air samples analysed for DNA concentrations of bacteria *Escherichia coli* and *Staphylococcus spp* were higher downwind from pig and poultry farms than upwind[10]. The difference in DNA concentration differs per farm type. DNA concentrations were still elevated at 200 m downwind from poultry farms. Elevated endotoxin concentrations have been measured at 30-250m downwind of farms[1,44,45]. High endotoxin levels were associated with higher microbial richness[38]. In a livestock dense area, livestock related characteristics - such as the number of livestock animals - explained spatial variance in ambient air concentrations of endotoxin[46]. As part of the VGO study, a large air measurement campaign was conducted in the study area. Based on results of this campaign, de Rooij *et al*. evaluated whether Land-Use Regression (LUR) modeling, in which farm characteristics were explored (farm density, type of farm etc.), can be used to explain spatial variation of endotoxin[47]. Ambient PM_{10} samples were collected at 61 residential sites and analysed for endotoxin concentrations. This study showed that spatial variation of annual endotoxin concentrations can be well explained by the spatial distribution of livestock farms (R^2 up to 0.64). In this thesis we used simple and general livestock characteristics as exposure proxies (e.g. number of farms in buffer, distance to nearest farm). De Rooij *et al.* found significant associations between general livestock characteristics and measured endotoxin concentrations. Especially, spatial variation of endotoxin explained by the number of farms was promising (R^2 up to 0.26). This provides a first scientific basis for the use of general farm characteristics as exposure proxies in this thesis and in general.

How to protect residents living in close proximity to farms from potentially harmful farm emissions?

In November 2012, the Health Council of the Netherlands published an advisory report on health risks associated with livestock farms[48]. One of the main topics was a recommendation to make an inventory of the usefulness and necessity of imposing a minimum distance between livestock farms and residential areas. The committee stated that nothing is known about the size of the area within which people in the local vicinity are at increased risk of health effects. The minimum distances used are based on odour standards that are in accordance with the provisions of the Odour Nuisance and Livestock Farming act. The committee stated that emission-related minimum distances need to be established which are not based on odour alone. In 2011, the Association of Public Health Services (GGD) advises municipalities to keep a distance of 250m between livestock farms and residents' houses by new spatial planning projects[49]. This distance was based on results of the IVG study which showed elevated levels of fine dust and endotoxin until 250m of a livestock farm[1]. However, this distance is not a statutory requirement, but only an advice.

Focusing on respiratory health risks, results of this thesis do not provide enough scientific basis for a distance-based policy. We showed associations with spirometric parameters of lung obstruction and the number of livestock farms within a 1,000m buffer from the home address (Chapter 4). However, no associations were found between lung function and other spatial exposure proxies such as minimal distance to the nearest farm. However, based on self-reported data we found higher risks of wheezing among COPD patients who live at shorter distances to the nearest farm[50]. COPD patients living at 290-450m (Q3) and 450-640m (Q2) from the nearest farm reported more wheezing compared to patients living >640m (Q1, reference) from a farm. In conclusion, associations with distance to the nearest farm were only observed among susceptible subjects based on self-reported data. This result needs to be confirmed and expanded before it can be used as input for establishing minimal distances. More generally speaking, the agents that may be responsible for some of the observed associations have not been identified. Therefore, causal inferences cannot be made yet. Once agent have been identified, it might be more feasible to consider reducing emissions instead of focusing on distance based approaches, which actually assume that pollutants emitted will have been diluted in the air with increasing distance, to concentrations that are considered not be lead to health effects.
To protect neighbouring residents from potentially harmful farm emissions, policy measures should especially be focused on areas with a high farm density. In this thesis a negative association was found between the number of livestock farms within a 1,000m buffer from the home address and maximum mid-expiratory flow (MMEF)[51]. This association with farm density was previously observed in a German study: living in an area with more than 12 stables within 500m of the home address

was associated with a lower FEV_1 in adults[6]. Taking both studies into account, there is evidence that living in rural areas with a high density of livestock farms can have adverse effects on respiratory health. To protect respiratory health of residents, provinces and municipalities should take public health into account in spatial planning. In rural areas with high livestock farm densities, the expansion of current farms or establishing of new livestock farms should be prevented. Furthermore, it is important that livestock farms lower their emissions, especially in areas with a high number of farms.

Another reason why we should not focus only on a distance-based policy is that our results also indicate associations with temporal livestock farm exposures. We found a lower lung function when measured after weeks with higher ambient ammonia levels. Almost all ammonia emissions result from agricultural activities and ammonia is mostly locally generated[52]. Ammonia contributes highly to atmospheric $PM_{2.5}$ through SIA formation[13]. The contribution of ammonia emissions through SIA formation often represents 10-20% of fine particle mass in populated areas in Europe, and even higher in areas with high livestock farm density[52]. $PM_{2.5}$ can be transported over long distances and has been identified as a major contributor to $PM_{2.5}$ in urban areas[13,14]. Lelieveld *et al.* assessed the effect of $PM_{2.5}$ to premature mortality on a global scale[53]. Results were obtained with an atmospheric chemistry-general circulation model which computes global air quality changes combined with population data, on country level health statistics and pollution exposure-response functions. The impact of seven main sources of $PM_{2.5}$ was calculated. Agriculture contributes one-fifth to premature mortality on a global scale and was the leading source category in Europe, East Asia and the eastern part of the USA. It should be noted that this model was based on the assumption that fine particles are equally toxic, without regard to their source. Experts suggests that carbonaceous particles are more toxic than crustal material, nitrates and sulfates[54]. But the evidence for different toxicity of particles is not consistent[55]. Lelieveld *et al.* conducted a secondary analysis assuming that carbonaceous $PM_{2.5}$ is five times more toxic than inorganic particles. As a result, the impact on mortality diminished as agricultural emissions mostly form inorganic $PM_{2.5}$. In Europe, the source categories with the largest impact on mortality became land traffic and residential energy use. Brandt *et al.* developed a model which was capable of calculating the health-related external costs from ten main specific atmospheric emission sources and sectors in Europe and Denmark[56]. The toxicity of particles was considered to be similar. Results showed that the major contributors from European emissions to the total health related external costs in Europe were power production, agriculture and road traffic, while in Denmark the number one dominating contributor to health costs was agriculture. Results of both studies show that reducing livestock farm emissions will not only positively contribute to respiratory health of neighbouring residents, but also to the health of people outside livestock farming areas.

