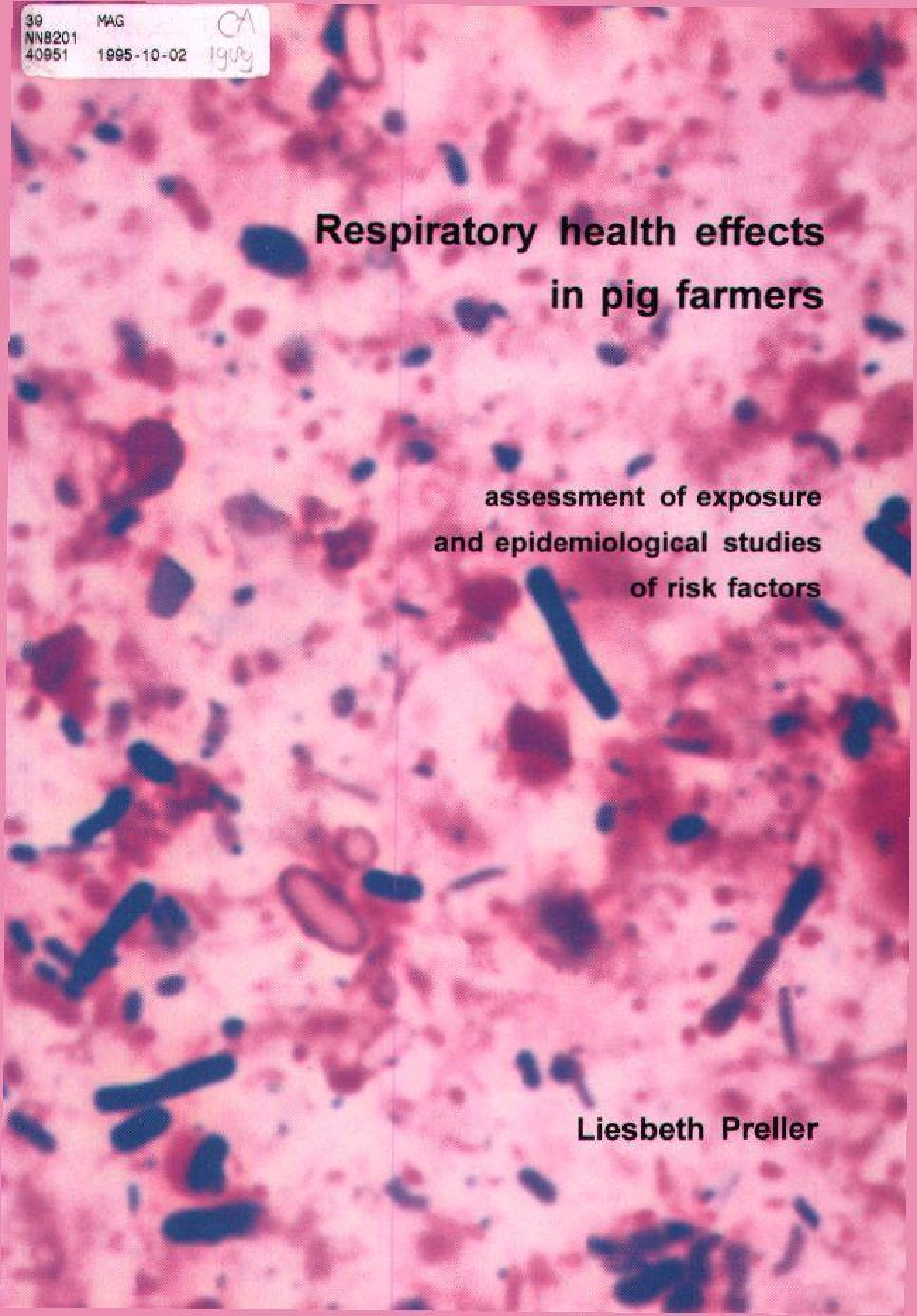

Respiratory health effects in pig farmers

assessment of exposure and epidemiological studies of risk factors

Liesbeth Preller

STELLINGEN

1. Eerdere studies naar de etiologie van chronische respiratoire aandoeningen bij varkenshouders waren gebaseerd op een veel te eenvoudige voorstelling van de daarbij betrokken immunologische mechanismen.
Dit proefschrift

2. Om de arbeidsomstandigheden van varkenshouders te verbeteren, daar waar het gaat om de blootstelling aan organisch stof, is het weinig zinvol dit te richten op het terugdringen van de stofblootstelling in het algemeen; op basis van de huidige kennis dienen deze maatregelen gericht te zijn op verlaging van de endotoxine-blootstelling.
Dit proefschrift

3. De ruime wetenschappelijke aandacht voor etiologisch onderzoek in arbeidssituaties staat in schril contrast met de beperkte wetenschappelijke interesse om effectieve beheersmaatregelen te ontwerpen.

4. De bewering in de Volkskrant 'Een groot aantal varkens hoest dagelijks, geeft slijm op en heeft last van astma of benauwdheid' naar aanleiding van het onderzoek dat de basis vormde van dit proefschrift doet vermoeden dat de uitdrukking 'varkens zijn net mensen' in toxicologisch opzicht letterlijk genomen kan worden.
De Volkskrant, 19 maart 1993

5. In de huidige arbeidsepidemiologie is het effect van blootstellingsvariabiliteit op de schatting van de blootstellings-effect relatie te vaak geen punt van discussie.

6. Ter wille van de veiligheid van fietsers zou langdurige en recente fietservaring een vereiste moeten zijn voor het verkrijgen van het auto-rijbewijs.

7. Het idee dat bijgeloof in Nederland weinig voorkomt is een mythe.

8. Zolang er belastingmaatregelen zijn die het kostwinnerschap begunstigen en er tegelijkertijd gekort wordt op subsidie voor kinderopvang, kunnen de inspanningen van de overheid voor een grotere en gelijkwaardige arbeidsparticipatie van vrouwen niet serieus genomen worden.

9. De strijd tussen zelf-1 en zelf-2 zorgt op de tennisbaan voor meer frustraties dan de strijd met de tegenstander.
Naar: W. Timothy Gallwey. Innerlijk Tennis, 1982

10. Of de uitspraak van Weiss 'Imperfect studies, properly interpreted, are far better than none at all' opgaat in een specifieke situatie kan alleen bewezen worden na het uitvoeren van de perfecte studie voor die situatie.
N.S. Weiss. Environ Health Perspect 1993; 101 (Suppl 4): 179-181.

11. Bij de afweging, of het opleggen van kostbare emissiereducerende maatregelen gerechtvaardigd is, dienen beleidmakers in ogenschouw te nemen of deze maatregelen een relevante reductie van de totale blootstelling kunnen bewerkstelligen.

Stellingen behorende bij het proefschrift 'Respiratory health effects in pig farmers: assessment of exposure and epidemiological studies of risk factors'.
Liesbeth Preller, Wageningen, 13 oktober 1995

Respiratory health effects in pig farmers

assessment of exposure
and epidemiological studies
of risk factors

Liesbeth Preller

Promotoren: dr J.S.M. Boleij, hoogleraar arbeidsomstandigheden in de land- en bosbouw

dr ir M.J.M. Tielen, hoogleraar huisvesting en verzorging der nutsdieren, Rijksuniversiteit Utrecht

Co-promotor: dr ir D.J.J. Heederik, universitair hoofddocent, Vakgroep Humane Epidemiologie en Gezondheidsleer

Liesbeth Preller

Respiratory health effects in pig farmers

assessment of exposure
and epidemiological studies
of risk factors

PROEFSCHRIFT
ter verkrijging van de graad van doctor
in de landbouw- en milieuwetenschappen
op gezag van de rector magnificus,
dr C.M. Karssen
in het openbaar te verdedigen
op vrijdag 13 oktober 1995
des namiddags om half twee in de Aula
van de Landbouwuniversiteit te Wageningen

Omslag: Gramkleuring van varkensmest. Foto Rudolf Gerrits

CIP-DATA KONINKLIJKE BIBLIOTHEEK, DEN HAAG

Preller, Liesbeth

Respiratory health effects in pig farmers: assessment of
exposure and epidemiological studies of risk factors/
Liesbeth Preller. - [S.l : s.n.]. -
Thesis Landbouwuniversiteit Wageningen. - With ref. - With summary in Dutch
ISBN 90 - 5485-427-8
Subject headings: respiratory health effects/ pig farmers/
epidemiological research

Voor mijn ouders

Abstract

Respiratory health effects in pig farmers

Assessment of exposure and epidemiological studies of risk factors

PhD Thesis. Department of Epidemiology and Public Health, Wageningen Agricultural University, Wageningen, the Netherlands, October 1995

Liesbeth Preller

This thesis describes a cross-sectional study of risk factors of chronic respiratory health effects in pig farmers working in the South of the Netherlands. The study population comprised 100 pig farmers with and 100 pig farmers without chronic respiratory symptoms. Base-line lung function, non-specific bronchial responsiveness, and specific antibodies to common and work-related antigens were determined as well. Exposure to dust, endotoxins and ammonia was measured by personal sampling during two days. Exposure to disinfectants was characterized by the type of disinfectant and procedure features.

As part of the study, long-term average exposure to endotoxins was modelled using information on task activity patterns during two weeks and on farm characteristics; exposure-response relationships would be seriously underestimated if measured exposure would be used due to the large day-to-day variability relative to variation between individuals.

Results of this epidemiological study suggest that the etiology of respiratory health effects in pig farmers is complex, involving different exposures and several mechanisms. The strongest evidence for a potential causal role of exposure was found for disinfectants and endotoxins, which may act either independently of atopy, or interact with atopic (IgE) sensitization to common allergens. It seems likely that IgE sensitization to common allergens can be induced by the use of disinfectants, specifically by those containing quaternary ammonium compounds. IgG_4 antibodies against work-related antigens were inversely related to respiratory impairment, potentially reflecting a protective effect of this type of antibodies.

Change of disinfection procedures, application of specific farm characteristics, and the use of personal protective equipment can be considered as control measures to reduce health hazards, but additional studies are required to estimate the actual effect.

Future epidemiological studies could provide evidence on the etiology of respiratory health effects when sufficient subjects are being studied in prospective studies and different etiological pathways are being considered. The exposure should be assessed on multiple occasions.

Contents

1. Introduction

Background

During the past decades, animal husbandry in several North American and European countries has become more and more an intensive agricultural activity with a high degree of specialization. In swine production, the number of piglets born per sow per year and the daily gain in weight of finishing pigs has increased strongly. This has brought about changes in working conditions and the occupational exposure of farmers. Current working methods require the use of large buildings with a high stocking density of animals, with the concomitant risk of increased exposure concentrations of several air pollutants. The cleaning and disinfection of the buildings and the frequent use of drugs are necessary for disease control among the animals. Early indications that the modern practice of swine confinement has led to an increase in human health hazards can be found in a small study carried out by Donham et al.[1]. They compared the respiratory symptoms of 24 swine confinement workers and 24 non-confinement swine workers who were comparable in age, smoking habits and the number of years they had worked in farming. Two to four times as many confinement workers reported symptoms of chronic cough, chronic phlegm and chronic wheezing, aside from during a cold. Later studies confirmed these high prevalence rates of predominantly chronic respiratory symptoms in swine confinement workers. However, the etiology has not yet been explained[2]. Also in the Netherlands, earlier studies indicated high prevalence rates of respiratory symptoms in pig farmers. A postal survey of 7 types of farming showed that chronic cough, chronic phlegm, chest tightness and clogged noses had the highest or second highest prevalence rates among pig farmers[3]. The first Dutch study on relationships between work place characteristics and respiratory health effects indicated that some farm characteristics, which were suggested to be surrogates for the exposure level of particular agents, were related to respiratory symptoms and base-line lung function[4].

Pig farming in the Netherlands

Intensive pig farming in the Netherlands is carried out at specialized farms, where usually the owner is the only full-time worker. Family members may be involved or employees may be hired to assist the farmer, so that task allocation can be applied. Of the total number of 17,938 workers at pig farms in 1993, 8,508 (47%) worked full-time in pig farming (38 hours or more a week)[5]. An additional 4,529 (25%) worked 20 to 38 hours a week, bringing the total work force working for at least 20 hours a week to 13,037. Of this workforce, 86% either own the farm or are a family member of the owner. The figure of 13,037 does not include workers at farms which combine pig farming with other types of animal or arable farming. 55 percent of the registered pig farm workers worked in the two southeastern provinces of the Netherlands, Noord-Brabant and Limburg[5].

Dutch pig farms are divided into three major types: breeding farms, finishing farms, and farrowing to finishing farms. In the first, breeding is the main activity. The piglets stay at the farm until they reach a weight of approximately 23 kg (about 10 weeks after farrowing), and are then taken to another type of farm, where the major activity is raising them to a slaughter weight of on average 106 kg, which takes 3-4 months. The farrowing to finishing farm is a combination of the above two types of farm.

Depending on the type of farm, there will be between one and four sections, each of which is divided into compartments. In the sow section, both pregnant and empty (non-pregnant) sows are housed; they comprise most of the sows present at the farm. In general, the number of sow compartments per farm ranges between 1 and 3. The week before farrowing, pregnant sows are moved to a compartment in the farrowing section, where they stay until their piglets have been weaned, about 4 weeks after farrowing. On most farms, farrowing compartments are small, with room for 6 to 8 sows. After weaning, piglets either stay in the same compartment or are transferred to another one, until they reach 23 kg. Compartments of weaned piglets house between 80 and 120 animals of the same age. The animals are then transferred to a finishing pig house at either the same or another farm, where they are put into compartments holding approximately 80 animals. For the latter three types of compartments, usually the 'all-in-all-out' system is maintained. This means that at the end of the cycle, before new pigs are placed in them, the compartments are completely empty. The empty compartments are cleaned, a process which is often followed by disinfection. The frequency of disinfection per compartment varies between farmers from never to each cycle.

At a farm, usually several pig houses of variable ages are used. The characteristics of compartments with respect to ventilation, type of flooring, feeding and other aspects depend partly on the type of section and on the age of the building. In modern farms, most compartments are ventilated mechanically with ventilation rates depending strongly on outdoor temperature and animal occupation. The manure is stored beneath the completely or partly slatted floor until its removal after several months.

Some activities performed on pig farms are carried out daily (such as feeding and inspecting the animals), whereas others are performed less frequently. Most farmers maintain some sort of weekly schedule of activities. The majority of these activities require farmers to be inside the pig houses.

Even on intensive pig farms, 2 out of 3 farmers perform other agricultural activities, such as cattle breeding, arable-, dairy- or poultry farming (Preller and Vogelzang, unpublished results). 27 percent spend 10 or more hours per week and 12% more than 20 hours per week on other agricultural activities. For the population selected for this study (n=200), these percentages were 25% and 7%, respectively.

Study setting and population

The population was selected for a study initiated by the NCB Pig Farmers Association. One of the major goals was to identify risk factors of chronic respiratory symptoms in pig farmers. To achieve this, a study population involved nearly full-time in pig farming was chosen, in order to minimize the confounding role of other occupational exposures. A farm size of more than 70 sows, more than 500 finishing pigs, or more than 50 sows in combination with more than 300 finishing pigs was considered to be the lower limit. Only the oldest owner of the farm was registered. In 1990 the owners of these farms in the provinces of Noord-Brabant and Limburg who were aged over 40 received a letter asking them to participate in the study. Positive responders at the farms where at least one of them worked more than 5 hours per day in pig farming, received a questionnaire. This group of 1,092 persons comprised owners and other farm workers. 1,341 farm owners in Noord-Brabant whose farm met the size requirement and who were younger than 40 received a questionnaire directly. Of these questionnaires, 1,504 were completed and returned. The questionnaire included questions on health, farm characteristics, working hours in and outside pig farming, and personal characteristics. For further study, farmers were selected from a group of 1,133 male pig-farm owners who worked at least five hours per day in pig farming and who were willing to participate in the further study. Further selection was based on chronic respiratory symptoms reported in the Dutch version of a shortened questionnaire on respiratory symptoms developed by the British Medical Research Council[6,7]. This questionnaire included questions on chronic cough, chronic phlegm, ever wheezing, persistent wheezing, shortness of breath, and attacks of chest tightness (asthma)[8] (Appendix 1.1). 200 randomly chosen asymptomatic farmers and 200 with one or more respiratory symptoms (171 with two or more, and 29 with one symptom) were invited to take part in a medical survey. These groups were further reduced to 100 each depending on the absence or presence, respectively, of symptoms of chronic non-specific lung disease[9] (Appendix 1.2) based on the medical survey. Farmers were classified as symptomatic if they answered positively to:

- 2 or more of the questions on: chronic cough, chronic phlegm, increase in chronic cough or chronic phlegm, ever wheezing, or shortness of breath (questions 1 to 5);

or to:

- 1 or more of the questions on: more than once episodes of (an increase in) chronic cough or chronic phlegm, shortness of breath while walking on level ground at a normal pace with people of a similar age, frequent wheezing, or asthma attacks during periods of rest (questions 6 to 9).

According to this classification, 115 farmers were symptomatic. The selected group of 100 symptomatic farmers comprised all 96 who had reported 2 or more symptoms in the first questionnaire, and 4 randomly chosen farmers with one symptom. Farmers were classified as asymptomatic if they answered questions 1-9 negatively (Appendix 1.2). Out of the 145 asymptomatic farmers, 100 were randomly selected.

Aims of the study and outline of the thesis

The main objectives of the study were (1) to characterize the exposure of pig farmers which potentially affects chronic respiratory functions and (2) to assess associations between occupational risk factors and chronic respiratory health effects. A secondary objective was to set priorities for the development of control measures to mitigate respiratory health risks to pig farmers. To realize these goals, the following exposures, health effects and working conditions were assessed:

- personal exposure to dust, endotoxins and ammonia was assessed during two days: one in winter and one in summer;
- exposure to disinfectants was evaluated by means of a questionnaire by phone and a walk-through survey;
- farm characteristics of all compartments on the farms were evaluated by inspection;
- time activity patterns were recorded by participants during two weeks: one in summer and one in winter;
- base-line lung function and non-specific bronchial responsiveness were assessed in the medical part of the study. Lung function tests were carried out by co-workers of the Occupational Health Service 'BGD Land van Cuyck en Noord Limburg' in Boxmeer, and non-specific bronchial responsiveness by co-workers of the Department of Occupational Medicine of the University of Nijmegen;
- specific IgE and IgG_4 antibodies were determined in the sera of the pig farmers.

Chapter 2 gives an overview of the current knowledge on exposure, respiratory health effects and exposure-response relationships in workers on pig farms. Chapter 3 describes the methodological problems encountered in assessing the long-term average exposure of pig farmers, as well as an estimation method for exposure to endotoxins. In Chapter 4, appropriate control measures to limit the inhalation of hazardous agents are presented, based on associations between exposure levels to dust and endotoxins, and working conditions. Chapter 5 gives an overview of the associations between chronic respiratory health effects and a variety of occupational exposures as found in our study population. The evaluated health effects include chronic respiratory symptoms and base-line lung function. The role of (1) the combination of atopic sensitization to common allergens and occupational exposure in respiratory health effects, and (2) the relationship between occupational exposure and atopic sensitization are described in Chapter 6. Associations between specific IgG_4 antibodies and respiratory health effects are presented in Chapter 7. Chapter 8 contains a summary of the main findings, which are discussed in view of the current knowledge.

References

1. Donham KJ, Zavala DC, Merchant JA. Respiratory symptoms and lung function among workers in swine confinement buildings: a cross-sectional epidemiological study. Arch Environ Health 1984;39:96-101.
2. Rylander R, Donham KJ, Hjort C, Brouwer R, Heederik D. Effects of exposure to dust in swine confinement buildings - a working group report. Scand J Work Environ Health 1989;15:309-12.
3. Willems H, Verlinden F, Biersteker K. Arbeid en gezondheid van de Nederlandse agrarier. Tijdschr Soc Gezondheidsz 1984;62:21-7.
4. Bongers P, Houthuijs D, Remijn B, Brouwer R, Biersteker K. Lung function and respiratory symptoms in pig farmers. Br J Ind Med 1987;44:819-23.
5. CBS Centraal Bureau voor de Statistiek. Landbouwtelling 1993. CBS (Statistics Netherlands), Voorburg/Heerlen, 1994.
6. BMRC. Standardized questionnaires on respiratory symptoms. Br Med J 1960;II -1665.
7. Biersteker K, Dijk WH van, Eissens JBMF, Geuns HA van. Ervaringen met geneeskundig onderzoek op CARA bij gemeentepersoneel te Rotterdam, 1970-1971. Tijdschr Soc Geneeskd 1974;52:158-62.
8. Heederik D, Brouwer R, Biersteker K, Boleij JSM. Relationship of airborne endotoxin and bacteria levels in pig farms with the lung function and respiratory symptoms of farmers. Int Arch Occup Environ Health 1991;62:595-601.
9. Lende R van der, Jansen-Koster EJ, Knijpstra S, Meinesz AF, Wever AMJ, Orie NGM. Definitie van CARA in epidemiologie en preventie. Ned Tijdschr Geneeskd 1975;119:1975-87.