There are several options to reduce emissions of livestock farms. In the Netherlands, since 2015 there are regulations that aims maximal emission factors on ammonia and fine dust in livestock housing facilities[57]. Measures to reduce emissions are focused on farm management inside stables (i.e. reduction of bioaerosol sources), removal of polluted air from stables, treatment of the ventilation air and measures outside stables. Air scrubbers are a commonly used approach and treat the ventilation air of a mechanically ventilated animal house[58]. Air scrubbers can reduce ammonia emission for 70-95%. However, only the two-stage air scrubber is able to reduce emissions from both ammonia and fine dust (PM_{10}). A Dutch study by Winkel *et al.* explored existing and new measures that can be taken to reduce emission of bioaerosols from animal stables[59]. Results show that the most effective existing measures are a biobed (biofilter, 80% reduction) and two types of air scrubbers namely: bioscrubber (60-75% reduction) and double-stage scrubber (80% reduction). All three measures also reduce the emission of ammonia. The study also explored reduction techniques which are currently used for poultry housing systems but which are promising for other animal housing systems. Most promising techniques to reduce bioaerosol emission are: electrostatic precipitators (57% reduction), dry filter (40% reduction), oil spraying method on floor (15-45% reduction) and negative ionisation of air in stables (49%)[60-62]. However, these techniques don't reduce ammonia emission. Promising measures which are available on the long-term basis are 'end of pipe' systems which can be connected to air scrubbers, electrostatic precipitators and filters. These systems are meant to inactivate or trap micro-organisms[61]. Fine dust emission can also be reduced by adding fat or oils to the diet of the animal[63].

Raised concerns about public health risks of exposure to emissions of livestock farms were reason for the Ministry of Economic Affairs (which includes Agriculture) and the Ministry of Health, Welfare and Sports to fund the VGO study. In particular the increasing number of large–scale farms ('mega-farms') and the Q-fever outbreak fuelled concerns among general practitioners and other people living in rural areas. Nevertheless, our study population had a relatively positive attitude towards farming (Chapter 6). This predominantly positive attitude was partly explained by familiarity with farming. Common risks are judged more acceptable than uncommon and unknown risks[64]. Since one third of our study population had grown up on a farm and 76% was born in the study area, the majority of the study population is familiar with agricultural activities since childhood. Risks of farm exposure might be underestimated by the study population. Findings of this thesis add to the existing body of evidence for a relationship between livestock farm proximity and respiratory health. In addition, we mentioned earlier the health effects[53,56] associated with elevated atmospheric $PM_{2.5}$ concentrations to which ammonia is the major contributor[12,13]. A more generic issue is raised by Vieno *et al.*, whom argued that despite scientific evidence there is a lack of public awareness about agricultural emissions of ammonia as a key contributor to high PM pollution events[14]. This lack of awareness may eventually result in a weaker policy mandate for any ammonia emission reduction targets.

Nevertheless, our results also indicate that part of our study population is having concerns about certain aspects of livestock farming in their living area (Chapter 6). Subjects who live in areas with a high number of livestock farms, and especially in close proximity of pig and goat farms, had a more negative attitude towards farming than subjects living in areas with fewer livestock farms. Also, the majority of the study population mentioned to be concerned about antibiotic use in livestock farming and zoonotic diseases. These concerns are remarkable since antimicrobial usage is reduced more than 60% in livestock farming since 2009 in the Netherlands[65], and surveillance systems are used to detect potential threats of zoonotic livestock diseases. However, during previous zoonotic infectious outbreaks in the Netherlands, the large economic interest of the agricultural sector prevailed above the interests of public health[66]. This may affect public trust in the government. Before the start of the IVG[1] and VGO study, the number of studies on the effect of livestock farm exposure on health of local residents was limited. Results of both studies gave more insight in potential health effects and revealed a number of positive and a number of negative effects of living in the proximity of livestock farms. These results can serve as input for a public debate on livestock farming in the Netherlands. The Dutch Health Council stated that such a public debate promotes that all involved parties can form a well-considered opinion which leads to a more trusting relationship between the government, the people and farmers[48]. In the long-run, this should contribute to more widespread support for potential measures.

Conclusion

The results presented in this thesis add to the existing body of evidence for a relationship between livestock farm proximity and respiratory health. Farm density (the number of farms within 1,000m) was associated with a reduced lung function level. In addition, a reduced lung function was observed when ambient ammonia concentrations were high. Results of our study replicates previous findings that livestock farm emissions affect in particular susceptible patients with a pre-existing lung disease. COPD patients who have a high exposure to livestock farm emissions report more respiratory symptoms and use of inhaled corticosteroids. We also found results that are indicative for beneficial health effects. People who live around livestock farms were less frequently found to have asthma, COPD and nasal allergies. Moreover, current exposure to a livestock environment seems to protect from atopy. This association was somewhat stronger in subjects who grew up on a farm.

Particulate matter, endotoxins, ammonia and H_2S are primary emitted pollutants that may affect respiratory health. Ammonia also contributes highly to atmospheric $PM_{2.5}$ by SIA formation, a PM fraction that can deeply penetrate into the lungs. Further epidemiological studies are needed to replicate and expand our findings and findings of other studies before we can infer causality. Moreover, more detailed characterization of livestock associated environmental exposures - including bioaerosol analysis and SIA formation - is needed.

Focusing on respiratory health risks, results of this thesis do not provide enough scientific basis for a distance-based policy (imposing a minimum distance between livestock farms and residential areas). To protect neighbouring residents from potentially harmful farm emissions, the expansion of current farms and / or establishing new livestock farms should be prevented in rural areas with high livestock farm densities. Furthermore, livestock farms should lower their emissions, in particular in areas with a high number of farms.

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English summary

English summary

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. In the Netherlands – a small country with one of the highest population densities in the world and one of the highest livestock farm densities - neighbouring residents are concerned about potential health risks of farm emissions. 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. Working in agriculture - and especially when working in livestock farms – poses a serious risk for development of respiratory diseases. On the other hand, exposure to a farming environment during childhood, but possibly also during adulthood, reduces the risk of atopic sensitisation.

People living at short distances from livestock farms are potentially exposed to air pollutants from livestock farms and may be at risk for respiratory health effects. Livestock farms 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. The level of exposure to these agents is considerably higher for farmers compared to subjects living in close proximity to farms. However, elevated levels of farm pollutants, such as endotoxin and ammonia, were associated with farm proximity. Also, ammonia is a precursor gas of secondary inorganic aerosols (SIA) and highly contributes to atmospheric PM concentrations of amongst others PM_{10} and $PM_{2.5}$, which can be transported over great distances.

Despite the substantial contribution of livestock farming to ambient air pollution, potential respiratory health risks among residents have been poorly studied. Previous studies on respiratory health of neighbouring residents show a large heterogeneity in methodology, and not all results are consistent. In the Netherlands, a considerable number of households are located in close proximity to livestock farms (around 355.000 houses are located within 250 m of a livestock farm), which may be a potential health risk for residents.

The aim of this thesis was to explore associations between air pollution from livestock farms and respiratory health of non-farming residents living in close proximity to farms in a rural area in the Netherlands.

The VGO study

This thesis was conducted as part of the Livestock Farming and Neighbouring Residents' Health Study (in Dutch: Veehouderij en Gezondheid Omwonenden, the VGO study) which aims to investigate health of residents living in the vicinity of livestock farms. The first part of the VGO study consisted of a questionnaire survey among patients from 21 general practitioner (GP) practices which were located in a rural area with the highest farm density of the Netherlands. In total 14,882 subjects completed the questionnaire (53.4% response). The second part of the VGO study consisted of a health examination survey. Questionnaire respondents who gave consent for a follow-up study, and who were not working or living on a farm were eligible. Between March 2014 en February 2015, 2,494 non-farming subjects (34.7% response) participated to the health examination which included the completion of a second and more extended questionnaire, length and weight measurements, a lung function measurement (pre- and post-bronchodilator spirometry) and collection of serum. Exposure to livestock farms was computed for each subject based on their home address and the location of farms in their vicinity.

Main results

Chapter 2 describes results of the first part of the VGO study: the questionnaire survey among 14,882 subjects. The questionnaire included respiratory health questions adopted from the European Community Respiratory Health Survey. Analyses were conducted on 12,117 responders, after excluding farmers (subject who reported to be living or working on a farm) and subjects who lived at their current home address for less than one year. Inverse associations were found between living in the proximity of livestock farms and self-reported respiratory health of residents. Prevalence of self-reported asthma, COPD, and nasal allergies was lower among residents living at a short distance from a farm compared to residents living further away (Q1 (<290m), compared to Q4 (>640m), asthma: OR 0.83, 95% CI 0.71–0.98, COPD: OR 0.71, 95% CI 0.56–0.91 and nasal allergies: 0.87, 95% CI 0.77–0.98). Nonetheless, COPD patients living in close proximity to livestock farms reported more respiratory symptoms. Wheezing and use of inhaled corticosteroids among COPD patients was positively associated with several indicators of livestock farm exposures. This may indicate an increased risk of exacerbations among COPD patients who live in close proximity to livestock farms.

In the VGO study, respiratory health of residents was assessed based on three data sources namely: self-reported data, GP diagnosis (based on Electronical Medical Records (EMR)), and spirometry results (pre- and post-bronchodilator (BD)). Epidemiological studies often rely on self-reports, medical records, or pre-BD spirometry. In Chapter 3, we compared four different definitions for determining COPD prevalence to get more insight into the possible effects of using various COPD-definitions on prevalence estimates and their associations with potential risk factors. COPD prevalence in 1,793 adults from the VGO study population was assessed based on self-reported data, EMR, and post-BD spirometry: using the Forced Expiratory Volume in 1 second (FEV_1)/ Forced Vital Capacity (FVC) below the lower limit of normal (LLN) and GOLD fixed cut-off (FEV_1/FVC <0.70). The operational definition used for COPD greatly influences prevalence estimates which varied from 2.9% to 10.9%. Self-reported or GP-diagnosed COPD identified less than 30% of all COPD cases based on persistent airflow limitations, which implies that a substantial number of subjects with COPD cannot be identified by questionnaires or medical records. However, the effect of the different COPD-definitions on associations with potential risk factors was limited, except for indicators of allergy, which were more strongly associated with self-reported COPD compared to the other definitions. The use of pre-BD instead of post-BD spirometry results led to higher prevalence estimates, but had minimal effect on associations with potential risk factors.

The aim of Chapter 4 was to explore associations between both spatial and temporal variation in air pollutant emissions from livestock farms and lung function of neighbouring residents. Respiratory health of the VGO study population was assessed with a pre- and post-BD pulmonary function test. Associations were found between lung function and both spatial and temporal livestock farm exposure estimates. A spatial association was found between the number of livestock farms within a 1,000 m buffer from the home address and lung function, which was statistically significant for the maximum mid-expiratory flow (MMEF). Subjects living in a 'hotspot' (≥17 farms within 1,000 m, cut-off based on smoothed plots) had a 4.5% lower MMEF (95% CI, -8.64 to -0.36) compared to participants from lower farm density areas (reference, <17 farms within 1,000 m). Also, associations with temporal livestock farm exposure estimates were observed. Lung function variables FEV1, FEV_1/FVC and MMEF were lower when measured after weeks with higher ammonia levels and PM_{10} levels. In a two-pollutant model, only ammonia remained statistically significantly associated. A 25 μg/m^3 increase in ammonia 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). Livestock production is the major contributor to ambient ammonia levels. Our results indicate that the spatial association was especially apparent in non-atopic subjects and in patients with COPD, while the temporal association with ammonia was observed in the whole population.