2. Occupational exposure and respiratory health effects in pig farming - a review of the literature

Introduction

Pig farmers are exposed to a large variety of air pollutants. Agents which have been mentioned to have a potential role in respiratory disease etiology include: dust, bacteria, bacterial endotoxins, moulds, yeasts, (1→3)-ß-D-glucans, hair, dander and excreta from the animals, feed particles, and ammonia and other gases[1]. The animals themselves and their feed are considered the major sources of these pollutants. Dust can be inhaled directly after being emitted from the original source, but also indirectly after re-suspension. Excreta of the animals flow partly to the pit beneath the slatted floor but some remains on the floor and can be re-suspended after drying. Bacteria, belonging to the natural gut microflora, are emitted from the excreta and from other sources. The gram-negative bacteria contain endotoxins in their cell wall. Moulds and yeasts may be present in the feed, and the large quantity of dust containing organic matter in the buildings promotes growth of moulds. Cell walls of moulds contain (1→3)-ß-D-glucans. Warm and humid conditions contribute to the growth of microorganisms present. Storage of the manure beneath the slatted floor as well as presence of varying quantities on the floor leads to relative high concentrations of decomposing gases in the buildings, of which ammonia is a main component. Dander particles and urine from the animals, feed and moulds contain proteins that may be antigenic or allergenic. Floor bedding, insect parts ad storage mites can contribute to the total load of organic dust and antigens in the buildings. In addition, pig farmers may be exposed to chemical substances like disinfectants and cleaning agents, pesticides, and to adverse climate conditions.

Exposure to dust and ammonia can be found in many occupational settings, and their potential health hazard has been recognized since long. Only in the past decades, more attention has been given to potential health effects of inhalation exposure to specific organic dust components and the use of disinfectants. This has not resulted yet in clearly defined health risks. Specifically endotoxins have been studied widely for its potential respiratory health effects, but evidence in epidemiological studies on associations with chronic respiratory symptoms remains limited. Recognition of disinfectants as potential health hazard has to a large extent been based on case studies described in the literature, but also for them epidemiological evidence on exposure-response relationships is limited. This chapter is therefore continued by background information on potential respiratory health effects of exposure to endotoxins and disinfectants. Subsequently, studies in pig farming reporting on exposure, on health effects, and on exposure-response relationships will be reviewed.

Background information

Endotoxins

The hypothesis that endotoxins are likely to cause respiratory health effects in pig farmers is merely based on strong indications found in other occupational settings and in human and animal experimental inhalation studies. These studies have recently been reviewed by Douwes and Heederik[2]. In summary, experimental studies showed an inflammatory reaction to endotoxin inhalation. The mechanisms underlying the response to inhalation of endotoxins involve in the first place activation of the alveolar macrophage in the distal portions of the lung. Endotoxins activate the macrophage, causing the cell to produce a host of mediators. In turn, the inflammatory mediators serve as chemo-attractants and activators of other cells, among others to release mediators in a secondary response. It is generally considered that endotoxins do not affect airways epithelium directly, but that they may alter both the para-cellular and trans-cellular permeability of the epithelium indirectly by mediators released by macrophages. Furthermore, the epithelium itself may respond to this secondary stimulation by producing its own specific mediators[3].

In endotoxin inhalation studies in human subjects, a variety of acute respiratory effects were observed, including dry cough, shortness of breath, decrease in Forced Expiratory Volume in one second (FEV_1) and decrease in lung diffusion capacity[4-7]. Castellan et al.[7] calculated a no-effect level for change in FEV_1 in healthy subjects of 9 ng/m^3. Rylander et al.[6] selected cotton workers who smoked for their experiment with endotoxin-containing cotton dust, because of the stronger expected reaction in smokers. They estimated a no-effect level of 33 ng/m^3 for a decrease in FEV_1 over the exposure period. Most often, human experimental studies were directed on acute or subacute effects in subjects that were previously not exposed to high endotoxin concentrations. In this way, differences in effects after single or infrequent exposure and chronic exposure due to a potential tolerance effect, could not be considered. An exception was the mentioned study among cotton workers[6]. Indications for development of tolerance originate from different fields. An experimental study in which mice were injected with lipopolysaccharides (LPS, chemically purified endotoxins) for four days, demonstrated a significantly reduced concentration of total cells, neutrophils, and tumour necrosis factor-alpha (TNF-alpha) after inhalation of LPS in bronchoalveolar lavage (BAL) fluid compared to control mice[8]. Each of the measures of inflammation was reduced by more than or equal to 50%. A second, indirect indication of tolerance may be derived from the difference in cell counts in BAL fluid after exposure to endotoxin-containing pig farm dust observed between pig farmers and previously unexposed subjects[9,10]. The mechanism which prevents further damage at chronic exposure may involve down regulation of the initial inflammatory mediators[11].

Observational studies in cotton and animal feed workers suggest that endotoxin exposure may induce chronic respiratory health effects. In a study performed among Dutch animal feed workers, the average exposure concentration was 67 ng/m^3 in the highest exposure group (arithmetic mean)[12]. This is lower than reported in pig farming by e.g. Heederik et al.[13] and Donham et al.[14]. In the animal feed workers, an increase in endotoxin exposure of 24.8 ng/m^3 was associated with a decrease in base-line Forced Vital Capacity (FVC) and FEV$_1$ of 112 ml and 122 ml, respectively. In an earlier study among cotton workers, a smaller effect on FEV$_1$ of 24-78 ml per 100 ng endotoxin/m^3 was reported[15].

Occupational threshold exposure levels have been proposed of 100 ng/m^3 by an international working group[16], and 30 ng/m^3 a few years later[17] for 8-hour time-weighted average exposure. It is clear that exposure concentrations exceed these suggested limit values frequently.

Disinfectants

Most disinfectants used by pig farmers in the southern part of the Netherlands contain chloramine-T, or quaternary ammonium compounds which are used alone or in combination with aldehydes (glutaraldehyde, glyoxal, formaldehyde). There are no studies reporting exposure concentrations to disinfectants in buildings housing animals. Some studies reported exposure levels to disinfectants in hospitals[18,19], but the different methods of application in hospitals and animal buildings makes it impossible to use these studies to estimate exposure levels in pig farming.

Glutaraldehyde has been recognized as a cause of occupational asthma in hospital workers[20,21]. In letters to the editor, Calder et al.[22] and Waldron[23] addressed the high prevalence rates of upper respiratory tract irritations and lower respiratory symptoms in groups of 165 and 150 hospital workers, respectively, who were exposed to glutaraldehyde. However, base-line lung function was not affected[23]. As far as known, Norbäck et al.[24] conducted the only epidemiological study on disinfectants using actual exposure measurements. The study was cross-sectional and compared 39 workers in medical services who were exposed to glutaraldehyde levels ranging from less than 0.01 mg/m^3 to 0.57 mg/m^3 (15 minutes average) to 68 unexposed controls. The exposure levels were below the Swedish occupational exposure limit of 0.8 mg/m^3 (15 min average). The authors reported differences in symptom prevalence of the upper respiratory tract, but not of the lower respiratory tract.

In various occupational settings, several effects following exposure to chloramine-T have been reported. The effects include respiratory symptoms such as cough, wheezing and dyspnoea, and immediate and late asthmatic reactions. Effects on eyes and the upper respiratory system are reported either together with or without symptoms of the lower respiratory system[25-28].

Recently, two cases of occupational asthma after exposure to quaternary ammonium compounds have been described; one case of a worker in a factory in which household cleaning products were produced and one case of a pharmacist who's working area was cleaned with quaternary ammonium compounds[29,30].

Formaldehyde as etiological factor in respiratory disease has been studied frequently in occupational and non-occupational settings, but has not been mentioned as cause of respiratory health effects when used as a disinfectant. The respiratory effects of exposure to formaldehyde include occupational asthma, effects on base-line lung function and associations with respiratory symptoms in some occupational groups, although other studies fail to show such associations[31-34]. Carcinogenic properties of formaldehyde are beyond the scope of this review and will not be discussed.

Only in a limited number of affected persons, occupational asthma or other respiratory symptoms caused by exposure to disinfectant components could be attributed to specific IgE sensitization. Specific IgE antibodies against chloramine-T have been shown in symptomatic persons[27,35]. It is unclear whether aldehydes cause IgE-mediated asthmatic symptoms, although formaldehyde-specific IgE antibodies have been demonstrated to be associated with skin problems and with discomfort in the upper airways[36,37]. In the production worker with occupational asthma due to quaternary ammonium compounds, no specific IgE could be found[29]. However, QAC can be type I allergens, as has been shown in muscle relaxant allergy[38]. The IgE binding epitope in the group of muscle relaxants containing quaternary ammonium compounds has been shown to be the QA functional group.

Exposures in pig farming

In this section, the main emphasis will be put on studies with a sufficient number of quantitative exposure measurements of dust, bacteria, endotoxins and ammonia to be useful for epidemiological purposes and in which information on sampling techniques was relatively complete. In addition, studies are mentioned which give information on other potential health hazards or which give insight into associations between exposure levels and working circumstances.

The number of epidemiological studies among pig farmers with quantitative exposure assessment is limited. The studies are summarized in Table 2.1. In most studies exposure was assessed by means of static sampling in the buildings. Donham et al.[14] and Heederik et al.[13] assigned outcomes of static measurements directly to all workers on the same farm. In this case differences in working circumstances between workers, leading to differences in personal exposure, is neglected. In two studies, staticly measured exposure concentrations of dust, endotoxins and ammonia and of ammonia only, respectively, were weighted for the time spent in different locations[39,40]. Holness et al.[41] and Heederik et al.[40] measured exposure to dust and to dust and endotoxins, respectively, by personal sampling over the work-shift. Donham et al.[14] presented also data on personal dust and endotoxin exposure, but the data were incomplete with respect to the number of measurements and sampling time. The number of farmers involved in the studies ranged from 27[40] to 177 farmers[13].

In the studies average total dust exposure ranged from 1.6 mg/m^3 to 4.0 mg/m^3, with individual measurements ranging from less than 1 to 23.5 mg/m^3. Averaging time was presumably several hours in all studies, although sampling time was given only by Heederik et al.[13] for static sampling (6-8 hours) and by Holness et al.[41] and Heederik et al.[40] for personal sampling (8-9 hours). Only few reported concentrations exceeded the Dutch occupational Threshold Limit Value of 10 mg/m^3 for exposure to total dust, which is based on a nuisance effect. The average ammonia concentration differed less than a factor two between the different studies: from 4.4 mg/m^3 to 7.6 mg/m^3, with single measurements varying between 0.2 mg/m^3 and 24.7 mg/m^3. Sampling time was several hours in the Canadian study[39], 6-8 hours in the Dutch study on 136 farms and not known in other cases. For the ammonia exposure reported by Donham et al.[14], it can not be inferred whether this was measured by grab sampling or by sampling with a larger averaging time. Depending on time dependent variation in ammonia exposure, this can cause large differences in average concentration and geometric standard deviations[42,43]. The presented results indicate that the Dutch occupational 8-hour time-weighted average threshold limit value of 18 mg/m^3 was exceeded only in few situations. For exposure to dust as well as ammonia, the largest ranges were reported for 136 Dutch pig farms[13]. This study included the largest number of measurements and by that, probably the largest sampling period of about 3 months, covering part of autumn and winter with potentially climatic effects.

Table 2.1 Exposure concentrations of dust, endotoxins and ammonia: Geometric Mean (Geometric Standard Deviation).

reference	country[*]	population	dust (mg/m^3)		endotoxins (ng/m^3)	ammonia (mg/m^3)
			total	respirable		
Holness[41]	C	54 workers	2.1[†] (2.5)	0.17[†] (3.7)	- -	- -
Donham[14]	S	57 workers 28 farms	3.9 (1.6)	- -	180 (2.1)	5.6 (1.7)
Heederik[40]	NL	27 workers 2 farms	1.6[†] (2.8)	- -	24[†] (4.0)	5.6[‡] (1.9)
Heederik[13]	NL	168-177 workers 136 farms	4.0 (1.7)	- -	130 (1.5)	4.4 (2.5)
Zejda[39]	C	54 workers[§] 50 buildings	2.8 (1.4)	0.12 (1.4)	740 (2.5)	7.6 (1.4)

[*] C = Canada, NL = Netherlands, S = Sweden

[†] personal sampling, time-weighted average

[‡] personal exposure estimated using time spent in buildings

[§] personal exposure estimated using average of concentrations in different barns and 2 seasons; endotoxin exposure known for 46 workers

Between studies, large differences existed in endotoxins exposure concentrations. The average concentration was in the same range in the studies of Donham et al.[14] with unknown sampling time and Heederik et al.[13] who measured during 6-8 hours, with 180 ng/m^3 and 130 ng/m^3, respectively. The range in both studies was also comparable, from approximately 30 ng/m^3 and 350 ng/m^3. Zejda et al.[39] (unknown sampling time) reported an average concentration which was a factor 5 higher than in these two other studies. The upper limit in their study was more than 4,000 ng/m^3. Since dust concentrations were somewhat lower than in both other studies, the presented endotoxin exposure seems very high. It is not likely that this was caused by the way in which exposure concentrations were assigned to individual farmers. Heederik et al.[40] measured exposure of 27 workers at two large scale Dutch farms by personal sampling during the work-shift of about 8 hours. Working conditions at these farms vary from those at the family farms which are usually encountered in the Netherlands. The low average concentration may be caused by specific working conditions at these farms. Also the average dust exposure was lower than in other studies. In all of the reviewed studies a *Limulus* Amoebocyte Lysate (LAL) test has been used, but variation in filters and in extraction and storage procedures may have caused differences between studies[44], apart from true differences in exposure and differences caused by variation in sampling time. Douwes et al.[44] showed for example that recovery of endotoxins can range a factor 7 between extraction procedures, with an otherwise similar procedure. In smaller studies among pig farmers[45-48], average and individual endotoxin concentrations ranged from 2 to 1,900 ng/m^3. It is clear that regardless the assay used, exposure concentrations exceed the earlier suggested occupational exposure limit values of 100 ng/m^3 and 30 ng/m^3 for endotoxin exposure[16,17] frequently.

The geometric standard deviations were larger for dust and endotoxin exposure measured by personal sampling compared to measured by static sampling (Table 2.1), suggesting that variation in personal exposure may be underestimated by static measurements.

Only Heederik et al.[13] quantified concentrations of airborne microorganisms systematically for epidemiological use. For sampling, they used the N6-modification of the Andersen sampler[49] with Plate Count Agar (PCA) media for total viable bacteria, and PCA with christalviolet media for viable gram-negative bacteria. In 62 farms they measured a geometric mean concentration of total viable bacteria of 1.07 * 10^5/m^3 (GSD 3.4, range 0.01-36 * 10^5/m^3), and of 0.08 *

$10^5/m^3$ of gram-negative bacteria (GSD 4.5, range 0.01-0.9 * $10^5/m^3$). These concentrations are in the same range as found in other studies, with a concentration of approximately $10^5/m^3$ to $10^6/m^3$ total viable bacteria. Usually 10% of the total viable bacteria was gram-negative[14,45,47,48,50]. Concentrations of viable mould particles averaged over several sampling sites varied between 300 to 30,000 per cubic meter, but in specific cases levels up to $100{,}000/m^3$ have been found[45,47,48,51].

To our knowledge, the study of Virtanen et al.[52] was the only one reporting antigen exposure levels of pig farmers. In samples taken by personal and static sampling, it seemed that pig epithelium antigens were much more abundant than feed and pig urinary antigens. However, according to the authors, quantitative comparisons between levels measured by immuno-chemical methods should be made cautiously.

Relation between exposure and working characteristics

Exposure levels may vary considerably between activities. In a study among 20 Swedish pig farmers, exposure to endotoxins was found to be more than eight times higher during feeding than during tending: 315 and 37 ng/m^3, respectively[9]. The difference in dust exposure was only a factor two, with exposure levels of 13.8 and 7.4 mg/m^3, respectively. Vinzents and Nielsen[53] suggested that distance to animals alone does not determine personal exposure levels during activities. In a small-scale study among 4 workers they did not find systematic differences in either dust or endotoxin exposure between activities performed close to and far from animals, but the distinction between the two groups of activities might have been to crude to yield large differences.

Statistically significant associations between type of feeding and personally or statically measured dust exposure have been reported[41,54]. Attwood et al.[54] found also statistically significant differences in dust concentrations between different sections, but not in concentrations of ammonia and endotoxins.

Validity of exposure measures

The validity of the exposure data in epidemiological studies among pig farmers, with chronic respiratory health effect as outcome variable, has hardly been evaluated. The validity may be affected in the first place by exposure variability, which has for instance been quantified by Zejda et al.[39]. They estimated a large intraindividual variation in exposure to endotoxins, which was assessed for two days, one day in summer and one day in winter. Given the exposure variability, the regression coefficient of base-line lung function on endotoxin exposure would be underestimated by 65%, using equations presented in the literature[55]. Since no overall difference in exposure between seasons was found, variation in total time spent in different sections may have contributed to a large extent to intraindividual variation. Exposure in other studies was based on only one measurement. This would, with similar exposure variability, cause even larger underestimation of exposure-response relationships.

Secondly, estimation of personal exposure by static measurements can lead to additional large bias in exposure-response relationships. In cotton and animal feed industries it has been shown that exposure assessed by static measurements and personal sampling correlates poorly[56,57], and personal exposure would be underestimated when based on static measurements. In the specific case of pig farming, factors such as re-suspension of settled dust by activity of animals after entrance of the farmer in a compartment[58], spatial variability within a compartment[59], and the effect of specific activities on personal exposure concentrations[9] may cause errors in personal exposure when based on static measurements.

For these reasons, it is likely that the exposure measurements used so far have limited value in the assessment of unbiased relationships between chronic health effects and exposure.

Health effects in pig farmers

Questionnaires and lung function testing are the most frequently used techniques to assess respiratory health effects in epidemiological studies among pig farmers. In some studies prevalence rates and average lung function parameters have been presented for a population which was selected on respiratory health effects. These studies will not be regarded in this overview. Since the early eighties, the possibility of an immunological mechanism as an explanation of observed health effects has been explored. In the nineties the emphasis in the literature shifted towards the use of advanced research techniques on the mechanisms underlying symptomatology. This involved assessment of Non-Specific Bronchial Responsiveness (NSBR) and qualification and quantification of inflammatory cells present in the respiratory tract by means of bronchoalveolar lavage (BAL). These research techniques have also been applied in some human experimental studies.

Chronic respiratory symptoms

Prevalence rates of chronic respiratory symptoms among pig farmers have been reported in many studies. In the larger studies, questionnaires based on the ATS[60] and the BMRC[61] questionnaire on chronic respiratory symptoms have been used most frequently. For this overview only studies have been regarded with a population exceeding 50 subjects and with questions on symptomatology that were more or less comparable to other studies.

Comparison of studies in different countries showed that Dutch and French pig farmers reported less chronic respiratory symptoms than Danish and Canadian pig farmers (Table 2.2). The relatively low prevalence rate in Dutch pig farmers still implies that 23% to 40% reported at least one chronic respiratory symptom. Similar prevalence rates have been reported in other occupational settings with exposure to organic dust and coal dust, in which symptoms were evaluated using the same questionnaire[62]. On average 20% of control office workers reported these symptoms in the studies. Because the influence of a potential healthy worker effect can not be excluded, no inferences can be made on the attributable risk of pig farming to respiratory symptoms.

In nearly all of the larger reviewed studies and other smaller studies using reference groups, a two to three fold higher prevalence rate (crude or adjusted) of cough, phlegm or chronic bronchitis in pig farmers compared to controls has been reported[14,41,63-69]. Occasionally, comparable prevalence rates have been observed in pig farmers and controls[65,70,71]. The reference groups consisted of farming and non-farming populations, including industrial workers. Ambiguous results have been reported on relative prevalence rates of other symptoms. For example, prevalence rates of shortness of breath were equal in French dairy and pig farmers[71], but were reported two times more often by Danish pig farmers than by Danish dairy farmers and farmers without animals[63]. Similar inter-study discrepancies existed for symptoms of chest tightness and wheezing[14,63,64,69,71,72].

In both large Dutch studies performed by the same research group, the same questionnaire was used and there were only few years between the studies. Comparable socio-economical criteria were used to select the farmers, but the response rate differed between the studies, with 81% and 51%, respectively, and farmers in the first study were on average 10 years older. The prevalence rate of chronic symptoms was nearly a factor two lower in the first study, suggesting that selection effects may cause large differences in study results. As a consequence, the use of questionnaires in cross-sectional epidemiological studies is limited for evaluating the attributable risk of occupational exposure to the incidence or prevalence of these symptoms. Since the two Dutch studies already showed large differences, it is not surprising that ambiguous results have been presented on relative prevalence rates in other studies as well.

Table 2.2 Prevalence rate of chronic respiratory symptoms in pig farmers.

					symptoms (%) †					
reference	country*	n	smokers (%)	age	C	P	S	W	T	B
Bongers[76]	NL	132	-	47	10	5	10	13	6	-
Heederik[13]	NL	183	38	37	16	12	9	22	10	-
Iversen[63]	DK	369	36	-	37	-	23	26	11	32
Iversen[65]	DK	124	20	43	32	-	30	21	-	-
Choudat[71]	F	102	28	40	13	-	12	7	3	-
Dosman[64]	C	504	-	42	-	17	32	29	-	13
Zejda[69]	C	249	15	38	21	29	-	25	-	25
Donham[67]	US	207	20	36	20	25	-	-	-	-

* C = Canada; DK = Denmark; F = France; NL = Netherlands; US = USA

† C = cough; P = phlegm; S = shortness of breath or dyspnea; W = wheezing; T = chest tightness/asthma; B = chronic bronchitis

Work-related respiratory symptoms and other symptoms

Work-related symptoms which have been reported, include lower and upper respiratory symptoms, eye irritation and symptoms that are characteristic for the Organic Dust Toxic Syndrome (ODTS). Symptoms are defined as work-related if they occur during work or up to several hours after work. ODTS comprises a variety of symptoms which are seen after exposure to high concentrations of organic dust. It may appear as an influenza like syndrome, which generally starts only 4 hours after exposure, with joint pains, myalgias, shivering, fever and flu-like symptoms. This syndrome can be distinguished from Hypersensitivity Pneumonitis by, among others, the absence of specific serum antibodies, no indication of progressive lung disease at repeated exposures, predominance of neutrophils rather than lymphocytes in material obtained in the acute phase by alveolar lavage, and negative findings in X-rays[73,74].

In the studies with a population exceeding 50 subjects, work-related cough has been reported by 17 to more than 80% of the pig farmers[14,39,47,65,67,75,76]. 8 to more than 60% of the pig farmers indicated that they experienced nose, throat or eye irritation, clogged nose, sneezing or rhinitis[14,41,47,67,71,75,76]. Work-related symptoms were generally more common in pig farmers than in control populations[63,65,67,71]. Prevalence rates of symptoms which can be indicative of ODTS ranged from 0 in 132 Dutch pig farmers (fever and shivering) to up to 17 and 21% in Swedish and Canadian pig farmers (joint pains and flu-like symptoms)[41,47,70,75,76]. Donham et al.[67] reported an unrealistic high prevalence rate of 34% for ODTS symptoms in US pig farmers, but their definition was not very specific since also farmers who experienced lower respiratory symptoms alone were regarded as having ODTS. As with the chronic respiratory symptoms, also the prevalence rate of ODTS-like symptoms were much higher in the later study among Dutch pig farmers; shivering was reported by 11%[13].

Base-line lung function

In pig farmers, Forced Vital Capacity (FVC) and Forced Expiratory Volume in one second (FEV_1) and to a lesser extent FEV_1/FVC, seem not to deviate largely from reference values (Table 2.3). The FEV_1/FVC ratio reported in the study of Donham et al.[14] seems very unlikely considering the values of FEV_1 and FVC. The population averages of the parameters varied several percent above or below reference values in six studies comprising more than 50 subjects (Table 2.3). In studies using control groups, similar patterns were observed as for comparison with reference values[41,64,66,69,77].

A reduction of 4% to 5% compared to predicted FVC or FEV_1 values as reported by Holness et al.[41] and Dosman et al.[64], suggests an average decrease of 200 to 250 ml in farmers who were working on average 20 years in pig farming. It has been shown that a small decrease in FEV_1 is associated with a higher risk of incidence of chronic respiratory symptoms and mortality from these symptoms[78].

Data in Table 2.3 suggest that particularly the flow-volume variables Maximum Mid Expiratory Flow (MMEF), and the Maximum Expiratory Flow when still x% remains to be exhaled (MEF_x) are decreased in pig farmers, with deviation from normal values up to 40%. These variables are less reproducible than FVC and FEV_1[79]. No consensus exists on the meaning of abnormalities in these variables in obstructive lung disease, although decrease in these parameters has been mentioned to show the earliest change associated with flow limitation, even if other parameters are not affected[80].

Table 2.3 Base-line lung function in pig farmers (percentage of predicted).

reference	n	smokers (%)	FVC	FEV_1	MMEF	MEF_{50}	MEF_{25}	FEV_1/FVC
Bongers[76] *,†	132	-	+0.16	-0.09	-0.71	-0.51	-0.40	-
Holness[41] ‡,§	53	15	98	95	-	93	64	97
Dosman[64] §	504	-	97	96	87	-	-	98
Donham[14] §	57	-	>95	>95	-	84	60	75
Zejda[69] ‡,§	249	15	106	103	98	108	88	97
Zejda[39] ‡	54	-	106	101	94	-	-	96

* residual values in L or L/s are given, in all cases statistically different from reference values
† ECCS reference values
‡ ATS reference values
§ other reference values or not reported

Lung function change over the working day

The across-shift changes in FVC, FEV_1, FEV_1/FVC and Peak Expiratory Flow (PEF) ranged from no or minor changes in Swedish pig farmers, to 5% in a study among workers in former Yugoslavia[9,14,47,68,81]. The studied populations were small, comprising 20 to 59 farmers. In the study in former Yugoslavia also a high prevalence rate of chronic and work-related respiratory symptoms was present, which may be related to the relatively large decrease in lung function. In a study among farmers with work-related symptoms, also larger across-shift decrements were found[82]. In none of these studies, results were adjusted for the circadian variation in lung function, which can vary largely between individuals from several percent decrease to several percent increase[83]. In one small study only, a comparison was made with an external control population[81]. The percentage of change in FEV_1 in 36 swine workers was not larger than in the 16 controls. Comparisons within the same population at days off work would have given better insight into the relative lung function changes over the working day.

Non-specific bronchial responsiveness (NSBR)

Studies reporting on prevalence of NSBR are not comparable, either because of difference in challenge procedure itself or by the definition of hyperresponsiveness. This resulted for example in a difference in average PC_{20} of a factor of 50 between two methacholine challenge studies with 20 subjects each[9,77]. The value of these studies remain therefore limited to intrastudy comparison. In studies among 36 to 167 pig farmers, they were found to be less[71], about equally[65,81] or more hyperresponsive[84] than the control (dairy and cattle) farmers. Larsson et al.[9] reported no difference in NSBR between 20 pig farmers and healthy controls, whereas in other studies pig farmers were found to be more hyperresponsive in tests with histamine, methacholine or acetylcholine than greenhouse workers[81], urban controls[77], and arable farmers[84], respectively. In 36 Swedish pig farmers, NSBR did not alter across work shift[81]. The ambiguous results of the studies do not allow to draw conclusions on NSBR in pig farmers. NSBR after exposure to pig farm dust has also been studied in an experimental setting, which will be discussed later on.

In several studies, the association between NSBR and symptomatology was evaluated. Briefly, NSBR was found to be related to the combination of chronic bronchitis with abnormal spirometry studied in a population of a total of 57 pig farmers (defined as $FEV_1/FVC < 95\%$)[85], to self-reported asthma (n=144)[86], to cough, asthma and rhinitis and conjunctivitis (n=167)[84], to inflammation of the bronchial mucosa (n=26)[87], and strongly or weakly to work-related symptoms (n=124 and n=65, respectively)[65,88]. In contrast, NSBR was not found to be related to: chronic bronchitis with normal spirometry, abnormal spirometry without chronic bronchitis (n=57)[85], and to work-related shortness of breath, wheezing or dry cough (n=144)[86]. Since NSBR is considered to be a hallmark of asthma, most of the positive associations with respiratory health were not surprising. The added value for studying the etiology of respiratory health effects in pig farmers seems so far limited.

Bronchoalveolar lavage (BAL)

BAL has been performed to study the presence of inflammatory cells in the respiratory tract in relation to the respiratory symptoms seen in pig farmers. Larsson et al.[9] found a statistically significantly elevated number of total cells (25% difference in median) and of neutrophils (factor 6 difference in median) in 20 pig farmers compared to controls. The number of eosinophils, alveolar macrophages and lymphocytes were not higher in the pig farmers. The small number of subjects in the study is plausible because of the unpleasant side effects, but it limits insight into the effects at population level.

Schwartz et al.[88] studied BAL in groups of swine confinement workers with and those without work-related respiratory symptoms, neighbourhood farmer controls and blue collar workers. Each group consisted of approximately 30 persons. They did not find differences between these groups. In another study inflammation of the bronchial mucosa in pig farmers, which was assessed by bronchoscopy, was related to increase in number of neutrophils but not to increase in total cell number[87]. Similar to the use in the latter two studies, future use of BAL in etiological studies can be useful when applied in subjects that are selected on specific clusters of respiratory symptoms. It does, however, require more insight into the existence of distinct clusters of symptoms.

Experimental studies on BAL in relation to exposure to pig farm dust will be discussed later on.

Immunology

An early case-study reported asthma in a student in agricultural sciences due to an IgE-response to pig urinary proteins[89]. Several years later a similar case of anti-pig urine allergy was described in a pig butcher[90]. These studies and the high prevalence rate of allergy to, for example, rat urinary proteins in animal laboratory workers, indicate that anti-pig urine IgE-mediated occupational asthma may be expected in farmers who are chronicly exposed to high levels of pig urinary proteins. In order to assess the role of IgE-mediated allergies in pig farmers, large studies are required since atopic reactivity can be expected in a minority of the population only.

The presence of an immunological component in the etiology of respiratory health effects has been studied by skin prick tests (SPT), and serological determination of specific IgE, IgG, and precipitating antibodies. The main emphasis in this review will be put on the first two parameters, since these are indicative of type I allergy. The prevalence rate of a positive SPT against work-related antigens varied from 0 to 78% (Table 2.4). Extracts of feed components, dander, epithelium or hair, pig urine, crude swine house dust or storage mites were used as allergen preparations. In two studies with a high prevalence rate of positive SPTs, also a relatively high percentage of controls showed a positive test, which makes the specificity of the tests questionable[66,72]. The large interstudy variation in these and other immunological parameters may be due to differences in allergen preparations and assay techniques. In most studies, the prevalence rate of positive IgE antibodies against work-related antigens was low. Only Brouwer et al.[91] and Crook et al.[48] found relatively high percentages of positive reactions to storage mites and feed antigens: 13% in 123 and 25% in 24 farmers, respectively. In the study of Brouwer et al.[91], 70% of all farmers with IgE against storage mites, also showed IgE to house dust mite allergens. The high prevalence rate of a positive test to storage mites could have been the result of cross reactivity between both types of mites[92]. In the small study of Crook et al.[48] 48 controls were included, but the percentage of positive reactions in them was not given. Overall, by their population size and selection criteria, only four studies give some insight into the potential role of IgE-mediated allergies in pig farmers[52,75,91,93,94].

Table 2.4 Prevalence of positive skin prick test (SPT) and specific IgE against work-related allergens: percentage (percentage in controls).

reference	n	allergen	SPT	IgE
Katila[95]	20	epithelium	-	0
Matson[93]	41	hog/feed/dust	0	2
Brouwer[75]	57	feed	-	1.8
		urine/hair	-	0
Brouwer[91]	123	storage mites	-	13
Cormier[66]	488	hog (large panel)	18 (10)	-
Zuskin[72]	32	settled dust/hair	44 (39)	-
		feed	78 (51)	-
		bacteria	9 (4)	-
		moulds	9 (3)	-
Crook[48]	24	microorganisms	-	0*
		skin/urine	-	12*
		feed (meal)	-	33*
Larsson[9]	20	dander/feed (2)/ dust/urine/plasma	0	-
Iversen[94]	247	airborne dust	-	2.5
		epithelium/urine	-	< 1

* 48 controls, prevalence rate unknown

Specific total IgG and IgG_4 antibodies against work-related allergens have been found[75,91,93,95]. The exact role of IgG or IgG_4 antibodies in respiratory disease is, however, not clear. Suggested effects of specific IgG_4 antibodies on respiratory health range from harmful to protective[96].

No associations were observed between IgE antibodies against work-related antigens and various respiratory health effects in populations of 20 and 41 farmers, respectively[95,93], whereas a positive SPT to hog allergens was not related to lung function or chronic bronchitis in a large study in 488 pig farmers[66]. Brouwer et al.[75] did not observe an association between base-line lung function and specific IgG_4 levels. A few other studies give weak indications for a positive association between an immunological response to work-related antigens and health effects. Farm workers with a positive skin prick test against work-related allergens had lower base-line lung function values but not more chronic respiratory symptoms, although the specificity of the test was questionable[72]. Elevated IgE levels against feed components were observed in symptomatic farmers, but not in asymptomatic farmers[66]. Of 6 farmers with weakly positive IgE reactions against airborne farm dust, 5 were asthmatic, but they also showed strong IgE reactions against mite species and grains[94].

In summary, the studies suggest that at the population level work-related type I allergy is not a predominant factor, but work-related allergies may, however, be present in individual farmers.

Experimental studies in the setting of pig farming

One research group reported experimental studies with six and 14 human subjects, respectively. The subjects were nonsmoking, non-farming, were previously not or hardly exposed to pig farm dust and did not experience respiratory symptoms. They were exposed for several hours to occupational air pollutants during weighing of pigs. Exposure concentrations during this activity were high. In the study with 14 subjects, average exposure to dust and endotoxins was 13.5 mg/m^3 (range 5.6 to 24 mg/m^3) and 600 ng/m^3 (80 to 1,300 ng/m^3), respectively. This is several factors higher than average exposure concentrations found for pig farmers in most studies (Table 2.1). Exposure induced strong reactions in these experimental subjects. Several experienced strong systemic reactions such as rise in body temperature up to 39°C, headache, malaise and nausea. A large increase in a variety of inflammatory cells was observed one day after exposure[10,97]. There was a 75-fold increase in neutrophilic granulocytes, a two- to threefold increase in alveolar macrophages, a fourfold increase in lymphocytes and total cells, an increase in eosinophilic granulocytes (all expressed in concentration in BAL fluid). The increase in neutrophils was significantly correlated with dust and endotoxin exposure concentration.

In six urban subjects experimentally exposed in the same way, working in pig farming induced within 6 hours a considerable increase of more than three doubling steps in bronchial responsiveness. Bronchial responsiveness appeared still not to be normal after one week[98].

These experimental studies showed that exposure to pig farm dust results in a clear inflammatory response in subjects that were previously not exposed to pig farm dust. The experimental exposure level to dust was higher than average reported for exposure levels in pig farmers, but still in the range of single exposure measurements. The average endotoxin level was higher than reported for individual exposure measurements by Donham et al.[14] and Heederik et al.[13]. The differences in BAL findings between farmers[9] and experimental subjects, in combination with exposure concentration to endotoxins that exceeded exposure of pig farmers in most cases, limits extrapolation of these results to pig farmers.

Association between exposure and health parameters in pig farming

Exposure levels

In this paragraph, basically only associations which were adjusted for smoking habits, age and standing height (lung function only) will be presented. Since in only few studies exposure measurements for epidemiological purposes were performed, the number of studies reporting on exposure-response relationships is low as well.

Measured dust exposure in four studies was not related to chronic respiratory symptoms, base-line lung function, and lung function change over the working day[13,14,39,41]. In contrast, two studies suggested an inverse association between endotoxin exposure levels and base-line lung function of several hundreds of millilitres over the range of exposure levels (FVC and FEV_1). Heederik et al.[13] found statistical significant associations between base-line lung function and endotoxin exposure only in a group of 62 farmers who worked at a farrowing to finishing farm, but not in the total population of 160 farmers. Zejda et al.[39]

observed an association when concentrations were multiplied by number of hours worked, but not with concentration alone. It is not clear whether this finding could be attributed to number of hours alone, which might be related to any type of exposure.

Donham et al.[14] reported a statistically significant association between endotoxin exposure and decrease in FEV_1 over the working day. The decrease was about 5% over the range of exposure levels, which equals on average 200 ml. In this study exposure levels were assessed by personal as well as static sampling. In their analysis, they used exposure based on static sampling which is expected to correlate poorly to personal exposure. They failed to give an explanation for the choice of the exposure measure. Statically measured exposure was assigned to all workers on the same farm. On the farm with the highest exposure concentrations, 7 farmers were working with on average a large decrease in across shift lung function, potentially contributing largely to the strength and magnitude of the exposure-response relationship. Such an analysis inhibits the danger of neglecting the potential effect of another health hazard present at one single farm. An effect of endotoxin exposure on across-shift change in lung function could not be confirmed in a study among 27 Dutch pig farm workers which were exposed to low concentrations of on average 24 ng/m^3, as measured by personal sampling[40].

Presence of several work-related symptoms was positively associated with statically measured endotoxin concentrations in the restricted population of 62 Dutch farmers[13]. The work-related symptoms included upper and lower respiratory tract symptoms and some flu-like symptoms. Similar associations existed with levels of gram-negative bacteria[13]. In the same study no statistically significant associations between chronic respiratory symptoms and exposure levels were observed. Zejda et al.[39] mentioned a statistically significant association between endotoxin exposure and chronic cough and chronic bronchitis. This association was not quantified, and not adjusted for age. In addition, their results are difficult to interpret since they used an unconventional statistical technique to assess these associations.

In the experimental study with 14 previously non-exposed subjects[10], the increase in neutrophils in BAL was significantly correlated with dust and endotoxin exposure during weighing of pigs. Since correlation between dust and endotoxin exposure in that study was very high, it can not be concluded which component was responsible for the increase in inflammatory cells.

An effect of ammonia exposure on across-shift change in several lung function parameters, but not in FVC, has been reported in a small study among 27 farm workers[40]. Ammonia exposure in that study was estimated, based on static measurements and time activity patterns. The associations were present on Tuesday but not on Monday and Friday. Since an effect of ammonia can be, unlike e.g. endotoxins, expected to be about similar on different days of the week, the associations may be spurious.

Associations between immunological parameters and exposure levels have rarely been reported. In a study among 44 pig farm workers, anti-swine urine IgG and anti-swine epithelium IgG correlated with exposure to pig urinary antigens, which was measured only once[52]. In this Finish study, a greater proportion of 37 pig farm workers showed high anti-pig urine IgG levels than 44 medical students. However, the distribution of IgG levels against epithelium and feed antigens between these two groups did not differ clearly. Brouwer et al.[91] observed an association between total dust exposure and specific IgG_4 against pig urinary proteins and pig hair among nonsmokers but not in smokers. Although Virtanen et al.[52] suggested that exposure to airborne epithelium antigen is higher than to feed and urinary antigens, findings in other studies do not suggest that this leads more frequently to atopic sensitization to epithelium derived allergens[75,94,95].