Exposure to farm environments during childhood and adult life seems to reduce the risk of atopic sensitization. Although the beneficial effect of farm exposure has mainly been shown in farming families, it may extend to inhabitants of rural areas. Chapter 5 shows that current exposure to a livestock farm environment, assessed as residential proximity to livestock farms, seems to protect against atopy in adults. Atopy was defined as specific serum IgE antibodies ≥0.35 IU/ml to one or more common allergens (grass pollen, house dust mite, cat, and dog) and/or a total IgE higher than 100 IU/ml. The prevalence of atopy was 29.8%. Subjects living at short distances from farms (<327 m, 1st tertile) had a lower odds for atopy compared to subjects living further away (>527 m, 3rd tertile) (OR 0.79, 95% CI 0.63-0.98). Significant associations in the same direction were found with distance to the nearest pig or cattle farm. The negative associations between atopy and livestock farm exposure were somewhat stronger in subjects who grew up on a farm.
Awareness bias is described as the propensity to report more illness and symptoms as a result of proximity to a potential hazard, in the absence of a biological effect. Attitude towards environmental risks – such as air pollution emitted by farms - may be a source of bias since concerns about environmental hazards may influence self-reported health outcomes. One of the findings of the VGO study was a higher risk of self-reported pneumonia for residents living in close proximity to goat farms. Since the studied health outcome was self-reported and therefore not objectively assessed, potential awareness bias may have resulted in confounding or effect modification by attitude towards farming. In Chapter 6, a score was developed to measure the attitude towards livestock farming in a residential environment. In general, the VGO study population had a positive attitude towards farming, in particular if participants were more familiar with farming. Awareness bias might have played a role since we observed associations between attitude and self-reported respiratory health, while objectively measured respiratory health was not associated with attitude. The attitude score was also associated with exposure to livestock farms and could therefore be a potential confounder. Characteristics of a confounder are: 1) it must be a risk factor for the disease, 2) it must be associated with the exposure under study and 3) it must not be an intermediate step in the causal path between the exposure and disease. However, we did not find any indication that the earlier observed associations between proximity to goat farms and self-reported pneumonia was confounded or modified by attitude.

Discussion and conclusion

The first part of Chapter 7 discusses the main results of this thesis.

The first main result showed that both spatial and temporal variation in livestock air pollution are associated with lung function deficits in non-farming residents. When this research project started, there were only three articles published in which respiratory health of neighbouring residents of livestock farms was measured objectively using spirometry. Most studies used self-reported health outcomes or diagnostic data from routinely collected medical records. Spirometry results are objective measurements and are not influenced by symptom perception, recall-bias, misdiagnosis and access to health care. Results of the current study confirmed and expanded some of the findings published earlier. A spatial association was found between farm density and MMEF. A spatial association between a large number of farms near the home address and a lower FEV_1 was earlier observed in a German study. Week-average PM_{10} and ammonia levels were negatively associated with FEV_1, FEV_1/FVC and MMEF. In a two-pollutant model, only ammonia remained associated. Such a temporal association with ammonia levels was also observed in a panel study among asthmatic children, but never before in a large population-based study. It is unlikely that ammonia alone caused a direct effect on respiratory health. Our hypothesis is that ammonia levels may serve as a marker for airborne emissions from livestock farms and that associated air pollutants affect respiratory health. Another explanation is that ammonia is associated with exposure to secondary particulate matter (mainly $PM_{2.5}$). Secondary particle formation takes time and $PM_{2.5}$ can be transported over long distances. However, without further studies of the local atmospheric chemistry we cannot support the possibility of these transformations happening locally.

The second main result showed more symptoms among COPD patients who are living in close proximity to livestock farms. Wheezing and use of inhaled corticosteroids among COPD patients was positively associated with several indicators of livestock farm exposure. Moreover, results of our health examination survey suggest that the negative association between farm density and lung function was restricted to patients with COPD. These results confirm previous findings that livestock farm emissions affect in particular susceptible patients with pre-existing lung disease.

The third main result indicates potentially beneficial health effects of living in close proximity to farms. Prevalence of self-reported asthma, COPD and nasal allergies was lower among residents living at a short distance from a farm. Moreover, current exposure to a livestock farm environment seems to protect against atopy in adults. The beneficial effect of farm exposure and atopic diseases has mainly been shown in farming families and in a few occupational studies on adult farmers. Two previous studies among non-farming populations found inverse associations between livestock farm proximity and allergic rhinitis based on self-reported data. The present study is the first to investigate this relationship with atopy based on objective markers. We assume that farm proximity is associated with a higher diversity of environmental microbial exposure which was put forward as an explanation of the protective effects on atopic diseases. We did not measure microbial diversity directly in this study but previous studies have shown associations with residential farm proximity and (proxies of) microbial agents. The observed negative association between farm proximity and self-reported COPD is not easily explained since IgE does not play a role in COPD. One explanation could be that self-reported COPD is associated with non-allergic asthma or with the asthma-COPD overlap syndrome. We found strong positive associations between self-reported COPD and indicators for allergy. These associations became weaker when subjects with current asthma were excluded. Some misclassification in self-reported COPD may be present due to overlap with asthma and might explain the observed protective effect on self-reported COPD.

Further epidemiological studies are needed to replicate and expand our findings and findings of other studies before we can infer causality. Moreover, more detailed characterization of livestock associated environmental exposures - including bioaerosol analysis and secondary inorganic aerosol formation - is needed. Furthermore, research into the impact of emissions from livestock farms on respiratory health of susceptible subgroups is warranted. In particular, children are vulnerable to the effects of air pollution because their lungs and immune system are not fully developed.

The second part of Chapter 7 discusses how to protect residents living in close proximity to farms from potentially harmful farm emissions. Focusing on respiratory health risks, results of this thesis do not provide enough scientific basis for a policy that is based on a minimum distance between livestock farms and residential areas. To protect neighbouring residents from potentially harmful farm emissions, policy measures should especially be focused on areas with a high farm density. In rural areas with already high livestock farm densities, the expansion of current farms or establishing of new livestock farms should be prevented. Furthermore, it is important that livestock farms lower their emissions, especially in areas with a high number of farms. Ammonia contributes to atmospheric secondary $PM_{2.5}$, and has been identified as a major contributor to $PM_{2.5}$ in urban areas. Therefore, reducing livestock farm emissions will not only positively contribute to respiratory health of neighbouring residents, but also to the health of people outside livestock farming areas.

Nederlandse samenvatting

Nederlandse samenvatting

Introductie

Recente studies onderstrepen de grote bijdrage van de landbouw aan luchtverontreiniging (met name fijnstof), en het mogelijke effect dat landbouw-emissies hebben op de volksgezondheid. In Nederland – een klein land met één van de hoogste bevolkingsdichtheden in de wereld en één van de hoogste veehouderijdichtheden – maken omwonenden zich ongerust over mogelijke gezondheidsrisico's van landbouwemissies. De lucht in veestallen bevat hoge concentraties (organische) stoffen waarvan bekend is dat deze bij beroepsmatige blootstelling kunnen leiden tot respiratoire gezondheidseffecten. Het werken in de landbouwsector – en met name het werken in de veehouderijsector – zorgt voor een hoger risico op respiratoire aandoeningen. Aan de andere kant brengt een agrarische omgeving tijdens de jeugd, en mogelijk ook op volwassen leeftijd, een verlaagd risico op atopische sensibilisatie met zich mee.

Mensen die dicht in de buurt wonen van veehouderijen worden potentieel blootgesteld aan luchtvervuilende stoffen vanuit die bedrijven en lopen daardoor mogelijk een risico op het ontwikkelen van respiratoire gezondheidseffecten. Veehouderijen stoten luchtvervuilende stoffen uit in de atmosfeer, die bestaan uit een mix van gassen zoals ammoniak (NH_3) en waterstofsulfide (H_2S), en daarnaast fijne en grove stofdeeltjes, micro-organismen en toxines (afkomstig van micro-organismen) zoals endotoxines: een component uit de celwand van Gram-negatieve bacteriën. Boeren worden aanzienlijk hoger blootgesteld aan deze stoffen dan mensen die in de buurt wonen van veehouderijen. Toch zijn verhoogde concentraties van vee-gerelateerde luchtvervuilende stoffen - zoals ammoniak en endotoxine – in verband gebracht met de nabijheid van veehouderijen. Ammoniak is een gasvormige voorloperverbinding (precursorgas) van secundaire anorganische aerosolen (SIA of secondary inorganic aerosols) en draagt daarmee substantieel bij aan de fijnstofconcentraties in de atmosfeer (PM_{10} en $PM_{2,.5}$), welke over grote afstanden getransporteerd kunnen worden.

Ondanks dat de veehouderij substantieel bijdraagt aan luchtverontreiniging, zijn de mogelijke respiratoire gezondheidseffecten onder omwonenden maar weinig onderzocht. Eerdere studies over respiratoire gezondheidseffecten onder omwonenden lieten een grote heterogeniteit zien in de methodologie, en de resultaten van deze studies waren niet consistent. Een groot aantal huishoudens (circa 355.000 woningen bevinden zich binnen een straal van 250 m van eev veehouderij) in Nederland bevindt zich in de buurt van veehouderijbedrijven, wat mogelijk een gezondheidsrisico kan zijn. Het doel van dit proefschrift was om associaties te onderzoeken tussen luchtvervuilende stoffen vanuit veehouderijbedrijven en de respiratoire gezondheid van mensen die in de buurt van veehouderijbedrijven wonen in een ruraal gebied in Nederland.

De VGO-studie

Dit proefschrift is tot stand gekomen als onderdeel van het Veehouderij en Gezondheid Omwonenden onderzoek (VGO). Deze studie had als doel om de gezondheid te onderzoeken van mensen die in de buurt van veehouderijbedrijven wonen. Het eerste deel van de VGO-studie bestond uit een vragenlijstonderzoek onder patiënten van 21 huisartsenpraktijken die gevestigd waren in een ruraal gebied met de hoogste vee-dichtheid van Nederland. In totaal hebben 14.882 mensen de vragenlijst ingevuld (53,4% respons). Het tweede deel van de VGO-studie bestond uit een gezondheidsonderzoek. Respondenten van de vragenlijst die aangaven mee te willen doen aan een vervolgstudie, en die niet werkten of woonden op een boerderij, waren geschikt voor het onderzoek. Tussen maart 2014 en februari 2015 deden 2.492 mensen (zonder agrarische achtergrond) mee aan het gezondheidsonderzoek, welke bestond uit het invullen van een tweede en meer uitgebreide vragenlijst, het meten van lengte en gewicht, een longfunctiemeting (pre- en post-bronchodilator spirometrie) en het verzamelen van serum. Blootstelling aan veehouderijbedrijven werd berekend voor elke deelnemer op basis van het woonadres en de locatie van de veehouderijbedrijven in de omgeving.