Surrogate exposure variables

Measures such as years in pig farming and farm characteristics have been used as a surrogate for measured exposure, to obtain information on potential exposure-response relationships. The associations with number of years in pig farming and age are difficult to separate, since high correlation between the two variables is likely. Several large scale studies reported a higher, but not always statistically significant, prevalence of symptoms of chronic cough, phlegm, and bronchitis in older farmers or those with a longer working history in pig farming[63,66,69]. Such associations could not be confirmed in another large study[64]. Wheeze was not positively associated with age or length of working history[64,69], whereas for asthma and shortness of breath positive associations have been reported[63,64]. It is likely that reported positive associations reflect associations between cumulative dose and at least symptoms of chronic bronchitis, since higher symptom prevalence rates were observed in pig farmers than in non-pig farming control groups of similar age.

Number of hours working per day or per year in pig farming were associated with chronic respiratory symptoms in Canadian and Swedish pig farmers[69,70], suggesting some sort of effect of currently received dose.

For base-line FEV_1, conflicting results were reported. Iversen et al.[65] estimated a decrease of 12 ml per year of pig farming in a group of 124 pig farmers, additional to the age effect. These two potentially highly correlated variables were included in the same model which can invalidate findings due to collinearity. In contrast, no clear differences in percentage of predicted FEV_1 and FVC existed between age categories of Canadian farmers[64]. In that study, there seemed to be an age trend for FEV_1/FVC and MMEF as percentage of predicted. However, this did not differ much from the trend observed in non-farming, rural controls, making a specific effect of pig farming less likely. Cormier et al.[66] failed to observe an association between base-line lung function and number of years in pig farming. In another study, an inverse association was present only in a subgroup with work-related symptoms[86], which is potentially a sensitive group. In two large Canadian studies statistically significant inverse associations between hours per day and base-line lung function were reported, but these were discordant with respect to the specific lung function parameters FVC and FEV_1[66,69]. Farm characteristics which are potentially related to exposure levels, such as flooring, feeding, and type of ventilation, were found to be related with FEV_1 and some flow-volume parameters[76].

Studies on NSBR report positive associations as well as absence of associations with length of working history[65,81,85,86]. Associations with number of hours per day in pig farming and number of raised pigs were absent[85]. These ambiguous results, next to those on the relative prevalence rate of NSBR as discussed before, makes it impossible to conclude whether pig farming induces NSBR.

Work-related symptoms were mostly not associated with age or number of years in pig farming, but some were with farm characteristics[65,76,99]. Inflammatory cells obtained by BAL or antibodies against swine antigens were not associated with number of years in pig farming[9].

Conclusion

It is striking that a large imbalance exists between the number of studies describing health effects and studies with quantification of occupational exposure for epidemiological purposes. The usefulness of the exposure data in all of these studies with chronic respiratory health effect as outcome variable was expected to be very limited due to unfavourable exposure variability and expected poor correlation between personal exposure and static measurements. As long as no validation study is carried out to support the underlying assumption that used exposure measures can be relevant for chronic respiratory health, it is doubtful whether reported exposure-response relationships reflect the true relationships. In most situations, however, nondifferential exposure misclassification will lead to underestimation of, or absence of, exposure-response relationships. If statistically significant relationships are found, the chance that in reality the true associations are stronger, is expected to be larger than that spurious relationships have been detected.

Combining literature on reported respiratory health effects, on associations with measures of exposure, and on human experimental studies the following pattern can be extracted. Chronic respiratory symptoms of cough, phlegm, and bronchitis are the most frequently reported chronic respiratory health effects in pig farmers. Most studies were primarily focused on finding and reporting these symptoms. Other chronic respiratory symptoms got less attention, thereby potentially underestimating the development of these symptoms in pig farmers. Despite relatively normal base-line values of FEV_1, FVC and FEV_1/FVC, effects of occupational exposure on these parameters are plausible because of reported associations with exposure surrogates. Effects of occupational exposure on lung function seem to be more clearly present in parameters such as MMEF and MEF_x, although the number of studies which incorporated these parameters in their lung function tests is limited. However, the significance of these abnormalities in chronic disease mechanisms is not clear. Despite the suggested health effects of working in pig farming, the etiological agent(s) has or have not been identified. Current knowledge obtained in observational as well as experimental studies suggests that endotoxin exposure, at concentrations found in pig farming, may be an important etiological factor in respiratory disease. Such a role for dust exposure in itself seems unlikely, and its use as marker for endotoxin exposure is not possible because of large variability in endotoxin content. Larsson et al.[9] showed that the difference in personal endotoxin exposure measured during different activities was a factor 4 larger than for personal dust exposure, and Attwood et al.[54] reported a variation in relative endotoxin content in dust (in ng/mg) sampled in farrowing and nursery buildings of a factor 90. Only very weak indications exist that ammonia can play a role in respiratory disease etiology. So far, disinfectants have not been suggested as etiological factors, but the literature on specific components indicates that they can not be excluded as causes of respiratory symptoms, most often of asthma-like symptoms.

Studies on a potential role of work-related allergy in chronic respiratory symptoms suggest that this can not explain the high prevalence rate of chronic symptoms. However, there are studies indicating that in individual farmers work-related allergy may be present. Reported prevalence rates of ODTS-like symptoms varied largely, but the presence of these symptoms due to pig farming is strengthened by exposure-response relationships. There is no consistency in the studies on NSBR in relation to pig farming. NSBR is considered to be a hallmark of asthma. Large effects on number of inflammatory cells in the respiratory tract were shown in human experimental studies. Contrarily, the cell counts of inflammatory cells in pig farmers were much closer to normal values. The differences between farmers and experimental subjects limits extrapolation of these results to pig farmers. Similarly, a strong effect on NSBR seems to be present in experimental subjects, but not in pig farmers. Therefore, pig farm dust does potentially have a large inflammatory effect, but at the population level the respiratory tract of pig farmers is most likely protected against large inflammatory reactions.

In summary, this overview suggests that poor characterization of exposure as discussed, limits insight into etiological factors. Better assessment of exposure is therefore required in the first place. Secondly, conclusions about health effects were generally based on cross sectional studies, in which selection processes could have biased the findings. In addition, the relative prevalence was often estimated by comparison with other occupational groups. Several of these groups were exposed to specific occupational agents as well. A better understanding of incidence of effects is needed, which can be obtained from prospective studies. Until now only one prospective study has been reported[100]. This study focused only on changes in lung function, but not on incidence of symptoms. This study has not been discussed, since poor description of applied statistical models, which could not be clarified by the authors (personal communication), hampered interpretation of the results. Thirdly, also characteristics of the study population and occupational setting may cause the diffuse view on health effects. Until now, the focus has been primarily on the potential role of endotoxins in relation to chronic airways obstruction and symptoms suggestive of chronic bronchitis. However, exposure is diverse and includes exposure to organic dusts with various potential harmful components, gases, and chemicals used such as cleaning agents. Different exposures may cause the existence of more than one disease entity which can potentially only to a limited extent be characterized by partly overlapping effects on e.g. base-line lung function. With a variety of different outcomes and different types of exposures, existing responses to one type of exposure are blurred. Combination of different research techniques to study health parameters may be used to define more clearly distinct disease entities.

Further, higher susceptibility of individuals by factors such as atopy and smoking have almost completely been ignored. For example, it has been suggested that much stronger effects of endotoxin exposure on acute lung function changes exist in asthmatics than in non-asthmatics[101]. The study designs and analyses used for description of exposure-response relationships which have been applied so far, may therefore have been to simple to detect them.

References

1. Donham KJ. Swine confinement buildings. In: Rylander R, Jacobs RR, eds. Organic dusts. Exposure, effects an prevention. Lewis Publishers, CRC Press, Boca Raton, 1994.
2. Douwes J, Heederik D. Health-based recommended occupational exposure limit for endotoxin (draft 1995, WHO).
3. Jacobs RR. Airborne endotoxins: an association with occupational lung disease. Appl Ind Hyg 1989;4:50-6.
4. Pernis B, Vigliani EC, Cavagna C, Finulli M. The role of bacterial endotoxins in occupational diseases caused by inhaling vegetable dusts. Br J Ind Med 1961;18:120-9.
5. Muittari AR, Rylander R, Salkinoja-Salonen M. Endotoxin and bathwater fever. Lancet 1980;89.
6. Rylander, R., Haglind, P. and Lundholm, M. Endotoxin in cotton dust and respiratory function decrement among cotton workers in an experimental cardroom. Am Rev Respir Dis 1985;131:209-15.
7. Castellan RM, Olenchock SA, Kinsley KB, Hankinson JL. Inhaled endotoxin and decreased spirometric values. N Eng J Med 1987;317: 605-10.
8. Schwartz, DA, Thorne PS, Jagielo PJ, White GE, Bleuer SA, Frees KL. Endotoxin responsiveness and grain dust-induced inflammation in the lower respiratory tract. Am J Physiol - Lung Cellular and Molecular Physiol 1994;11:L609-L617.
9. Larsson K, Eklund A, Malmberg P, Belin L. Alterations in bronchoalveolar lavage fluid but not in lung function and bronchial responsiveness in swine confinement workers. Chest 1992;101:767-74.

10. Larsson, KA, Eklund, AG, Hansson, LO, Isaksson, BN, Malmberg, PO. Swine dust causes intense airways inflammation in healthy subjects. Am J Respir Crit Care Med 1994;150:973-7.
11. Rylander R. Symptoms and mechanisms - Inflammation of the lung. Am J Ind Med 1994;25:19-23.
12. Smid T, Heederik D, Houba R, Quanjer PH. Dust- and endotoxin-related respiratory effects in the animal feed industry. Am Rev Respir Dis 1992;146:1471-9.
13. Heederik D, Brouwer R, Biersteker K, Boleij JSM. Relationship of airborne endotoxin and bacteria levels in pig farms with the lung function and respiratory symptoms of farmers. Int Arch Occup Environ Health 1991;62:595-601.
14. Donham K, Haglind P, Peterson Y, Rylander R, Belin L. Environmental and health studies of farm workers in Swedish swine confinement buildings. Br J Ind Med 1989;46:31-7.
15. Kennedy SM, Christiani DC, Eisen EA, Wegman DH, Greaves IA, Olenshock SA, Ye TT, Lu PL. Cotton dust and endotoxin exposure-response relationships in cotton textile workers. Am Rev Respir Dis 1987;135:194-200.
16. Clark S. Report on prevention and control. Am J Ind Med 1986;10: 267-73.
17. Palchak RB, Cohen R, Ainslie M, Hoerner CL. Airborne endotoxin associated with industrial-scale production of protein products in gram-negative bacteria. Am Ind Hyg Assoc J 1988;44:420-1.
18. Binding N, Witting U. Exposure to formaldehyde and glutardialdehyde in operating theatres. Int Arch Occup Environ Health 1990;62:233-8.
19. Leinster P, Baum JM, Baxter PJ. An assessment of exposure to glutaraldehyde in hospitals: typical exposure levels and recommended control measures. Br J Ind Med 1993;50:107-11.
20. Benson WG. Case Report. Exposure to glutaraldehyde. J Soc Occup Med 1984;34: 63-4.
21. Chan-Yeung M, McMurren T, Catonio-Begley C, Lam S. Occupational asthma in a technologist exposed to glutaraldehyde. J Allergy Clin Immunol 1993;91:974-8.
22. Calder IM, Wright LP, Grimstone D. Glutaraldehyde allergy in endoscopy units. Lancet 1992;339:433.[letter].
23. Waldron HA. Glutaraldehyde allergy in hospital workers. Lancet 1992;339:880 [letter].
24. Norbäck D. Skin and respiratory symptoms from exposure to alkaline glutaraldehyde in medical services. Scand J Work Environ Health 1988;14:366-71.
25. Bourne MS, Flindt MLH, Miles Walker J. Asthma due to industrial use of chloramine. Br Med J 1979;2:10-2.
26. Dijkman JH, Vooren PH, Kramps JA. Occupational asthma due to inhalation of chloramine-T. I. Clinical observations and inhalation-provocation studies. Int Arch Allergy Appl Immunol 1981;64:422-7.
27. Wass U, Belin L, Eriksson NE. Immunological specifity of chloramine-T-induced IgE antibodies in serum from a sensitized worker. Clin Exp Allergy 1989;19:463-71.
28. Blomqvist AM, Axelsson IGK, Danielsson D, Kiviloog J, Ulander A, Zetterström O. Atopic allergy to chloramine-T and the demonstration of specific IgE antibodies by the radioallergosorbent test. Int Arch Occup Environ Health 1991;63:363-5.
29. Bernstein JA, Stauder T, Bernstein DI, Bernstein IL. A combined respiratory and cutaneous hypersensitivity syndrome induced by work exposure to quaternary amines. J Allergy Clin Immunol 1994;94:257-9.
30. Burge PS, Richardson MN. Occupational asthma due to indirect exposure to lauryl dimethyl benzyl ammonium chloride used in a floor cleaner. Thorax 1994;49:842-3.

31. Burge PS, Harries MG, Lam WK, O'Brien IM, Patchett PA. Occupational asthma due to formaldehyde. Thorax 1985;40:255-60.
32. Nordman H, Keskinen H, Tuppurainen-M. Formaldehyde asthma--rare or overlooked? J Allergy Clin Immunol 1985;75:91-9.
33. Horvath EP, Anderson H, Pierce WE, Hanrahan L, Wendlick JD. Effects of formaldehyde on the mucous membranes and lungs. A study of an industrial population. JAMA 1988;259:701-7.
34. Malaka T, Kodama AM. Respiratory health of plywood workers occupationally exposed to formaldehyde. Arch Environ Health 1990;45:288-94.
35. Kramps JA, Toorenenbergen AW van, Vooren PH van, Dijkman JH. Occupational asthma due to inhalation of chloramine-T. Int Arch Allergy Appl Immunol 1981;64:428-38.
36. Bernstein RS, Stayner LT, Elliott LJ, Kimbrough R, Falk H, Blade L. Inhalation exposure to formaldehyde: an overview of its toxicology, epidemiology, monitoring, and control. Am Ind Hyg Assoc J 1984;45:778-85.
37. Wilhelmsson B, Holmstrom M. Possible mechanisms of formaldehyde-induced discomfort in the upper airways. Scand J Work Environ Health 1992;18:403-7.
38. Didier A, Cador D, Bongrand P, Futoss R, Fourneron P, Senft M, Philip-Joet F, Charpin D, Charpin J, Vervloet D. Role of the quaternary ammonium ion determinants in allergy to muscle relaxants. J Allergy Clin Immunol 1987;79:578-84.
39. Zejda JE, Barber E, Dosman JA, Olenshock SA, McDuffie HH, Rhodes C, Hurst T. Respiratory health status in swine producers relates to endotoxin exposure in the presence of low dust levels. J Occup Med 1994;36:49-56.
40. Heederik D, Zwieten R van, Brouwer R. Across-shift lung function changes among pig farmers. Am J Ind Med 1990;17:57-8.
41. Holness DL, O'Blenis EL, Sass-Kortsak A, Pilger C, Nethercott JR. Respiratory effects and dust exposures in hog confinement farming. Am J Ind Med 1987;11:571-80.
42. Coker DT. Bodily uptake rate differences between real life fluctuating concentrations and steady state laboratory conditions. XXI International Congress on Occupational Health, Dublin, Ireland 1994.
43. Spear RC, Selvin S, Francis M. The influence of averaging time on the distribution of exposures. Am Ind Hyg Assoc J 1986;47:365-8.
44. Douwes J, Versloot P, Hollander A, Heederik D, Doekes G. Influence of various dust sampling and extraction methods on the measurement of airborne endotoxin. Appl Environ Microbiol 1995;61:1763-9.
45. Clark S, Rylander R, Larsson L. Airborne bacteria, endotoxin and fungi in dust in poultry and swine confinement buildings. Am Ind Hyg Assoc J 1983;44:537-41.
46. Attwood P, Versloot P, Heederik D, Wit R de, Boleij JSM. Assessment of dust and endotoxin levels in the working environment of Dutch pig farmers: a preliminary study. Ann Occup Hyg 1986;30:201-8.
47. Haglind P, Rylander R. Occupational exposure and lung function measurements among workers in swine confinement buildings. J Occup Med 1987;29:904-7.
48. Crook B, Robertson JF, Travers Glass SA, Botheroyd EM, Lacey J, Topping MD. Airborne dust, ammonia, microorganisms, and antigens in pig confinement houses and the respiratory health of exposed farm workers. Am Ind Hyg Assoc 1991;52:271-9.
49. Jones W, Morring K, Morey P, Sorenson W. Evaluation of the Andersen viable impactor for single stage sampling. Am Ind Hyg Assoc J 1985;46:294-8.
50. Cormier Y, Tremblay G, Meriaux A, Brochu G, Lavoie J. Airborne microbial contents in two types of swine confinement buildings in Quebec. Am Ind Hyg Assoc J 1990;51:304-9.

51. Donham KJ, Popendorf W, Palmgren U, Larsson L. Characterization of dusts collected from swine confinement buildings Am J Ind Med 1986;10:294-7.
52. Virtanen T, Kalliokoski P, Vilhunen P, Taivainen A, Mäntyjärvi R. Concentrations of specific dusts in swineries and the humoral response of swinery workers. Allergy 1990;45:1-9.
53. Vinzents P, Nielsen BH. Variations in exposures to dust an endotoxin in Danish piggeries. Am Ind Hyg Assoc J 1992;53:237-41.
54. Attwood P, Brouwer R, Ruigewaard P, Versloot P, Wit R de, Heederik D, Boleij JSM. A study of the relationship between airborne contaminants and environmental factors in Dutch swine confinement buildings. Am Ind Hyg Assoc J 1987;48:745-51.
55. Liu K, Stamler J, Dyer A, McKeever J, McKeever P. Statistical methods to assess and minimize the role of intra-individual variability in obscuring the relationship between dietary lipids and serum cholesterol. J Chron Dis 1978;31:399-418.
56. Cinkotai FF, Gibbs ACC, Sharpe TC. A comparison of exposure to airborne dust in cotton processing plants estimated from personal and workzone samples. Ann Occup Hyg 1984;28:347-52.
57. Smid T, Heederik D, Mensink G, Houba R, Boleij JSM. Exposure to dust, endotoxins, and fungi in the animal feed industry. Am Ind Hyg Ass J 1992;53:362-8.
58. Zeitler-Feicht MH, Praml GJ, Riedel H. Die zeitliche Variation der Feinstaubbelastung der Luft im Mastschweinestall. Tierhygienische und arbeitsmedizinische Aspekte. (in German) 'The temporal variation of airborne fine dust concentration in pig fattening houses. Hygienic and medical aspects'. Berl Münch Tierärztl Wschr 1991;104:84-9.
59. Barber EM, Dawson JR, Battams VA, Nicol RAC. Spatial variability of airborne and settles dust in a piggery. J Agric Engng Res 1991;50:107-27.
60. American Thoracic Society. Epidemiology Standardization Project. Am Rev Respir Dis 1978;118 (suppl):7-53.
61. BMRC. Standardized questionnaires on respiratory symptoms. Br Med J 1960;II -1665.
62. Post W, Burdorf L, Heederik D. Chronische Aspecifieke Respiratoire Aandoeningen als bedrijfsgezondheidskundig probleem. T Soc Gezondheidsz 1993;71:103-11.
63. Iversen M, Dahl R, Korsgaard J, Hallas T, Jensen EJ. Respiratory symptoms in Danish farmers: an epidemiological study of risk factors. Thorax 1988;43:872-7.
64. Dosman JA, Graham BL, Hall D, Pahwa P, McDuffie HH, Lucewicz M, To T. Respiratory symptoms and alterations in pulmonary function tests in swine producers in Saskatchewan: Results of a survey of farmers. J Occup Med 1988;30:715-20.
65. Iversen M, Pedersen B. Relation between respiratory symptoms, type of farming, and 5ung function disorders in farmers. Thorax 1990;45:919-23.
66. Cormier Y, Boulet LP, Bedard G, Tremblay G. Respiratory health of workers exposed to swine confinement buildings only or to both swine confinement buildings and dairy barns. Scan J Work Environ Health 1991;17:269-75.
67. Donham KJ, Merchant JA, Lassise D, Popendorf WJ, Burmeister LF. Preventing respiratory disease in swine confinement workers: Intervention through applied epidemiology, education, and consultation. Am J Ind Med 1990;18:241-61.
68. Zuskin E, Zagar Z, Schachter E, Mustajbegovic J, Kern J. Respiratory symptoms and ventilatory capacity in swine confinement workers. Br J Ind Med 1992:49:435-40.
69. Zejda JE, Hurst TS, Rhodes CS, Barber EM, McDuffie HH, Dosman JA. Respiratory health of swine producers. Focus on young workers. Chest 1993;103:702-9.
70. Wilhelmsson J, Bryngelsson IL. Ohlson CG. Respiratory symptoms among swedish swine producers. Am J Ind Med 1989;15:311-8.