Resultaten

In hoofdstuk 2 worden de resultaten beschreven van het eerste deel van de VGO-studie: het vragenlijstonderzoek onder 14.882 mensen. De vragenlijst bevatte vragen over onder andere de respiratoire gezondheid. Er zijn onder 12.117 respondenten analyses uitgevoerd, nadat boeren (mensen die aangaven te wonen of werken op een boerderij) en respondenten die korter dan 1 jaar in hun huidige woning woonden waren uitgesloten. Er is een omgekeerd verband gevonden tussen het wonen in de buurt van veehouderijen en zelf-gerapporteerde respiratoire gezondheid. De prevalente van zelf-gerapporteerde astma, COPD en allergische rhinitis (neusallergieën) was lager onder deelnemers die op kortere afstand woonden van een boerderij vergeleken met deelnemers die verder weg woonden (Q1 (<290m), vergeleken met Q4 (>640m), astma: OR 0,83, 95% CI 0,71–0,98, COPD: OR 0,71, 95% CI 0,56–0,91 en allergische rhinitis: 0,87, 95% CI 0,77–0,98). Maar COPD patiënten die dichtbij veehouderijen woonden rapporteerden meer respiratoire symptomen, dan COPD patiënten die verder weg woonden. Zo was een piepende ademhaling en het gebruik van inhaleerbare corticosteroïden onder COPD patiënten positief geassocieerd met verschillende indicatoren van blootstelling aan veehouderijen. Dit impliceert dat COPD-patiënten die in de buurt van veehouderijen wonen een verhoogd risico hebben op complicaties van hun ziekte (exacerbaties).

Binnen de VGO-studie is de respiratoire gezondheid van omwonenden in kaart gebracht op basis van drie data bronnen namelijk: zelf-gerapporteerde data, diagnose van de huisarts (op basis van elektronische patiëntendossiers (EPD's)), en spirometrie (pre- en post broncodilator (BD)). Epidemiologische studies zijn vaak gebaseerd op zelf-gerapporteerde data, EPD's of pre-BD spirometrie. In hoofdstuk 3 zijn vier verschillende definities voor COPD met elkaar vergeleken op basis van prevalentieschattingen en associaties met potentiële risicofactoren. Onder 1.793 volwassenen van de VGO-studiepopulatie was de COPD-prevalentie bepaald op basis van zelf-gerapporteerde data, EPD's en post-BD spirometrie gebaseerd op de geforceerde één seconde volume (FEV_1)/geforceerde vitale capaciteit (FVC) lager dan de 'Lower limit of normal' (LLN-definitie) en de GOLD-definitie (FEV_1/FVC <0,70). De definitie die gebruikt wordt voor COPD had een grote invloed op de prevalentieschattingen, deze varieerden van 2,9% tot 10,9%. Zelf-gerapporteerde COPD, of COPD op basis van een huisartsendiagnose identificeerde minder dan 30% van alle COPD-gevallen gebaseerd op EPD's, dit betekent dat een groot aantal deelnemers met COPD niet geïdentificeerd kan worden met vragenlijsten of EPD's. Toch was het effect van de verschillende COPD-definities op de associaties met potentiële risicofactoren minimaal. Alleen de allergie-indicatoren waren sterker geassocieerd met zelf-gerapporteerde COPD dan met de andere drie definities. Het gebruik van pre-BD in plaats van post-BD spirometrie resulteerde in hogere prevalentieschattingen, maar dit had maar een minimaal effect op de relatie met potentiële risicofactoren.

Het doel van hoofdstuk 4 was om associaties te onderzoeken tussen zowel ruimtelijke als temporele variatie in de emissies van veehouderijen en de longfunctie van omwonenden. De respiratoire gezondheid van de VGO-studiepopulatie is vastgesteld met een pre- en post-BD longfunctietest. Er zijn zowel ruimtelijke als temporele relaties gevonden tussen de longfunctie en veehouderij-gerelateerde blootstelling. Een ruimtelijk verband werd gevonden tussen het aantal veehouderijen binnen een straal rond de woning van 1,000 meter en de longfunctie, deze was statistisch significant voor de maximale mid-expiratoire volumestroom (MMEF). Deelnemers die in een 'hotspot' woonden (≥ 17 veehouderijbedrijven binnen 1,000 m rond de woning, afkappunt gebaseerd op smooth plots) hadden een 4,5% lagere MMEF (95% CI, -8,64 tot -0,36) ten opzichte

van deelnemers met minder dan 17 veehouderijbedrijven binnen 1,000 m rond de woning (referentiegroep). Ook zijn er verbanden gevonden met temporele variatie in veehouderij-gerelateerde blootstelling en longfunctie. De longfunctie variabelen FEV_1, FEV_1/FVC en MMEF waren lager wanneer deze gemeten waren na weken met gemiddeld hoge niveaus ammoniak of PM_{10}. Wanneer ammoniak en PM_{10} samen in een regressiemodel werden opgenomen, dan bleef het effect van ammoniak statistisch significant. Een toename van 25 $\mu g/m^3$ ammoniak was geassocieerd met een 2,22% lagere FEV_1 (95%CI -3,69 - -0,74), FEV_1/FVC: -1,12% (-1,96 - -0,28) en MMEF: -5,67% (-8,80 - -2,55). Ammoniakniveaus worden in zeer hoge mate bepaald door de aanwezigheid van veehouderijbedrijven. Het ruimtelijke verband was met name gevonden onder niet-atopische deelnemers en bij COPD-patiënten, terwijl het temporele verband met ammoniak in de hele populatie werd gevonden.
Blootstelling aan een agrarische omgeving tijdens de jeugd, maar mogelijk ook op volwassen leeftijd, verlaagt het risico op atopische sensibilisatie. Hoewel dit beschermende effect voornamelijk is gevonden onder agrarische families, zou dit effect ook kunnen bestaan bij bewoners van rurale gebieden. Hoofdstuk 5 laat zien dat blootstelling aan een veehouderij-gerelateerde omgeving - op basis van de nabijheid van veehouderijen – lijkt te beschermen tegen atopische sensibilisatie. Atopische status is als volgt gedefinieerd: een positieve test tegen minstens één van de geteste specifieke allergenen (graspollen, huisstofmijt, kat, en hond, positief wanneer >0,35 IU/ml), en/of een totaal IgE >100 IU/ml. De atopie prevalentie was 29,8%. Deelnemers die op een korte afstand woonden van veehouderijen (<327 m, 1e tertiel) waren minder vaak atopische gesensibiliseerd vergeleken met deelnemers die verder weg woonden (>527 m, 3de tertiel) (OR 0,79, 95% CI 0,63-0,98). Vergelijkbare significante verbanden zijn gevonden wanneer werd gekeken naar de afstand tot het eerste varkensbedrijf en het eerste rundveebedrijf. Dit omgekeerde verband tussen atopische sensibilisatie en vee-gerelateerde blootstelling was iets sterker onder deelnemers die opgegroeid waren op een boerderij.
'Awareness bias' wordt omschreven als de neiging van deelnemers om meer symptomen te rapporteren wanneer zij beseffen dat ze worden blootgesteld aan een potentieel omgevingsrisico, zonder dat daar een biologische verklaring voor is. Hoe mensen aankijken tegen risico's in hun omgeving – zoals luchtvervuilende uitstoot van veehouderijen – kan zorgen voor vertekening van resultaten omdat bezorgdheid van deelnemers over mogelijke gevaren in de omgeving invloed kan hebben op het rapporteren van symptomen. Eén van de bevindingen van de VGO-studie was dat deelnemers die in de buurt van geitenbedrijven wonen meer pneumonie rapporteerden. De gezondheidsuitkomst pneumonie was in deze studie zelf-gerapporteerd en niet gebaseerd op objectief gemeten data. De houding van

mensen tegenover de veehouderijsector kan hier zorgen voor awareness bias, en mogelijk zorgen voor confounding of effect modificatie. In hoofdstuk 6 is een score ontwikkeld om de houding van deelnemers tegenover de veehouderij in hun omgeving te meten. In het algemeen had de VGO-studiepopulatie een positieve houding tegenover veehouderij, met name deelnemers die meer bekend waren met de veehouderijsector. Awareness bias zou mogelijk kunnen hebben opgetreden aangezien er wél verbanden werden gevonden tussen de houding van deelnemers en zelf-gerapporteerde gezondheid, terwijl er geen verband werd gevonden tussen de houding van deelnemers en objectief gemeten gezondheid (longfunctie). De houdings-score was ook geassocieerd met veehouderij-gerelateerde blootstelling, de score zou daarom een potentiële confounder kunnen zijn. Kenmerkend van een confounder is dat het: 1) een risicofactor voor de ziekte moet zijn, 2) geassocieerd moet zijn met de bestudeerde blootstellingsmaat, en 3) geen intermediair mag zijn in het causale verband tussen blootstelling en ziekte. Er is geen aanwijzing gevonden dat het eerder gevonden verband tussen blootstelling aan geiten-bedrijven en zelf-gerapporteerde longontsteking werd beïnvloed door confounding of effect modificatie door de houding van deelnemers tegenover veehouderij.

Discussie en conclusie

Het eerste deel van hoofdstuk 7 bediscussieert de belangrijkste resultaten van dit proefschrift.

Ten eerste laat het onderzoek in dit proefschrift zien dat zowel ruimtelijke als temporele variatie in veehouderij-gerelateerde emissies geassocieerd zijn met longfunctieverlies onder omwonenden. Toen dit onderzoeksproject begon, waren er maar drie studies gepubliceerd waarin respiratoire gezondheid van omwonenden van veehouderijen objectief was gemeten door middel van spirometrie. De meeste studies gebruikten zelf-gerapporteerde gezondheids-uitkomsten of data van EPD's. Spirometrie-uitkomsten zijn objectief gemeten data en worden niet beïnvloed door symptoom-perceptie, herinneringsbias (recall-bias), misdiagnose, en de toegang tot zorg. De huidige studie heeft enkele bevindingen van eerdere studies kunnen bevestigen en uitbreiden. Er is een ruimtelijk verband gevonden tussen de dichtheid van veehouderijen rondom de woning en longfunctie-variabele MMEF. Zo'n ruimtelijk verband tussen het aantal stallen dichtbij de woning en FEV_1 was eerder ook al gevonden in een Duitse studie. Week-gemiddelde PM_{10} en ammoniakniveaus waren negatief geassocieerd met FEV_1, FEV_1/FVC en MMEF. In een regressiemodel waarin zowel PM_{10} en ammoniak zijn opgenomen, blijft ammoniak het meest geprononceerd aanwezig. Dergelijke temporele associaties met ammoniakniveaus zijn eerder gevonden in een

panelstudie onder astmatische kinderen, maar niet eerder in een grote populatiestudie. Het is niet te verwachten dat ammoniak zelf tot acute effecten op de luchtwegen leidt. Onze hypothese is dat ammoniak als een marker dient voor veehouderij-gerelateerde emissies en dat deze geassocieerde luchtverontreinigende stoffen leiden tot effecten op de luchtwegen. Een andere verklaring is dat ammoniak samenhangt met blootstelling aan secundair fijnstof (voornamelijk $PM_{2,5}$). Vorming van secundair fijnstof neemt tijd in beslag en $PM_{2,5}$ kan vervolgens worden getransporteerd over grote afstanden. Zonder verdere studies over de lokale atmosferische chemie, kunnen we niet uitsluiten dat de formatie van secundair fijnstof mogelijk ook op lokaal niveau gebeurt.
Het tweede hoofdresultaat liet zien dat COPD patiënten die dicht in de buurt van veehouderijbedrijven woonden meer symptomen rapporteerden. Dit impliceert dat COPD-patiënten die in de buurt van veehouderijen wonen een verhoogd risico hebben op complicaties van hun ziekte (exacerbaties). Daarnaast lieten de resultaten van het gezondheidsonderzoek zien dat de negatieve associatie tussen veehouderijdichtheid en longfunctie vooral was toe te schrijven aan patiënten met COPD. Deze resultaten bevestigen bevindingen van eerdere studies dat veehouderij-gerelateerde emissies voornamelijk gevoelige patiënten met bestaande longziekten treffen.
Het derde hoofdresultaat laat zien dat het wonen in de buurt van veehouderijen ook positieve effecten heeft op de gezondheid. De prevalentie van zelf-gerapporteerde astma, COPD en allergische rhinitis was lager onder deelnemers die op een korte afstand van een veehouderij woonden. Daarnaast was er een beschermend effect gevonden van veehouderij-gerelateerde blootstelling op atopische sensibilisatie onder volwassenen. Het gunstige effect van veehouderij-gerelateerde blootstelling en allergieën is eerder aangetoond in agrarische families en in een aantal studies onder boeren. Twee eerdere studies onder niet-agrarische populaties vonden een omgekeerd verband tussen de nabijheid van veehouderij en allergische rhinitis op basis van zelf-gerapporteerde data. De huidige studie was de eerste die deze relatie onderzocht op basis van objectief gemeten markers (atopie). We veronderstellen dat de nabijheid van veehouderijbedrijven geassocieerd is met een hogere diversiteit aan microbiële blootstelling. Dit zou het beschermende effect op atopische sensibilisatie mogelijk kunnen verklaren. Hoewel we microbiële diversiteit niet direct hebben gemeten in deze studie, laten eerdere studies verbanden zien tussen de nabijheid van veehouderij en (proxy 's van) microbiële componenten. Het gevonden negatieve verband tussen de nabijheid van veehouderijen en zelf-gerapporteerde COPD is lastiger te verklaren omdat IgE geen rol speelt bij COPD. Een mogelijke verklaring zou kunnen zijn dat zelf-