71. Choudat D, Goehen M, Korobaeff M, Boulet A, Dewitte JD, Martin MH. Respiratory symptoms and bronchial reactivity among pig and dairy farmers. Scan J Work Environ Health 1994;20:48-54.
72. Zuskin E, Kanceljak B, Schachter EN, Mustajbegovic J, Goswami S, Maayani S, Marom Z, Rienzi N. Immunological and respiratory findings in swine farmers. Environ Res 1991;56:120-30.
73. doPico GA. Report on diseases. Am J Ind Med 1986;10:261-5.
74. May JJ, Marvel LH, Pratt DS. Organic dust toxic syndrome: A follow-up study. Am J Ind Med 1990;17:111-3.
75. Brouwer R, Biersteker K, Bongers P, Remijn B, Houthuijs D. Respiratory symptoms, lung function, and IgG_4 levels against pig antigens in a sample of Dutch pig farmers. Am J Ind Med 1986;10:283-5.
76. Bongers P, Houthuijs D, Remijn B, Brouwer R, Biersteker K. Lung function and respiratory symptoms in pig farmers. Br J Ind Med 1987;44:819-23.
77. Zhou C, Hurst TS, Cockcroft DW, Dosman JA. Increased airways responsiveness in swine farmers. Chest 1991;99:941-4.
78. Ebi-Kryston KL. Respiratory symptoms and pulmonary function as predictors of 10-year mortality from respiratory disease, cardiovascular disease, and all causes in the whitehall study. J Clin Epidemiol 1988;41:251-60.
79. Morgan WKC. Epidemiology and occupational lung disease. In: WKC Morgan & A Seaton: Occupational lung diseases. Saunders, Philadelphia, 1984.
80. American Thoracic Society: Lung function testing: selection of reference values and interpretative strategies. Am Rev Respir Dis 1991;144:1202-18.
81. Rylander R, Essle N, Donham KJ. Bronchial hyperreactivity among pig and dairy farmers. Am J Ind Med 1990;17:66-9.
82. Donham KJ, Zavala DC, Merchant J. Acute effects of the work environment on pulmonary functions of swine confinement workers. Am J Ind Med 1984;5:367-75.
83. Ghio AJ, Castellan RM, Kinsley KB, Hankinson JL. Changes in forced expiratory volume in one second and peak expiratory flow rate across a work shift among unexposed blue collar workers. Am Rev Respir Dis 1991;143:1231-4.
84. Scheefeldt M, Wilfert A, Lehnigk B, Wosnitzka H. Bronchiale Hyperreaktivitat bei Beschaftigten in der Schweine- und Rinderproduktion. Z Erkrank Atm Org 1990;174:131-6.
85. Bessette L, Boulet LP, Tremblay G, Cormier Y. Bronchial responsiveness to methacholine in swine confinement building workers. Arch Environ Health 1993;48:73-7.
86. Iversen M, Dahl R, Jensen EJ, Korsgaard J, Hallas T. Lung function and bronchial reactivity in farmers. Thorax 1989;44:645-9.
87. Pedersen B, Iversen M, Dahl R. Bronchoalveolar lavage of pig farmers. Am J Ind Med 1990;17:118-9.
88. Schwartz DA, Landas SK, Lassise DL, Burmeister LF, Hunninghake GW, Merchant JA. Airway injury in swine confinement workers. Ann Internal Med 1992;116:630-5.
89. Harries MG, Cromwell O. Occupational asthma caused by allergy to pigs' urine. Br Med J 1982;284:867.
90. Brennan NJ. Pig Butcher's Asthma - case report and review of the literature. Irish Med J 1985;78:321-2.
91. Brouwer R, Heederik D, Swieten P van. IgG_4 antibodies against pig-derived antigens. Am J Ind Med 1990;17:96-8.

92. Johansson E, Borga A, Johansson SGO, Hage-Hamsten M van. Immunoblot multi-allergen inhibition studies of allergenic cross-reactivity of the dust mites *Lepidoglyphus destructor* and *Dermatophagoides pteronyssinus.* J Immunol 1991;147:972-9.
93. Matson SC, Swanson MC, Reed CE, Yunginger JW. IgE and IgG-immune mechanisms do not mediate occupation-related respiratory or systemic symptoms in hog farmers. J Allergy Clin Immunol 1983;72:299-304.
94. Iversen M, Dahl R. Specific antigens in dust from swine confinement buildings. Am J Ind Med 1994;25:49-51.
95. Katila ML, Maentyjaervi RA, Ojanen TH. Sensitisation against environmental antigens and respiratory symptoms in swine workers. Br J Ind Med 1981;38:334-8.
96. Aalberse RC, Milligen F van, Tan KY, Stapel SO. Allergen-specific IgG_4 in atopic disease. Allergy 1993;48:559-69.
97. Larsson K, Malmberg P, Eklund A. Acute exposure to swine dust causes airway inflammation and bronchial hyperresponsiveness. Am J Ind Med 1994;25:57-8.
98. Malmberg P, Larsson K. Acute exposure to swine dust causes bronchial hyperresponsiveness in healthy subjects. Eur Respir J 1993;6:400-4.
99. Husman K, Terho EO, Notkola V. Organic dust toxic syndrome among Finnish farmers. Am J Ind Med 1990;17:79-80.
100. Schwartz DA, Donham KJ, Olenchock SA, Popendorf WJ, Vanfossen DS, Burmeister LF, Merchant JA. Determinants of longitudinal changes in spirometric function among swine confinement operators and farmers. Am J Respir Crit Care Med 151;1995:47-53.
101. Michel O, Duchateau J, Sergysels R. Effects of inhaled endotoxin on bronchial reactivity in asthmatic and normal subjects. J Appl Physiol 1989;66:1059-64.

3. Modelling long-term average exposure - an application in occupational exposure-response analysis

Liesbeth Preller, Hans Kromhout, Dick Heederik, Martin J. M. Tielen

Abstract

Estimates of long-term average exposure to occupational hazards are often imprecise because intraindividual variability in exposure can be large, and exposure is usually based on one or few measurements. This potentially results in bias of exposure-response relationships. We evaluated whether a more valid measure of exposure could be obtained by modelling exposure and consequently increasing the number of days with exposure estimates, using simple measurable exposure surrogates. In a group of 198 Dutch pig farmers, exposure to endotoxins was measured during one working day in summer and one day in winter. Farmers recorded activity patterns during one week in both seasons, and farm characteristics were evaluated. Relationships between farm characteristics and activities, and log-transformed measured exposure levels, were quantified in a multiple regression analysis. Exposure was estimated for 14 days with known activity patterns. The ratio of intraindividual and interindividual variance in log-transformed measured exposure was 4.7. Given this ratio, the true regression coefficient of lung function on exposure would potentially be attenuated by 70%. The variance ratio for predicted exposures was only 1.2, and potential attenuation by variation in exposure estimates was decreased to 8%. A relationship between lung function and measured exposure was absent. Modelled long-term average exposure was inversely related to base-line lung function and reached statistical significance for asymptomatic farmers. Results suggest that the presented strategy offers a possibility to minimize measurement effort in occupational epidemiological studies, without apparent loss of statistical power.

Accepted for publication in the Scandinavian Journal of Work, Environment and Health

Introduction

Validity and precision of the measures of exposure used in statistical analyses are key issues in studying exposure-response relationships. On a conceptual level, validity refers to the biological relevance of the measure of exposure. More practically, validity is dependent on biases occurring in the measurement process. Precision refers to the random error in the exposure estimate. A regression coefficient of a continuous outcome variable as base-line lung function on exposure will generally be underestimated when exposure is measured with nondifferential, random error[1-3]. This hampers detection of weak associations that are more often studied in occupational epidemiology. Because of practical reasons, it is common practice in studies on chronic effects to estimate long-term average occupational exposure by a limited number of measurements. The resulting measure of exposure is therefore generally subject to considerable measurement error as a result of intraindividual exposure variability. An example in which this lead to underestimation of exposure-response relationships, or so called attenuation, was demonstrated empirically in a population of 1,105 coal miners[4]. On average 34 dust exposure measurements were available per worker. With three randomly chosen measurements per individual, the regression coefficient of base-line lung function on exposure was only 40% of the regression coefficient when using all available measurements. However, three measurements per individual still involved more than 3,000 exposure measurements. This illustrates that reduction in attenuation by increasing the number of measurements per individual might require substantial investments in field work.

The magnitude of potential bias in the regression coefficient depends on the ratio of the intraindividual and interindividual components of variance and the number of repeated measurements, according to the formula[2]:

$$b = \beta\ (1 + \lambda\ /\ n)^{-1} \qquad (1)$$

where:

b = observed value of the empirical regression coefficient of Y on *ln*(X), while X measured with error
β = true value of the regression coefficient of Y on *ln*(X)
λ = ${}_wS_y^2\ /\ {}_bS_y^2$
${}_wS_y^2$ = estimate of intraindividual variance in *ln*(exposure)
${}_bS_y^2$ = estimate of interindividual variance in *ln*(exposure)
n = number of repeated measurements per individual

Application of formula (1) assumes absence of autocorrelation between repeated measurements. Although autocorrelation of repeated 8-hr TWA exposure measurements in occupational settings is usually low and decreases with time between measurements[5], repeated measurements should preferably not be taken on consecutive days in order to estimate the day-to-day variability correctly. Formula (1) refers to stationary processes. If this is violated, e.g. due to changes over time in tasks involved etcetera, bias can not be predicted. With a single or few exposure measurements per individual in stationary processes, a large ratio λ will result in considerable underestimation of the exposure-response relationship. Underestimation of a relationship can be reduced by either increasing the interindividual variability in exposure, or decreasing the influence of intraindividual variability by increasing the number of measurements per individual[2,6]. The interindividual variance, or contrast in exposure between individuals, can be optimized in the design stage of the study by choosing a population with large differences in exposure between subjects. The interindividual variance can also be increased by introducing an external group with extremely low or high exposure. Reducing attenuation by only increasing the number of measurements per individual is a method which will often not be feasible, since costs and logistics of large field work campaigns are restrictive factors. Correction for attenuation of the regression coefficient can be applied in specific cases if λ is known. It does, however, not increase the power of the study and therefore can only be useful in situations where exposure-response relationships are expected to be strong.

Empirical modelling of exposure can be used to predict exposure in situations where limited numbers of exposure measurements are available, using simple measurable exposure surrogates in the models[7,8]. So far, some researchers have reported the use of statistical models to predict occupational exposures. Several studies were undertaken for industrial hygiene purposes[9-12]. In some studies historic exposure was modelled by job, department and production process etcetera for application in epidemiological exposure-response analyses[8,13-18]. Application of this technique to obtain an estimate of individual, current long-term average exposure, has to our knowledge not been reported.

Working conditions of Dutch pig farmers are characterized by large day-to-day variability in activities and in time spent in different locations with distinctive exposures. Long-term average exposure could not be assessed by a large number of actual exposure measurements for each individual, since field work was logistically complex and costly. In this study, statistical models were used to estimate long-term average exposure to endotoxins of pig farmers. Endotoxins are regarded as a major respiratory health hazard of pig farmers[19]. First, farm characteristics and daily activities were related to measured exposure to endotoxins. Factors affecting exposure were identified and their association with exposure was quantified. Subsequently, information on activity patterns collected during one week in summer and one week in winter and information on farm characteristics was used to estimate exposure for each day of these two weeks. The average of the estimated 14 daily exposures was used as a measure of long-term average exposure. The number of repeated observations for each individual increased from two for measured exposure to 14 for estimated exposure, covering most of the variability in time activity patterns. The different measures of exposure were applied in exposure-response relationship analyses.

Materials and methods

Population and health data

The study population comprised 198 male pig-farm owners in the southern part of the Netherlands, who worked at least five hours per day in pig farming. 98 farmers had one or more respiratory symptoms (chronic cough, chronic phlegm, shortness of breath, ever wheezing, frequent wheezing, chest tightness), and 100 farmers did not have these symptoms. The respiratory symptoms were investigated with a shortened version of a questionnaire on respiratory symptoms developed by the British Medical Research Council[20,21]. Lung function was determined during a medical examination. Spirometry was performed according to methods and procedures of the ECCS as described elsewhere[22,23]. Lung function tests were taken between 8.30 a.m. and 15.15 p.m..

Measurement strategy

Exposure to inhalable dust containing endotoxins was determined by means of personal sampling. The procedure for measuring dust levels is described in detail elsewhere[24]. Endotoxins were analyzed in the dust sample with a kinetic *Limulus* Amoebocyte Lysate test[25].

Exposure was assessed for each participant during one work shift in summer (June 4 until July 3 1991), and during one work shift in winter (January 27 until February 27 1992). Average measurement time was 8.3 hours (standard deviation 0.6, range 5.2-10.4 hours). Measurements were performed from

Monday through Thursday with the day of the week randomly chosen. Meteorological data were obtained from a monitoring station in the South of the Netherlands.

In summer and winter farmers were requested to complete a diary on time spent on different activities during the day of exposure measurement, and the following six days. Time (in quarters of an hour) spent on 21 pre-selected activities in pig farming had to be recorded. Thus for each participant a two week record of daily activity patterns was obtained.

In a subgroup of six farmers, exposure measurements were performed nearly monthly during a one year period. Procedures were similar as described for measurements taken in summer and winter.

Pig farms in the study are characterized by presence of a large number of compartments in several buildings. Farm characteristics are generally very heterogeneous within one farm, and depend on type of compartment and period of construction of the buildings. The 198 farms were visited to record farm characteristics of all compartments present by means of a walk-through survey. Data were recorded on: number of animals, feeding methods, heating and ventilation, type of floor and bedding material, and degree of contamination. The data set contained 95 distinct variables.

Computational and statistical methods

Data on exposure levels on two days, activity patterns on 14 days, farm characteristics, and outdoor temperature were used to estimate long-term average exposure to endotoxins. First, associations between factors affecting exposure were quantified. First, variation in log-transformed personal time-weighted average exposure (*ln*(TWA)) was explained by time spent on activities during sampling, farm characteristics (either as an average percentage of time dealing with the characteristic, or as a dummy variable (0/1)), and outdoor temperature in classical stepwise regression analysis. All of the 95 characteristics and 21 activities which showed some association with endotoxin exposure in univariate regression analysis, were included in the stepwise regression analysis. Potential confounding factors were evaluated. Analyses were based on log-transformed exposure levels to standardize variance and to obtain normally distributed residuals. All measurements were used as independent observations in the analysis, since the correlation between repeated measurements taken in summer and winter was low (0.19). Each independent variable had to meet a significance level of at least 0.50 for entry in the model and was kept in the model at a significance level below 0.10. Model adequacy was tested with standard regression techniques such as residual plots and outlier detection.

Regression equations derived were subsequently used to estimate the *ln*(exposure) during sampling (two days) and *ln*(exposure) for all 12 to 14 days for which activity patterns were known. Estimates were based on models using activities as recorded by the individual farmers in the diaries and farm characteristics, derived from walk-through surveys. For modelling exposure, an outdoor temperature of 17°C in summer and 5°C in winter was used, which were the average outdoor temperatures in the field work periods. The average of the *ln*(exposure)'s was taken as measure of long-term average exposure. Intraindividual and interindividual variance components were estimated by applying a one-way random effect ANOVA model in a similar way as described by Kromhout et al.[26]. Variance components were estimated for measured exposure (two measurements), predicted exposure during sampling (two estimates), and predicted long-term average exposure ($\geq$ 12 estimates), all on the log scale. An exposure-response relationship was estimated for predicted exposure during sampling for demonstration of the effect of an increased number of exposure estimates per individual, when using information on surrogate variables. Intra- and interindividual variability are expressed as ${}_{w}\hat{R}_{0.95}$ and ${}_{b}\hat{R}_{0.95}$, the ratios between the 97.5th and 2.5th percentiles of the log-normal within and between worker exposure distributions and are computed as *exp*[3.92 * variance component$^{0.5}$][27]. Attenuation of exposure-response relationships due to intraindividual variability relative to interindividual variability was estimated and represented as attenuation ratio. The attenuation ratio is the ratio between the empirically estimated regression coefficient (b) and the true regression coefficient (ß), computed as $(1 + \lambda / n)^{-1}$, in which λ is the ratio of the intra- and interindividual variance components, and n is the number of repeated measurements per individual[2].

The relationship between base-line lung function (Forced Expiratory Volume in one second (FEV$_1$)) and measured and modelled endotoxin exposure was evaluated by means of multiple linear regression analysis, adjusting for smoking habits (pack years), standing height and age. The following measures of exposure were used in the analyses: average measured exposure, average predicted exposure during sampling, and predicted long-term average exposure. All exposure measures were based on log-transformed values since also exposure variability was assessed for log-transformed exposure measure. Statistical tests were one-sided.

Statistical analyses were performed with SAS/PC version 6.04 using PROC REG and PROC NESTED.

Results

Exposure levels

350 endotoxin measurements were available, after excluding 46 samples (12%) in which failure of equipment during sampling or failure in endotoxin analysis occurred.

Table 3.1 gives a summary of the exposure levels found. The mean exposure in winter was significantly higher than in summer (paired t-test, $p < 0.05$).

Table 3.1 Personal time-weighted average exposure concentration to endotoxins (ng/m^3) of 198 Dutch pig farmers.

	No. of measurements	arithmetic mean	geometric mean	geometric standard deviation	range
summer 1991	182	111	78	2.4	5.6 - 825
winter 1992	168	150	109	2.3	10.6 - 1503
all*	350	129	92	2.4	5.6 - 1503

* 154 farmers with 2 measurements

Modelling exposure

Outdoor temperature, 12 farm characteristics and eight activities in pig farming explained 37% (adjusted R^2 = 33%) of the variation in *ln*(TWA) exposure to endotoxins (Table 3.2). Activities and farm characteristics explained an equal amount of the variation. Activities in the model were in general (almost) daily activities (feeding, controlling, re-penning), and activities with close contact to very active animals which are usually done once every few days only (iron injection, castrating, teeth cutting, ear tagging). Flooring (convex floor, fully slatted floor, fully slatted floor with piglet mat, synthetic grid, combined concrete and metal grid, floor heating, floor heating in combination with delta heating tubes), and to a lesser extent feeding methods (manual dosage dry feeding, pig starter, automated dry feeding in trough) were the major groups of farm characteristics in the model.

Table 3.2 Multiple linear regression analysis of farm characteristics, activities and outdoor temperature significantly related to log-transformed personal endotoxin concentrations of 198 pig farmers (348 observations).

	regression coefficient	standard error	p
intercept	+ 4.44	0.18	
outdoor temperature*	- 0.35	0.05	< 0.01
FARM CHARACTERISTICS			
feeding			
manual dosage dry feeding †	- 0.37	0.12	< 0.01
pig starter †	+ 0.35	0.16	0.03
automated dry feeding in trough ‡	- 0.06	0.03	0.02
flooring			
convex floor †	- 0.22	0.09	0.01
fully slatted floor ‡	+ 0.08	0.02	< 0.01
fully slatted floor with piglet mat ‡	- 0.08	0.03	< 0.01
synthetic grid ‡	- 0.13	0.06	0.03
concrete and metal grid ‡	- 0.15	0.05	< 0.01
floor heating ‡	+ 0.07	0.02	< 0.01
floor heating in combination with delta heating tubes ‡	+ 0.10	0.05	0.03
other			
overall very dusty ‡	+ 0.12	0.05	0.01
air exhaust via pit †	- 0.33	0.10	< 0.01
ACTIVITIES §			
feeding	+ 0.08	0.02	< 0.01
controlling	+ 0.07	0.03	0.02
re-penning	+ 0.07	0.04	0.05
floor sweeping	+ 0.17	0.06	< 0.01
iron injection	+ 0.17	0.08	0.03
castrating	+ 0.14	0.06	0.03
teeth cutting	+ 0.51	0.18	< 0.01
ear tagging	+ 0.43	0.08	< 0.01

* per 10°C

† dummy variable: characteristic present vs. not present

‡ per 10% time spending with characteristic

§ per half hour

164 farmers had recorded time activity patterns during at least two periods of six days. For them, long-term average exposure could be estimated, using predictive models and data on activity patterns during 12 to 14 days. Further analyses were undertaken for the group of 125 farmers with complete data on measured as well as modelled endotoxin exposure. In this group, Pearson correlation between individual average, predicted long-term average exposure on the one hand and average measured exposure and predicted exposure during sampling on the other hand was 0.53 and 0.81, respectively.