gerapporteerde COPD geassocieerd is met niet-allergische astma of met het astma-COPD overlap syndroom (ACOS). Zelf-gerapporteerde COPD en zelf-gerapporteerde allergie-indicatoren waren onderling sterk geassocieerd. Deze associaties werden zwakker wanneer de deelnemers met zelf-gerapporteerde huidige astma werden uitgesloten. Mogelijk is door overlap met astma missclassificatie opgetreden bij zelf-gerapporteerde COPD, wat het gevonden beschermende effect op zelf-gerapporteerde COPD deels zou kunnen verklaren.
Verdere epidemiologische studies zijn nodig om de huidige bevindingen en die van eerdere studies te repliceren en verder uit te breiden voordat een oorzakelijk verband kan worden vastgesteld. Ook is een meer gedetailleerde typering nodig van veehouderij-gerelateerde blootstellingen – inclusief analyse van bioaerosolen en formatie van secundair fijnstof. Er wordt aangeraden om verder onderzoek te doen naar de impact van veehouderij-gerelateerde emissies op de luchtwegen van gevoelige groepen. Met name kinderen zijn gevoelig voor de effecten van luchtverontreiniging omdat de longen en het immuunsysteem op jonge leeftijd nog niet volledig ontwikkeld zijn.
Het tweede deel van hoofdstuk 7 bediscussieert hoe mensen die in de buurt van veehouderijbedrijven wonen beschermd moeten worden tegen potentieel schadelijke emissies. Wanneer gekeken wordt naar risico's voor de respiratoire gezondheid dan geven de resultaten van dit proefschrift weinig wetenschappelijke basis voor een beleid dat is gericht op een minimum afstand tussen veehouderijbedrijven en dorpskernen. Om omwonenden te beschermen voor mogelijk schadelijke emissies van veehouderijen, zou het beleid vooral gefocust moeten zijn op gebieden met een hoge dichtheid aan veehouderijbedrijven. In deze gebieden zou voorkomen moeten worden dat huidige veehouderijen verder uitbreiden en/ of dat nieuwe bedrijven zich gaan vestigen. Daarnaast is het belangrijk dat de emissies van veehouderijen worden verlaagd, en zeker in gebieden met een hoge vee-dichtheid. Ammoniak draagt bij aan secundair $PM_{2,5}$ in de atmosfeer en draagt daardoor ook aanzienlijk bij aan $PM_{2,5}$ in meer verstedelijkte gebieden. Het verlagen van emissies van veehouderijbedrijven zal daarom niet alleen positief bijdragen aan de respiratoire gezondheid van omwonenden maar ook aan de gezondheid van mensen die buiten deze rurale gebieden wonen.

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Curriculum vitae

Curriculum vitae

Floor Borlée werd geboren op 4 juni 1986 in Alphen aan den Rijn. In 2004 heeft ze haar VWO diploma behaald aan het Ashram College in Alphen aan den Rijn. Daarna heeft ze één jaar gestudeerd aan de Kunstacademie Minerva aan de Hanzehogeschool Groningen waar ze haar Propedeuse heeft behaald. In 2005 besluit ze te kiezen voor een andere richting en is ze Biologie gaan studeren aan de Universiteit van Groningen. Na het behalen van haar bachelor in 2008, begint ze aan haar master Communicatie en Educatie in de Wiskunde en Natuurwetenschappen. Tijdens deze master loopt ze stage bij het VPRO programma Labyrint waar ze o.a. artikelen over wetenschap heeft geschreven voor de website van het programma. Voor haar scriptie heeft ze met behulp van een frame-analyse onderzocht op welke manier Q-koorts in Nederlandse krachtenberichten is neergezet. In 2011 rond ze haar master af en gaat ze aan de slag bij het Institute for Risk Assessment Sciences (IRAS) als junior onderzoeker waar ze o.a. een luchtmeetnetwerk heeft opgezet om Q-koorts te meten in de buurt van geitenbedrijven. Ook heeft ze een onderzoek gecoördineerd naar de blootstelling van MRSA bij varkenshouders. In 2012 begon ze aan haar promotieonderzoek onder begeleiding van Prof. dr.ir. Dick Heederik en Dr. ir. Lidwien Smit van het IRAS en Prof. dr. François Schellevis en Dr. Joris Ijzermans van het NIVEL. Dit promotieonderzoek is beschreven in dit proefschrift. Tijdens haar promotieonderzoek begint ze aan de master Epidemiologie met specialisatie milieu-epidemiologie aan de Universiteit van Utrecht. Deze master rond ze af in 2015. Momenteel werkt zij bij de gemeente Utrecht op de afdeling Volksgezondheid als adviseur Gezonde Leefomgeving. In deze functie geeft zij advies over gezondheid in ruimtelijke projecten.

List of publications

List of publications

This thesis

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