Table 3.3 gives an overview of the different exposure indices, and results of the analyses of variance. The interindividual variance in endotoxin exposure was small for measured exposure, with a ratio of 4.1 between the 97.5th and 2.5th percentiles of the exposure distribution (${}_{b}\hat{R}_{0.95}$). The intraindividual variance was considerably larger than the interindividual variance: 82% of the total variability in exposure appeared to be day-to-day variability. The large ratio of intraindividual and interindividual variance would result in largely underestimated exposure-response relationships. With two measurements per individual available, the empirical regression coefficient of base-line lung function on exposure is expected to be only 30% of its true value.

Table 3.3 Measured and modelled exposure to endotoxins (ng/m^3) of 125 pig farmers, with variance components and estimated attenuation of regression coefficients in exposure-response relationships.

	No. of observations	AM*	geometric mean	var_t †	var_w †	${}_{w}\hat{R}_{0.95}$ ‡	var_b †	${}_{b}\hat{R}_{0.95}$ ‡	λ	attenuation ratio
measured	250	142	98	0.729	0.601	20.9	0.128	4.1	4.7	0.30
predicted during sampling	250	109	97	0.232	0.154	4.7	0.078	3.0	2.0	0.50
predicted long-term average	1739	122	106	0.288	0.159	4.8	0.129	4.1	1.2	0.92

* maximum likelihood arithmetic mean

† estimated variance components of the total (var_t), intraindividual (var_w) and interindividual (var_b) exposure distribution

‡ ratio of the 97.5th and 2.5th percentiles of the intraindividual (${}_{w}\hat{R}_{0.95}$) and interindividual (${}_{b}\hat{R}_{0.95}$) exposure distribution

As a consequence of modelling with constant variables for farm characteristics, the intraindividual variance for modelled exposure during sampling was much smaller than for measured exposure. The interindividual variance was slightly smaller than for measured exposure. Predicted long-term average exposure yielded a similar intraindividual variance as for predicted exposure during sampling, but the interindividual variance was increased. This resulted in a substantial reduction in the variance ratio λ from 2.0 for modelled exposure during sampling, to 1.2 for modelled exposure during 14 days. Potential attenuation of the regression coefficient of lung function on modelled endotoxin exposure, was reduced by this decrease in λ, and was further reduced by using all data on activity patterns during 12 to 14 days from 50% to less than 10%.

Lung function and exposure

Four people with incomplete data on smoking habits were excluded from the analysis, leaving 121 respondents with complete data. Associations between exposure to endotoxins and base-line FEV_1 are presented in Table 3.4. Results are given for the entire population with complete data, for ever smokers, and for farmers without chronic respiratory symptoms.

Table 3.4 Relationship between base-line FEV_1 (L) and log-transformed average measured and modelled exposure to endotoxins for all pig farmers (n=121), ever smokers (n=71), and asymptomatic farmers (n=62). Adjusted for standing height, age, and smoking habits (pack years).

	all			ever smokers			asymptomatic farmers*		
	b	se (b)	p-value †	b	se (b)	p-value †	b	se (b)	p-value †
measured	-0.03	0.09	0.40	-0.04	0.15	0.39	0.05	0.12	0.34
predicted during sampling	-0.12	0.16	0.22	-0.27	0.22	0.11	-0.18	0.21	0.20
predicted long-term average	-0.21	0.16	0.10	-0.28	0.22	0.10	-0.41	0.21	0.03

* no chronic respiratory symptoms (chronic cough, chronic phlegm, shortness of breath, ever wheezing, frequent wheezing, chest tightness)

† tested one-sided

There appeared to be a considerable decrease in base-line lung function with increase in predicted long-term average exposure. P-values clearly reduced when regression models with predicted (long-term) average exposure are being compared to regression models with measured exposure. Within the total population, reduction in FEV_1 was 210 ml when exposure to endotoxins increased with a factor 2.72. Larger reductions were seen among ever smokers and asymptomatic farmers. The estimated reduction of 410 ml in FEV_1 in asymptomatic farmers was statistically significant ($p < 0.05$, tested one sided). Other presented associations between modelled long-term average exposure were borderline statistically significant ($0.05 \leq p < 0.10$). Among never smokers and symptomatic farmers (not shown), and in all other cases using predicted exposure during sampling or measured exposure, no exposure-response relationship was observed.

Discussion

Exposure to endotoxins is generally regarded as one of the major respiratory health hazards for pig farmers[19]. Our study clearly shows that, despite the considerable measurement effort compared to other studies, it is difficult to detect associations between endotoxin exposure and chronic health effects. For example, in the only previous study among pig farmers, applying personal exposure monitoring to evaluate associations between chronic respiratory health effects and exposure to dust, exposure was based on a single exposure measurement[28]. In other studies endotoxin exposure was assessed by static sampling during one day among 168 farmers[23] or during two days among 46 farmers[29]. The large ratio between intraindividual and interindividual variance in exposure in our study (4.7) implied that the true regression coefficient of lung function on average measured endotoxin exposure was expected to be attenuated by 70%. With the observed value of λ, 42 endotoxin exposure measurements would be required to obtain a 10% attenuated regression coefficient[1,2], which need not to be uncommon[4].

The large value of λ was not caused by an exceptionally large intraindividual variability in exposure. The intraindividual variability compares relatively well with that reported for 81 groups of industrial workers exposed to total particulate matter[26]. The large value of λ can mainly be attributed to a small interindividual variability in exposure. The ratio between the 97.5th and 2.5th percentiles of the exposure distribution (${}_b\hat{R}_{0.95}$) was 4.1. Kromhout et al.[26] reported that in 80 out of 165 industry groups which were based on job title and factory, this ratio exceeded 4. For comparison, in a population of 120 workers in eight different job categories in the Dutch animal feed industry, this ratio was 82 for dust and 234 for endotoxin exposure[6]. In that study λ was about 0.9 for dust and endotoxin exposure, and 30% attenuation would be expected with only two exposure measurements per individual.

Modelled exposure during sampling and modelled long-term average exposure showed clearly less intraindividual variance than measured exposure. The ratio of the 97.5th and 2.5th percentiles of the distribution of individual mean exposure (${}_w\hat{R}_{0.95}$), was about a factor 4.5 smaller for modelled long-term average endotoxin exposure and modelled exposure during sampling than for measured exposure. Intraindividual variability in modelled exposure reflects primarily variation due to performance of activities present in the models. Day-to-day variability due to time spent on locations with different characteristics was not taken into account. This explains, together with unmodelled variables, the reduction in intraindividual variance. Interindividual variance of modelled exposure was only slightly affected compared to measured exposure. Clearly, the intraindividual variance in modelled exposure underestimates the true intraindividual variance in exposure, and λ for modelled exposure is no precise estimate of λ for actual exposure.

By modelling, the effect of time spent in locations with different exposures was reduced to zero. However, the variability in modelled exposure due to activities (and season) would still result in considerable attenuation, about a factor two, of exposure-response relationships when applying modelled exposure during the two days of sampling. Long-term average exposure was therefore estimated using predicted exposure levels for 14 days, covering most activities performed by the pig farmers. For this approach, attenuation of exposure-response relationships due to intraindividual relative to interindividual variance was estimated to be 8%. The reduction in attenuation, compared to modelled exposure during sampling, is partially caused by a smaller variance ratio for estimated long-term average exposure which was most likely caused by taking into account working in pig farming during the weekend and before and after the exposure measurements. The largest decrease, however, is most likely a consequence of an increase in the number of repeated exposure estimates per individual, which increased the chance that rarely occurring activities are included. This clearly shows the beneficial use of estimating exposure in situations in which no actual exposure measurements can be performed, and which differ in working conditions that presumably affect exposure levels.

In theory it is possible that by modelling, other types of exposure have been modelled which may be causally related to lung function, but this seems unlikely to be the case. Particularly farm characteristics of flooring were found to be related to exposure levels, which are most likely causally related to endotoxin concentrations. Manure is an important source of gram-negative bacteria, which in turn contain endotoxins. The amount of manure staying on the floor as well as the possibility of exchange of air between the pit beneath the floor and the space above, depends on the type of flooring. In addition, activities which explained variation in dust exposure, were similar to those explaining variation in endotoxin exposure. Modelled dust exposure was, however, not related to any health outcome (not shown).

A large reduction in FEV_1 of several hundred millilitres was expected over the range of endotoxin exposure levels when modelled long-term average exposure was applied. The stronger associations between modelled long-term average exposure and response sharply contrast with the observed associations when using average measured exposure or predicted exposure during sampling. Among ever smokers larger associations between lung function and long-term average exposure were observed, suggesting an interaction effect between exposure and smoking as found in some other studies[30,31]. In addition, relationships between lung function and long-term average exposure estimated on current working conditions seems to correlate better within the group of asymptomatic farmers than the group of symptomatic farmers. The estimated effect of endotoxin exposure on lung function was larger for all reported associations and p-values were all equal to or below 0.10. Standard errors of regression coefficients were larger with modelled exposure than with measured exposure, most likely due to error in modelled exposure (R^2 was 37%). These results demonstrate that an exposure-response relationship can be detected by applying exposure modelling techniques in combination with a limited number of exposure measurements per individual, even when the population is homogeneously exposed and day-to-day variability in exposure is relatively large.

In general, several epidemiological concepts determine the deviation of the estimated exposure-response relationship from its true value, which may be either attenuation, inflation or change of direction. These concepts include misclassification of disease, measurement error being differential, selection bias, residual confounding, and the extent to which the applied exposure measure deviates from the etiological relevant type of exposure. Focusing on the latter, precision as well as validity aspects determine the strength of the correlation between applied and relevant exposure measure. The main objective of our study was to obtain a more valid measure of long-term average exposure for epidemiological purposes by modelling. This could not be achieved by increasing the number of actual exposure measurements for each individual to a sufficient number, since field work was logistically complex and costly. The actual effect of modelling, with the effect of time spent in locations with different exposure reduced to zero, can not be assessed, but the effect by increasing the number of days with different working conditions to estimate long-term average exposure seems evident. No positive evidence can directly be derived from the data, which measure of exposure correlates best with the biologically relevant exposure, implying type of agent and exposure measure, e.g. peak exposure, long-term average exposure, or cumulative exposure, since the biologically relevant exposure is not known. In our study long-term average exposure was regarded to be the biologically relevant measure of exposure. A study performed among Dutch animal feed workers, support this idea[32]. In that study similar procedures and techniques were applied for lung function testing and exposure measurements. The average exposure level among animal feed workers was 67 ng/m^3 (AM) in the highest exposure group, which was a factor two lower than the average exposure level of 129 ng/m^3 among pig farmers. A statistically significant association of 122 ml for FEV$_1$ with an increase in endotoxin exposure of 24.8 ng/m^3 was observed in this population, which comprised 80% ever smokers. These associations compare very well with those between estimated long-term average exposure and lung function observed in our study. The difference of 97 ng/m^3 between 10 and 90 percentile of the exposure distribution is almost a factor 2.72, and corresponds with a reduction in lung function of 200 to 400 ml. In an earlier study among cotton workers, a much smaller effect of endotoxin exposure of 24 to 78 ml per 100 ng/m^3 on FEV$_1$ was reported[33]. The exposure-response relationships in our study indicate that modelled long-term average exposure probably correlates better with the biologically relevant exposure, than the average of the measured exposure, despite the large proportion of about 65% of unexplained variance in the models. An indication supporting this observation can be found in the repeated monthly measurements in the subpopulation of six farmers. The number of valid exposure measurements per individual ranged from six to nine, and its average was considered to be the best estimate of long-term average exposure. The correlation (R) of the average of two exposure measurements in summer and winter with this best estimate was 0.61, whereas the correlation of the modelled long-term average exposure with the best estimate was 0.80.

The presented modelling strategy gives an instrument to minimize effort in exposure sampling in occupational epidemiology, without apparent loss of statistical power. Without increasing exposure monitoring effort, it allows an increase in the number of exposure estimates. The central idea is that empirical models which are based on a limited number of measurements, can be applied to estimate exposure in other cases, using simple measurable exposure surrogates in the models. Information on these surrogates can easily be obtained by interview or questionnaire. A possible application can be an increase of the study population in large cohort studies, if exposure is measured in a representative sample of the cohort and estimated for other cohort members. This application will particularly be effective when dealing with small working units, which are often encountered in agriculture or small industries. In these situations complex field work logistics lead to high costs per measurement. A second application of the strategy is increasing the number of exposure estimates per individual, which can be applied in retrospective and prospective studies, as well as in cross-sectional studies as was done in the presented study. This can result into a more accurate estimate of long-term average exposure than when performing few actual exposure measurements. This application can be very effective in working situations where large day-to-day variability in exposure levels results into a large value of λ. Recently, it was shown that large day-to-day variability can be expected among specific groups of workers such as those working outdoors, those in places without local exhaust ventilation, those with an intermittent production process or with a local source of exposure, and among mobile workers[26].

Based on this study it seems that the gain in information on exposure by applying empirical models can by far outweigh the loss of information due to unmeasured factors affecting exposure.

Acknowledgements

The authors wish to thank Dr. Stijn Gielen for testing lung function of the farmers.

References

1. Cochran WG. Errors of measurement in statistics. Technometrics 1968;10:637-66.
2. Liu K, Stamler J, Dyer A, McKeever J, McKeever P. Statistical methods to assess and minimize the role of intra-individual variability in obscuring the relationship between dietary lipids and serum cholesterol. J Chron Dis 1978;31:399-418.
3. Armstrong BG. The effects of measurement errors on relative risk regressions. Am J Epidemiol 1990;132:1176-84.
4. Heederik D, Kromhout H, Attfield M. Occupational exposure assessment in epidemiology - Principles in the design of an optimal exposure assessment strategy. Proceedings of the 9th International Symposium on Epidemiology in Occupational Health (ICOH); 1992 Sept 23-25; Cincinnati, Ohio. DHHS (NIOSH) Publ. no. 94-112, 1994:29-53.
5. Symanski E, Rappaport SM. An investigation of the dependence of exposure variability on the interval between measurements. Ann Occup Hyg 1994;38;361-72.
6. Heederik D, Boleij JSM, Kromhout H, Smid T. Use and analysis of exposure monitoring data in occupational epidemiology: an example of an epidemiological study in the Dutch animal food industry. Appl Occup Environ Hyg 1991;6:458-64.
7. Buringh E, Lanting RW. Models predicting airborne concentrations in workplaces. Staub - Reinhalt der Luft 1991;51:97-104.
8. Hornung RW, Greife AL, Stayner LT, Steenland, NK, Herrick RF, Elliott LJ, Ringenburg VL and Morawetz J. Statistical model for prediction of retrospective exposure to ethylene oxide in an occupational mortality study. Am J Ind Med 1994;25:825-36.
9. Hansen DJ, Whitehead LW. The influence of task and location on solvent exposure in a printing plant. Am Ind Hyg Assoc J 1988;49:259-65.
10. Kalliokoski P. Estimating long-term exposure levels in process-type industries using production rates. Am Ind Hyg Assoc J 1990;51:310-2.
11. Kromhout H, Swuste P, Boleij JSM. Empirical modelling of chemical exposure in the rubber manufacturing industry. Ann Occup Hyg 1994;38:3-22.
12. Woskie SR, Smith TJ, Hammond SK, Hallock MH. Factors affecting worker exposures to metal-working fluids automotive component manufacturing. Appl Occup Environ Hyg 1994;9:612-21.
13. Dement JM, Harris RL, Symons MJ, Shy CM. Exposures and mortality among chrysotile workers. Part I: exposure estimates. Am J Ind Med 1983;4:399-419.
14. Dement JM, Harris RL, Symons MJ, Shy CM. Exposures and mortality among chrysotile workers. Part II: Mortality. Am J Ind Med 1983;4:421-33.
15. Eisen EA, Smith TJ, Wegman DH, Louis TA, Froines J. Estimation of Long Term Dust Exposures in the Vermont Granite Sheds. Am Ind Hyg Assoc J 1984;45:89-94.
16. Dodgson J, Cherrie J, Groat S. Estimates of past exposure to respirable man-made mineral fibres in the European insulation wool industry. Ann Occup Hyg 1987;31:567-82.
17. Simonato L, Fletcher AC, Cherrie JW, Andersen A, Bertazzi P, Bolander AM et al. Updating lung cancer mortality among a cohort of man-made mineral fibre production workers in seven European countries. Cancer Lett 1986;30:189-200.
18. Smith TJ, Hammond SK, Wong O. Health effects of gasoline exposure. I.Exposure assessment for U.S. distribution workers. Environ Health Perspect 1993;101 (suppl 6):13-21.
19. Rylander R, Donham KJ, Hjort C, Brouwer R, Heederik D. Effects of exposure to dust in swine confinement buildings - a working group report. Scand J Work Environ Health 1989;15:309-12.

20. BMRC (British Medical Research Council). Standardized questionnaires on respiratory symptoms. Br Med J 1960:1665.
21. Biersteker K, Dijk WH van, Eissens JBMF, Geuns HA van. Ervaringen met geneeskundig onderzoek op CARA bij gemeentepersoneel te Rotterdam, 1970-1971. Tijdschr Soc Geneeskd 1974;52:158-62.
22. Quanjer PH (editor). Standardized lung function testing. Report of the Working Party "Standardization of Lung Function Tests". Bull Eur Physiopathol Respir 1983;19 (suppl 5):1-95.
23. Heederik D, Brouwer R, Biersteker K, Boleij JSM. Relationship of airborne endotoxin and bacteria levels in pig farms with the lung function and respiratory symptoms in farmers. Int Arch Occup Environ Health 1991;62:595-601.
24. Smid T, Heederik D, Mensink G, Houba R, Boleij JSM. Exposure to dust, endotoxins, and fungi in the animal feed industry. Am Ind Hyg Assoc J 1992;53:362-8.
25. Hollander A, Heederik D, Versloot P, Douwes J. Inhibition and enhancement in the analysis of airborne endotoxin levels in various occupational environments. Am Ind Hyg Assoc J 1993;54:647-53.
26. Kromhout H, Symanski E, Rappaport SM. A comprehensive evaluation of within- and between-worker components of occupational exposure to chemical agents. Ann Occup Hyg 1993;37:253-70.
27. Rappaport SM. Review: Assessment of long-term exposures to toxic substances in air. Ann Occup Hyg 1991;35:61-121.
28. Holness DL, O'Blenis EL, Sass-Kortsak A, Pilger C, Nethercott JR. Respiratory effects and dust exposures in hog confinement farming. Am J Ind Med 1987;11:571-80.
29. Zejda JE, Barber E, Dosman JA, Olenshock SA, McDuffie HH, Rhodes C, Hurst T. Respiratory health status in swine producers relates to endotoxin exposure in the presence of low dust levels. J Occup Med 1994;36:49-56.
30. Sheridan D, Deutscher C, Tan L, Maybank J, Gerrard J, Horne S et al. The relationship between exposure to cereal grain dust and pulmonary function in grain workers. In: Dosman JA, Cotton DJ, eds. Occupational pulmonary disease: focus on grain dust and health. New York: Academic Press,1979:196-213.
31. Cotton DJ, Graham BL, Li KYR, Froh F, Barnett GD, Dosman JA. Effects of smoking and occupational exposure on peripheral airway function in young cereal grain workers. Am Rev Respir Dis 1982;126:660-5.
32. Smid T, Heederik D, Houba R, Quanjer PH. Dust- and endotoxin-related respiratory effects in the animal feed industry. Am Rev Respir Dis 1992;146:1471-9.
33. Kennedy SM, Christiani DC, Eisen EA, Wegman DH, Greaves IA, Olenshock SA, Ye TT, Lu PL. Cotton dust and endotoxin exposure-response relationships in cotton textile workers. Am Rev Respir Dis 1987;135:194-200.

4. Determinants of dust and endotoxin exposure of pig farmers: development of a control strategy using empirical modelling

Liesbeth Preller, Dick Heederik, Hans Kromhout, Jan S.M. Boleij,
Martin J.M. Tielen

Abstract

Personal exposure to dust and endotoxins was measured among 198 Dutch pig farmers. For each participant, 8-hour measurements were made during two days, one in summer 1991 and one in winter 1992. Mean Time-Weighted Average (TWA) exposure to dust was 3.0 mg/m^3 (arithmetic mean, range 0.3-27 mg/m^3) and mean TWA exposure to endotoxins was 129 ng/m^3 (arithmetic mean, range 6-1503 ng/m^3). Empirical statistical modelling was applied to identify activities and farm characteristics associated with exposure. In a multiple least squares regression analysis, aspects of hygiene and feeding were major characteristics associated with dust exposure. Flooring and feeding were predominant characteristics explaining variation in endotoxin exposure. Activities performed frequently like feeding and controlling, cleaning activities, and activities in which very active animals were involved, such as teeth cutting and ear tagging, were associated with exposure to dust and endotoxins. The models were used to set priorities for the development of control measures to eliminate the dust and endotoxin hazard of pig farmers.

Annals of Occupational Hygiene, *in press*

Introduction

Among pig farmers in several countries in Europe and North America, high prevalence rates of chronic as well as acute respiratory symptoms have been reported[1-8]. In general, exposure to high concentrations of organic dust, and particularly to endotoxins present in dust, are thought to cause respiratory health effects[9]. Endotoxins are constituents of the cell wall of gram-negative bacteria, and can be biologically active when still present in bacteria, as well as in free form after lysis of bacteria. Animal feed and excreta from animals are potential sources of endotoxins in pig farms.

Despite high prevalence rates of respiratory health effects among pig farmers, and exposure to high concentrations of dust and endotoxin, no systematic studies have been undertaken to identify the determinant factors of exposure of farmers. Insight into associations between personal exposure and determinant factors associated with working conditions would be useful in developing practical strategies for reducing exposure. So far, some associations between dust exposure concentrations and farm characteristics, most often type of feeding or aspects of ventilation, have been reported. Studies have usually been based on ambient air measurements, which do not allow extrapolation towards personal exposure of pig farmers. Holness et al.[3], who applied personal sampling, found an average dust exposure of 2.0 mg/m^3 among Canadian farmers using automated feeding, whereas among farmers applying other feeding methods the exposure was 3.5 mg/m^3. This difference was statistically not significant. In Dutch farms, Attwood et al.[10] measured the lowest total dust concentrations in compartments with wet feeding (2.9 mg/m^3), and higher concentrations when feed pellets or meal was supplied (4.5 and 5.4 mg/m^3, respectively). Chiba et al.[11] reported a reduction of dust exposure of 50% when fat was added to the feed. Studies conducted in countries with variation in outdoor climatic conditions showed higher dust concentrations with lower outdoor temperature, clearly due to restricted ventilation[12,13].

Task-specific sampling[14] and exposure modelling[15] can be used to develop control measures, but the first of these is not feasible in pig farming. Time per activity is too short for gravimetric sampling, which is required for assessment of endotoxin exposure. Moreover, task-specific exposure depends on many preconditions, which hampers extrapolation to general practical situations. In this study, we therefore applied empirical modelling techniques to identify and quantify factors affecting personal exposure levels. This paper describes how this strategy was used in setting priorities in developing control measures.

Materials and methods

Pig farming and population

Three different types of Dutch pig farms can be distinguished. A breeding farm comprises several small farrowing compartments, each with about eight sows, several piglet compartments with about 80 weaned piglets per compartment, and one or a few large sow compartments with empty (non-pregnant) and pregnant sows. A finishing farm consists of several finishing compartments with some 80 finishing pigs or gilts per compartment. The third type is a combination of breeding and finishing farm, and is the most common type. Activities done in, and time spent in separate compartments differ considerably. Often more than one pig house is present at one farm, dating from different time periods. As a consequence, large differences in farm characteristics can be found at one farm, even within the same type of compartment.

The study took place in two southeastern provinces of the Netherlands, where 6.6 million sows and finishing pigs are housed[16]. This is 55% of the total pig population in the Netherlands. The study population comprised 198 male owners of pig farms, which were nearly all family farms. On such a farm the owner is sometimes assisted by family members, or by an employee. Most pig farmers also perform other agricultural activities. The participants were selected out of a group of 1,504 pig farmers who had completed a questionnaire on respiratory health and farm characteristics. Only farmers who worked at least five hours a day in pig farming were selected for participation, to minimize confounding effects due to other working activities.

Measurement strategy

Exposure to inhalable dust and endotoxins was determined by means of personal sampling. The inhalable fraction is the fraction of total airborne particles which is inhaled through nose and mouth. Dust was collected using a dust sampler with a 6 mm diameter inlet opening (PAS6) and an airflow of 2 l/min[17]. Teflon filters with a pore size of 1 μm (Millipore) were used. The procedure for measuring dust concentrations has been described in detail elsewhere[18]. Endotoxins in the dust samples were analyzed with a modified kinetic *Limulus* Amoebocyte Lysate test[19].

Exposure was assessed for each participant during one day in summer (between June 4 and July 3, 1991), and during one day in winter (between January 27 and February 27, 1992). Measurement equipment was handed out to each farmer in the morning, and collected on average 8.3 hours later (standard deviation 0.6, range 5.2-10.4 hours). The day of measurement was randomly chosen between Monday and Thursday.

Meteorological data were obtained from a monitoring station in the South of the Netherlands.

In both seasons the farmers were requested to complete a diary on time spent on activities during the day of exposure measurement, and during the following six days. Time (in quarters of an hour) spent on 21 pre-selected activities in pig farming had to be recorded.

Farm characteristics

From January until March 1992, all farms were visited to record farm characteristics by means of a walk-through survey. Data were collected separately for each compartment. Data were recorded on:

- number of animals;
- feeding method;
- heating and ventilation system;
- type of floor and bedding material;
- degree of contamination, by visual inspection.

A data set of 95 farm characteristics in 16 groups was obtained.

Computational and statistical methods

The average time a farmer spent in compartments with a specific characteristic was estimated, based on the average number of pigs present in these compartments. Average time was calculated as a percentage of total time the farmer spends in pig farming. Differences in time spent per animal in the farrowing, sow, piglet and finishing compartments were allowed for by weighting factors, 18:5:1:1, respectively. These weights were based on information from all farmers on the number of animals and the time spent in different types of compartments.

Data on exposure concentrations in summer and winter, on farm characteristics, on activities performed during sampling, and on outdoor temperature were used to identify and quantify factors affecting exposure. Analyses were based on log-transformed exposure data in order to stabilize variance and to obtain normally distributed residuals. Using classical stepwise regression analysis, the variation in log-transformed Time-Weighted Average exposure (TWA) was explained by activities performed during sampling (discretely in quarters of an hour), by farm characteristics (either as percentage of time dealing with the characteristic or as a dummy variable: present or not present), and by outdoor temperature. All measurements were considered as independent observations in the analysis, since the correlation between repeated measurements taken in summer and winter was low (0.25 for dust and 0.19 for endotoxin exposure). Each independent variable had to meet a statistical significance level of at least 0.50 for entry in the model and was kept in the model at a statistical significance level below 0.10. Model adequacy was tested with standard least squares regression techniques such as residual plots and outlier detection.

Statistical analyses were performed with SAS/PC version 6.04 using PROC REG.

Results

Exposure levels and farm characteristics

A total of 360 dust measurements and 350 endotoxin measurements were available, after excluding samples in which equipment failed during sampling or in endotoxin analysis (10% and 12%, respectively).

Average maximum outdoor temperature was 5.1°C in winter (range -0.9-12.9°C) and 17.1°C in summer (range 12.1-28.7°C). Table 4.1 gives a summary of the exposure levels found. Mean exposure to dust and endotoxins was higher in winter than in summer (16% and 39%, respectively). These differences were statistically significant (paired t-test on log-transformed values, $p < 0.05$).

The average number of compartments per farm was 19 (range 5-55). On five farms there was only one building and on 165 farms there were two, three or four buildings. 15 farms had five buildings, and 13 farms had six or more different buildings. The presence of farm characteristics, which were found to be related to exposure to dust or endotoxin, is given in Table 4.2. The number of farms with given characteristics ranged from 5 (air exhaust via other compartments) to 166 (manual dosage dry feeding). Median percentage of time spent in compartments with these characteristics, if present, ranged from 2 to 68%.

Table 4.1 Personal time-weighted average exposure to inhalable dust and endotoxins of 198 pig farmers in the Netherlands, summer 1991 and winter 1992.

	No. of measurements	arithmetic mean	geometric mean	geometric standard deviation	range
dust (mg/m^3)					
summer	187	2.7	2.2	1.9	0.5 - 11.2
winter	173	3.3	2.6	2.0	0.3 - 26.6
all*	360	3.0	2.4	1.9	0.3 - 26.6
endotoxin (ng/m^3)					
summer	182	111	78	2.4	5.6 - 825
winter	168	150	109	2.3	10.6 -1503
all †	350	129	92	2.4	5.6 -1503

* 162 farmers with 2 measurements

† 154 farmers with 2 measurements

For 164 farmers activity patterns were known for 12 to 14 days. Table 4.2 gives the number of farmers who spent time on activities related to exposure concentrations. For these farmers also the median of average daily time is given. During the days on which the farmers recorded their activities, 51 cleaned the food storage and all 164 fed their animals: the numbers doing the other listed activities fell between these two figures. If performed, the average time spent on an activity ranged from two minutes to two hours per day (averaged over 12-14 days). Since most activities were not performed daily, more time was spent on an activity on occasional days. The usual time spent on feeding and controlling was about two hours and about one hour, respectively. Most other activities took in general about half an hour each day they were performed. However, the maximum time spent on one day exceeded these values by a factor two to four.

Modelling exposure

Outdoor temperature, 10 farm characteristics and 10 specified activities were significantly related to log-transformed TWA dust exposure. They accounted for 34% (adjusted R^2 = 30%) of the variation in dust exposure. In Table 4.3 the results of the multiple regression analysis are given. Regression coefficients (ß) give the estimated change in *ln*(exposure) per change in standardized units of the independent variable.

Table 4.2 Farm characteristics* on 198 Dutch farms, and time spent on activities by 164 farmers: number of observations with positive value, and median values for positive observations.

	number of observations	median
CHARACTERISTICS		(%)
hygiene		
dusty feeding path	156	30
little dry manure	127	6
overall very dusty	67	6
overall slightly dusty	151	13
feeding		
automated mobile feeding trolley	14	23
pig starter	14	14
wet feeding	24	42
manual dosage dry feeding	166	68
automated dry feeding in trough	36	32
flooring		
convex floor	75	17
fully slatted floor	125	19
fully slatted floor with piglet mat	44	34
synthetic grid	32	14
concrete and metal grid	14	24
floor heating	165	49
floor heating in combination with delta heating tubes	29	17
complete concrete floor	11	5
other		
air exhaust via pit	53	39
air exhaust via other compartments	5	2
gas heaters	37	27
ACTIVITIES †		(minutes)
frequently performed		
feeding	164	114
controlling	163	57
re-penning	156	18
cleaning		
floor sweeping	135	9
cleaning food storage	51	2
removing dry manure	78	6
other, involving active animals		
castrating	114	6
teeth cutting	71	6
ear tagging	102	4
iron injection	138	8
pig marking	59	4

* expressed as percentage of total time spent in pig farming

† based on daily averages during 12-14 days

Table 4.3 Farm characteristics and activities related to personal dust exposure (mg/m^3) (log-transformed) in multiple regression analysis, and estimated factor* for change in exposure for common population levels (10°C, median values, or present vs. not present).

	regression coefficient	se	p-value	factor for common population levels
intercept	0.77	0.13	< 0.01	
outdoor temperature (per 10°C)	-0.20	0.04	< 0.01	0.82
FARM CHARACTERISTICS				
hygiene				
dusty feeding path (per 10%)	0.03	0.01	0.02	1.10
little dry manure (1/0)	-0.16	0.07	0.02	0.85
overall very dusty (1/0)	0.17	0.07	0.01	1.18
overall slightly dusty (1/0)	-0.16	0.07	0.04	0.85
feeding				
automated mobile feeding trolley (1/0)	-0.27	0.13	0.04	0.76
pig starter (1/0)	-0.21	0.12	0.08	0.81
wet feeding (1/0)	-0.23	0.10	0.02	0.79
other				
air exhaust via other compartments (1/0)	-0.55	0.23	0.02	0.58
gas heaters (1/0)	-0.17	0.08	0.04	0.85
complete concrete floor (1/0)	-0.24	0.14	0.09	0.79
ACTIVITIES (per 10 minutes)				
frequently performed				
feeding	0.02	0.01	< 0.01	1.27
controlling	0.02	0.01	0.01	1.11
re-penning	0.03	0.01	< 0.01	1.06
cleaning				
floor sweeping	0.07	0.02	< 0.01	1.07
cleaning food storage	0.06	0.04	0.10	1.02
removing dry manure	0.04	0.02	0.07	1.03
other, involving active animals				
castrating	0.06	0.02	< 0.01	1.04
teeth cutting	0.11	0.05	0.02	1.07
ear tagging	0.08	0.02	< 0.01	1.04
pig marking	0.04	0.02	0.10	1.02

RSD = 0.56

(1/0) dummy variable: present vs. not present

* median values for observations > 0, as given in Table 4.2. Change in exposure level is estimated by multiplying exposure by given factor.

Since models were based on log-transformed values, exp^{β} represents the factor by which the estimated exposure is changed per unit change in characteristic or activity. These factors are given in Table 4.3 for realistic values of independent variables in the population. For dummy farm characteristics, the results represent the difference in estimated exposure when the characteristic is present versus that for when it is not. For continuous variable characteristics and for activities, median values (see Table 4.2) were taken to estimate the factor by which exposure changes, and thus the results give the difference in estimated exposure when the value of the independent variable is increased by this median value. By choosing the median values as unit, insight is given in the way a variable potentially affects long-term average exposure. The use of results in Table 4.3 is illustrated in the following examples. If a farmer spent no time on ear tagging on the first day, and 12 minutes on the second day (3 times median value), the other variables remaining constant, his TWA dust exposure is estimated to be a factor $(1.04)^3 = 1.12$ higher than on the first day. If on the first day his exposure was 3 mg/m^3, then the estimated increase is about 0.35 mg/m^3. In another example, farmer A did not use wet feeding and spent the median time on feeding, and his exposure was 3 mg/m^3. Farmer B used wet feeding and spent half of the median time on feeding, and all other variables were the same for both farmers. The exposure of farmer B is estimated to be $(0.79 * 1.27^{-0.5}) * 3 = 2.1$ mg/m^3.

Specific activities in pig farming explained twice as much variance in exposure as farm characteristics, 21% and 9%, respectively. In the population under study, feeding was associated with the largest estimated effect on long-term average exposure. Spending median time (114 minutes) on feeding yields an estimated exposure which is higher by a factor 1.3 than when no time is spent on feeding. With an average exposure of 3 mg/m^3 this implies an increase in TWA exposure of nearly 1 mg/m^3. Control was associated with an increase of more than 10%, whereas all other activities were associated with an increase in long-term average exposure of less than 10%. However, on specific days when half an hour is spent on activities such as ear tagging or teeth cutting, the estimated increase in exposure is considerable. The estimated increase is about 25% to 40% (increase in exposure with factor $exp^{3*0.08}=1.27$ and $exp^{3*0.11}=1.39$). Such an increase is similar to the increase associated with feeding during approximately two hours.

General hygiene (dusty feeding path in compartments, little dry manure in pens, overall very dusty or slightly dusty compartments), was related to a positive or negative change in exposure of 10% to 20%. The presence of several ways of feeding (automated mobile feeding trolley, pig starter, wet feeding) was associated with a decrease in exposure of about 20% to 25%. For farms with gas heaters, air exhaust via other compartments, or complete concrete floors, the estimated exposure level was lower.

Outdoor temperature, 12 farm characteristics and eight activities in pig farming explained 37% (adjusted R^2 = 33%) of the variation in log-transformed TWA exposure to endotoxin. In Table 4.4 the results are presented in the same way as they are for dust in Table 4.3. Activities and farm characteristics explained equal amounts of the variance (13% and 15%, respectively). Of activities, the estimated effect of feeding was largest with an increase of about 40%. This implies an average increase in TWA exposure of some 40 ng/m^3. Next, only controlling and teeth cutting were associated with an increase that exceeded 10%. However, on occasional days, when spending half an hour on floor sweeping or on activities with active animals, each of these activities is associated with an increase in exposure ranging from 15% to more than 60%.

Seven characteristics describing different aspects of flooring were associated with exposure. Convex floor, fully slatted floor with piglet mat, synthetic grid, and combined concrete and metal grid, were associated with a reduction in exposure ranging from about 15% to 30%. Fully slatted floor, floor heating, and floor heating in combination with delta heating tubes, were associated with an increase of about 15% to 40%. The use of manual dry feeding and automated dry feeding in a trough were associated with reduced concentrations (30% and 20%), and the use of pig starter with an increase (40%). Air exhaust via a pit was associated with lower, and overall poor hygiene with higher concentrations.

Discussion

In our study among 198 pig farmers we found exposures to high mean concentrations of dust and of endotoxins (3.0 mg/m^3 and 129 ng/m^3, respectively (AM)). Personal exposure was of the same order of magnitude as found in other studies[3,5,20-23].

Castellan et al.[24] reported a no-effect-level of 9 ng/m^3 airborne endotoxins in people with chronic respiratory symptoms, whereas among healthy subjects a no-effect-level of 33 ng/m^3 was assessed in experiments with cotton dust containing endotoxins[25]. In both studies change in lung function was used as a measure of effect.

Table 4.4 Farm characteristics and activities related to personal endotoxin exposure (ng/m^3) (log-transformed) in multiple regression analysis, and estimated factor* for change in exposure for common population levels (10°C, median values, or present vs. not present).

	regression coefficient	se	p-value	factor for common population levels
intercept	4.44	0.18		
outdoor temperature (per 10°C)	-0.35	0.05	< 0.01	0.71
FARM CHARACTERISTICS				
feeding				
manual dosage dry feeding (1/0)	-0.37	0.12	< 0.01	0.69
pig starter (1/0)	0.35	0.16	0.03	1.41
automated dry feeding in trough (per 10%)	-0.06	0.03	0.02	0.82
flooring				
convex floor (1/0)	-0.22	0.09	0.01	0.80
fully slatted floor (per 10%)	0.08	0.02	< 0.01	1.16
fully slatted floor + piglet mat (per 10%)	-0.08	0.03	< 0.01	0.75
synthetic grid (per 10%)	-0.13	0.06	0.03	0.84
concrete and metal grid (per 10%)	-0.15	0.05	< 0.01	0.70
floor heating (per 10%)	0.07	0.02	< 0.01	1.38
floor heating combined with delta heating tubes (per 10%)	0.10	0.05	0.03	1.19
other				
overall very dusty (per 10%)	0.12	0.05	0.01	1.07
air exhaust via pit (1/0)	-0.33	0.10	< 0.01	0.72
ACTIVITIES (per 10 minutes)				
frequently performed				
feeding	0.03	0.01	< 0.01	1.37
controlling	0.02	0.01	0.02	1.14
re-penning	0.02	0.01	0.05	1.04
cleaning				
floor sweeping	0.06	0.02	< 0.01	1.05
other, involving active animals				
iron injection	0.09	0.04	0.03	1.04
castrating	0.05	0.02	0.03	1.03
teeth cutting	0.17	0.06	< 0.01	1.11
ear tagging	0.14	0.03	< 0.01	1.07

RSD = 0.71

(1/0) dummy variable: present vs. not present

* median values for observations > 0, as given in Table 4.2. Change in exposure level is estimated by multiplying exposure by given factor.

Threshold exposure concentrations of 100 ng/m^3 have been proposed by an international working group[26], and a few years later of 30 ng/m^3 by Palchak et al.[27]. In our study 50%, 90% and 99% of the valid endotoxin observations exceeded 100, 30 and 9 ng/m^3, respectively, indicating that, whatever the threshold, acute respiratory effects seem likely.

Respiratory health hazards of exposure to organic dust depend largely on its composition. For total dust, a maximum 8-hour TWA occupational exposure level of 10 mg/m^3 based on nuisance effect is accepted in the Netherlands. For exposure to grain dust, with partly comparable composition, a Threshold Limit Value of 4 mg/m^3 was set for the 8-hour TWA in the U.S.. Two and 20% of our observations exceeded 10 and 4 mg/m^3, respectively. Since the sampled dust fraction was somewhat smaller than total dust, on which threshold values are based, the limit values will be exceeded in more cases than estimated.

Given these high exposure levels, a major objective of the study was to develop control measures. 8-hour TWA exposure levels were used in empirical modelling analyses, with farm characteristics, activities, and outdoor temperature as explanatory variables. In several other studies dealing with occupational exposure, empirical modelling techniques have been used either for similar goals[28-30], or to estimate historic exposure for use in epidemiological analyses[31-35]. Performance of our models, with Residual Standard Deviation (RSD) of 0.56 (R^2=34%) and 0.71 (R^2=37%) in dust and endotoxin exposure models, respectively, was comparable to the model used by Eisen et al.[32] with an RSD of 0.58 (R^2=46%). They explained variation in log-transformed dust exposure by job, shed, season, survey year and several interaction terms. In other studies using similar modelling techniques, no information was given on RSD.

In our study, dust exposure levels of pig farmers were found to be primarily related to aspects of hygiene and feeding. Major characteristics found to be associated with endotoxin levels were aspects of flooring, and to a lesser extent of feeding. In one other study an association of farm characteristics (type of feed) with personal dust exposure levels was reported[3]. In our study, 11 distinct activities were associated either with dust and/or with endotoxin levels. We found large differences between activities in estimated increase in exposure per unit of time. These findings conflict with those of a Danish study[22]. In that study among four workers, no differences in personal dust and endotoxin exposure levels were observed between tasks performed close to animals and far from them. The negative results in that study are most likely caused by its small-scale, and by applying inappropriate grouping. For example, in the Danish study repenning and insemination were regarded as activities close to animals, with presumably high exposure levels. In our study these activities were associated respectively with a small, and with no increase in dust or endotoxin exposure, per unit of time. On the other hand, in our study, the usual time of half an hour spent on cleaning activities, performed at a distance from animals, was associated with a considerable increase in exposure.

Different mechanisms can be considered as potential explanations for associations found in our study. Microscopic analysis of dust in pig farms showed that feed and faeces were the major constituents[36]. Both feed and faeces are a source of endotoxins. These observations can explain associations with feeding, flooring, and air exhaust via the pit. Warm and humid conditions related to some aspects of flooring and feeding may play a role in increasing the growth of endotoxin-containing gram-negative bacteria. Re-suspension of settled dust can potentially explain associations between exposure concentrations, and activities and aspects of hygiene. It has been shown by real-time measurements that much higher concentrations of inhalable dust existed in compartments when animals were disturbed by the presence of the farmer than at times of rest. Close to the animals, peak exposure concentrations, with averaging times of seconds, were higher by a factor up to 100 when they were disturbed than at times of little activity[37]. A positive correlation between exposure concentrations and activities could also be a result of the release of dust particles from animals.

Results of the analyses can be used to develop control measures. However, with respect to this goal, empirical modelling has several limitations. Evaluation is restricted to explanatory variables with sufficient variation in the population. In observational studies, variation in variables potentially affecting exposure may be absent, and for example in our study there was little variation in the use of some bedding materials. Secondly, if there are two correlated variables in the model, their effects can not be separated, as for example in our study the correlation between application of wet feeding and the way of feeding may affect the estimated effects of both variables. Interpretation of results is therefore always conditional. On the other hand, if a modelled variable is correlated with an unmodelled variable with an effect on exposure, the effect can erroneously be ascribed to the modelled variable. In our study this is potentially true for, for example, air exhaust via other compartments, and complete concrete flooring. If present, there were only very few compartments with these characteristics, and no possible mechanisms can be suggested as to why these characteristics themselves would reduce dust exposure levels. Limited explained variance of models can further limit the value of interpolations made based on outcomes. Additionally, to obtain normally distributed residuals, models were based on log-transformed exposure concentrations. Using log-transformed values has an added advantage that there are no negative estimates of exposure concentrations, when used in prediction[38]. Models based on log-transformed values estimate a larger effect on exposure concentrations at higher values of independent variables. An effect which is far from proportional does not seem plausible, although there are probably factors in the working environment that induce non-proportional effects. In our study, within the range of independent variables, the estimated effect does not deviate to any great extent from proportionality since regression coefficients are small. However, in other situations the use of models based on log-transformed exposure concentrations may be restricted.

Despite these limitations, it is expected that at this stage for many pig farmers working circumstances with exposure levels considerably lower than those currently encountered can be created. This study suggests that such a goal can be achieved by applying flooring and feeding characteristics which are interpretively associated with a low exposure, and by paying attention to maintaining good hygiene. Restricting time spent on specific activities can not be regarded as a control measure. Alternatively, the use of personal protective equipment may be considered to minimize inhaled amount of dust and endotoxins. Results suggest that, on average, the use of personal protection equipment during feeding will have the largest effect on long-term average exposure. However, on days on which infrequent activities with high estimated effect on exposure are performed, inhaled dose can be considerably reduced by wearing protective equipment. A respirator may yield a reduction in effective exposure of 75% (CEN, EN 140/EN 143). Taken a situation in which median time is spent on feeding, and four times median time on castrating and teeth cutting, the use of a respirator during feeding yields an estimated reduction of effective exposure of 17%, whereas use during both other activities yields a corresponding reduction of 27%.

The value of empirical modelling strategies for the development of control measures can in general be validated in several ways. Cross-validation of models on a data set which is not used for building models can be applied, and the opinions of occupational hygienists or other experts on models can be asked for[38]. In our study the opinion of specialists in pig farming was used to evaluate the findings. Experimental studies, and evaluation of exposure before and after small-scale interventions with respect to selected factors, can also verify findings. In general, real-time monitoring is an effective method of evaluating exposure under different circumstances[39]. Real time monitoring is not applicable for all hazardous exposures because for some, such as endotoxins, correlation with dust exposure is low. Further, analysis of dust from personal samples can help to explain mechanisms responsible for empirically found associations. When dealing with organic dust, techniques designed to characterize proteins, such as immunoassays, are appropriate[40]. As shown, microscopic analysis can sometimes be used to determine dust sources[36].

The strategy followed, based on empirical modelling, made it possible to obtain information on the role of activities and of farm characteristics simultaneously. For occupational hygiene purposes, task- or site-specific ambient air sampling strategies would have been possible alternatives, but activities generally took too little time for gravimetric sampling. The large number of types of compartment with more or less similar characteristics, the large variation in concentration with time, the mobility of farmers, and the generally poor correlation between ambient air measurements and personal sampling[18,41,42] made ambient air sampling inappropriate.

The modelling strategy appeared useful for getting a first impression of associations between the exposure of pig farmers, and the large number of factors encountered in the complex working circumstances of the farmers and potentially affecting exposure. Priorities could be set for development of control measures. Most factors showing up in the models are thought to be causally related to exposure concentrations and will be used to develop appropriate control measures to reduce the dust and endotoxin hazard of pig farmers.

References

1. Donham K, Zavala D, Merchant J. Respiratory Symptoms and Lung Function among Workers in Swine Confinement Buildings: A Cross-Sectional Epidemiological Study. Arch Environ Health 1984;39:96-101.
2. Brouwer R, Biersteker K, Bongers P, Remijn B, Houthuijs D. Respiratory symptoms, lung Function, and IgG_4 levels against pig antigens in a sample of dutch pig farmers. Am J Ind Med 1986;10:283-5.
3. Holness D, O'Blenis E, Sass-Kortsak A, Pilger C, Nethercott, J. Respiratory Effects and Dust Exposures in Hog Confinement Farming. Am J Ind Med 1987;11:571-80.
4. Dosman J, Graham B, Hall D, Pahwa P, McDuffie HH, Lucewicz M, To T. Respiratory Symptoms and Alterations in Pulmonary Function Tests in Swine Producers in Saskatchewan: Results of a Survey of Farmers. J Occup Med 1988;30:715-20.

5. Donham K, Haglind P, Peterson Y, Rylander R, Belin L. Environmental and health studies of farm workers in Swedish swine confinement buildings. Br J Ind Med 1989;46:31-7.
6. Heederik D, Brouwer R, Biersteker K, Boleij J. Relationship of airborne endotoxin and bacteria levels in pig farms with the lung function and respiratory symptoms of farmers. Int Arch Occup Environ Health 1991;62:595-601.
7. Cormier Y, Boulet L, Bedard G, Tremblay G. Respiratory health of workers exposed to swine confinement buildings and dairy barns. Scand J Work Environ Health 1991;17:269-75.
8. Zuskin E, Zagar Z, Schachter E, Mustajbegovic J, Kern J. Respiratory symptoms and ventilatory capacity in swine confinement workers. Br J Ind Med 1992;49:435-40.
9. Rylander R, Donham KJ, Hjort C, Brouwer R, Heederik D. Effects of exposure to dust in swine confinement buildings - a working group report. Scand J Work Environ Health 1989;15:309-12.
10. Attwood P, Brouwer R, Ruigewaard P, Versloot P, Wit R de, Heederik D, Boleij JSM. A study of the relationship between airborne contaminants and environmental factors in Dutch swine confinement buildings. Am Ind Hyg Assoc J 1987;48:745-51.
11. Chiba LI, Peo ER, Lewis AJ, Fritschen RD, Crenshaw JD. Effect of dietary fat on pig performance and dust levels in modified-open-front and environmentally regulated confinement buildings. J Anim Sci 1985;61:763-81.
12. Crook B, Robertson JF, Travers Glass SA, Botheroyd EM, Lacey J, Topping MD. Airborne dust, ammonia, microorganisms, and antigens in pig confinement houses and the respiratory health of exposed farm workers. Am Ind Hyg Assoc 1991;52:271-79.
13. Zejda J, Barber E, Dosman JA, Olenchock SA, McDuffie HH, Rhodes C, Hurst T. Respiratory health status in swine producers relates to endotoxin exposure in the presence of low dust levels. J Occup Med 1994;36:49-56.
14. Nicas M, Spear RC. A task-based statistical model of a worker's exposure distribution. Part 1, Description of the model. Am Ind Hyg Ass J 1993;54:211-20.
15. Buringh E, Lanting RW. Models predicting airborne concentrations in workplaces. Staub - Reinhalt Luft 1991;51:97-104.
16. CBS Centraal Bureau voor de Statistiek. Landbouwtellingen 1989. (Statistics Netherlands) Voorburg/Heerlen.
17. Kuile WM ter. Vergleichsmessungen mit verschiedenen Geraeten zur Bestimmung der Gesamtstaubkonzentration am Arbeitsplatz-Teil II. (in German) 'Comparative measurements of the total dust concentration at the work place with different samplers - Part II'. Staub - Reinhalt Luft 1984;44:211-6.
18. Smid T, Heederik D, Mensink G, Houba R, Boleij JSM. Exposure to dust, endotoxins, and fungi in the animal feed industry. Am Ind Hyg Ass J 1992;53:362-8.
19. Hollander A, Heederik D, Versloot P, Douwes J. Inhibition and enhancement in the analysis of airborne endotoxin levels in various occupational environments. Am Ind Hyg Assoc J 1993;54:647-53.
20. Haglind P, Rylander R. Occupational Exposure and Lung Function Measurements among Workers in Swine Confinement Buildings. J Occup Med 1987;29:904-7.
21. Louhelainen K, Vilhunen P, Kangas J, Terho EO. Dust exposure in piggeries. Eur J Respir Dis 1987;71 (suppl 152):80-90.
22. Vinzents P, Nielsen BH. Variations in exposures to dust an endotoxin in Danish piggeries. Am Ind Hyg Assoc J 1992;53:237-41.
23. Heederik D, Zwieten R van, Brouwer R. Across-shift lung function changes among pig farmers. Am J Ind Med 1990;17:57-8.

24. Castellan RM, Olenchock SA, Kinsley KB, Hankinson JL. Inhaled endotoxin and decreased spirometric values. N Eng J Med 1987;317:605-10.
25. Rylander R, Haglind P, Lundholm M. Endotoxin in cotton dust and respiratory function decrement among cotton workers in an experimental cardroom. Am Rev Respir Dis 1985;131:209-15.
26. Clark S. Report on prevention and control. Am J Ind Med 1986;10:267-73.
27. Palchak RB, Cohen R, Ainslie M, Hoerner CL. Airborne endotoxin associated with industrial-scale production of protein products in gram-negative bacteria. Am Ind Hyg Assoc J 1988;44:420-1.
28. Hansen DJ, Whitehead LW. The influence of task and location on solvent exposure in a printing plant. Am Ind Hyg Assoc J 1988;49:259-65.
29. Kalliokoski P. Estimating long-term exposure levels in process-type industries using production rates. Am Ind Hyg Assoc J 1990;51:310-2.
30. Kromhout H, Swuste P, Boleij JSM. Empirical modelling of chemical exposure in the rubber manufacturing industry. Ann Occup Hyg 1994;38:3-22.
31. Dement JM, Harris RL, Symons MJ, Shy CM. Exposures and mortality among chrysotile workers. Part I: exposure estimates. Am J Ind Med 1983;4:399-419.
32. Eisen EA, Smith TJ, Wegman DH, Louis TA, Froines J. Estimation of Long Term Dust Exposures in the Vermont Granite Sheds. Am Ind Hyg Assoc J 1984;45:89-94.
33. Dodgson J, Cherrie J, Groat S. Estimates of past exposure to respirable man-made mineral fibres in the European insulation wool industry. Ann Occup Hyg 1987;31:567-82.
34. Woskie SR, Smith TJ, Hammond SK, Schenker MB, Garshick E, Speizer FE. Estimation of the diesel exhaust exposures of railroad workers II. National and historical exposures. Am J Ind Med 1988;13:395-404.
35. Smith TJ, Hammond SK, Wong O. Health effects of gasoline exposure. I. Exposure for U.S. Distribution workers. Environ Health Perspect 1993;101:13-21.
36. Donham KJ, Popendorf W, Palmgren U, Larsson L. Characterization of dusts collected from swine confinement buildings. Am J Ind Med 1986;10:294-97.
37. Zeitler-Feicht MH, Praml GJ, Riedel H. Die zeitliche Variation der Feinstaubbelastung der Luft im Mastschweinestall. Tierhygienische und arbeitsmedizinische Aspekte. (in German) 'The temporal variation of airborne fine dust concentration in pig fattening houses. Hygienic and medical aspects'. Berl Münch Tierärztl Wschr 1991;104:84-9.
38. Hornung RW, Greife AL, Stayner LT, Steenland, NK, Herrick RF, Elliott LJ, Ringenburg VL and Morawetz J. Statistical model for prediction of retrospective exposure to ethylene oxide in an occupational mortality study. Am J Ind Med 1994;25:825-36.
39. Woskie SR, Shen P, Eisen EA, Finkel MH, Smith TJ, Smith R, Wegman DH. The real-time dust exposures of sodium borate workers: examination of exposure variability. Am Ind Hyg Assoc J 1994;55:207-17.
40. Virtanen T, Kalliokoski P, Vilhunen P, Taivainen A, Mäntyjärvi R. Concentrations of specific dusts in swineries and the humoral response of swinery workers. Allergy 1990;45:1-9.
41. Zhang J, Billiet J, Nagels M, Dams R. Survey of total and respirable suspended particulate matter in an iron foundry : comparison of stationary sampling and personal monitoring. Sci Total Environ 1983;30:167-80.
42. Cinkotai FF, Gibbs ACC, Sharpe TC. A comparison of exposure to airborne dust in cotton processing plants estimated from personal and workzone samples. Ann Occup Hyg 1984;28:347-52.

5. Lung function and chronic respiratory symptoms of pig farmers: focus on exposure to endotoxins and ammonia and use of disinfectants

Liesbeth Preller, Dick Heederik, Jan S.M. Boleij, Peter F.J. Vogelzang,
Martin J.M. Tielen

Abstract

The prevalence rate of chronic respiratory symptoms among pig farmers is known to be high, but the etiology of these symptoms is not yet uncovered. We evaluated whether long-term average exposure to dust, endotoxins and ammonia and the use of disinfectants were associated with chronic respiratory symptoms and base-line lung function. A cross-sectional study was performed among 198 Dutch pig farmers, of whom 100 without and 98 with chronic respiratory symptoms. Exposure-response relationships were evaluated using multiple logistic and linear regression analysis. Estimates of long-term average exposure were based on two personal exposure measurements, taken during one day in summer and one day in winter. Information on the use of disinfectants and disinfection procedures was assessed by a walk-through survey and interview by telephone. Exposure to dust, endotoxins and ammonia were not related to chronic respiratory symptoms. Duration of the disinfection procedure and pressure used at disinfection were strongly and positively related to chronic respiratory symptoms. A statistically significantly inverse association between base-line lung function and endotoxin exposure was observed only among asymptomatic farmers. Ammonia exposure and duration of the disinfection procedure were statistically significantly associated with base-line lung function in the entire population. Results suggest that the use of disinfectants is an important etiological factor in chronic respiratory health effects on pig farmers. This factor has not been studied before. Results also suggest an etiological role for exposure to endotoxins and ammonia in development of chronic respiratory health effects, but longitudinal studies with detailed exposure assessment strategies are required to assess their role.

Accepted in revised form for publication in Occupational and Environmental Medicine

Introduction

Studies performed since 1977 in countries in Northern America and Europe show high prevalence rates of acute and chronic respiratory symptoms among pig farmers. The same studies suggest that major base-line lung function parameters (FVC and FEV_1) seem not to differ largely from expected values[1-5]. The etiology of these respiratory health effects remains until now indistinct. Pig farmers are exposed to a variety of health hazards such as organic dust and its constituents, gases, chemicals used for disinfection of buildings, and climatic conditions. Recent epidemiological studies were focused on the potential health hazard of exposure to high levels of endotoxins, and to a lesser extent to other organic dust components. Reported relationships between quantified exposure levels and chronic respiratory health effects are sparse. Heederik et al.[6] and Zejda et al.[7] reported associations between base-line lung function and exposure to endotoxins. In their studies exposure was based on static measurements in swine confinement buildings during one or two days.

In our study we assessed personal exposure to inhalable dust, endotoxins and ammonia and evaluated their association with base-line lung function and chronic respiratory symptoms. In this study we also investigated disinfectants as potential etiological factor. Several case studies have been described in which disinfectants induced respiratory symptoms, including asthma[8-14]. These agents have, however, been overlooked as possible cause of respiratory health effects in pig farmers. The questionnaire based evaluation preceding this study showed that farmers reported more asthma-like symptoms with larger frequency of disinfection (unpublished data).

This study describes associations between chronic respiratory symptoms and base-line lung function and mentioned determinants of exposure.

Material and methods

Population and health data

The population existed of 198 pig farmers living in the two southeastern provinces of the Netherlands. In the selected population 100 farmers were included without chronic respiratory symptoms (chronic cough, chronic phlegm, ever wheezing, frequent wheezing, shortness of breath, and chest tightness (asthma)), and 98 with one or more of the mentioned symptoms. The population was derived from a group of 1,133 male pig-farm owners who had completed a self administered questionnaire and who worked at least five hours per day in pig farming. Presence of chronic respiratory symptoms was investigated with the Dutch shortened questionnaire on respiratory symptoms of the British Medical Research Council[15], as part of the self administered questionnaire. In a medical survey held in winter 1990/1991, presence of symptoms was checked and forced expiratory lung function measurements were conducted using a Vicatest-V spirometer (Mijnhardt, Bunnik, the Netherlands). The procedure of testing and selecting of variables was according to standards of the ECCS[16]. Lung function tests were taken between 8.30 a.m. and 15.15 p.m.. Parameters included in the study were: Forced Vital Capacity (FVC), Forced Expiratory Volume in one second (FEV_1), Maximum Mid Expiratory Flow (MMEF): Forced Expiratory Flow between 25% and 75% of FVC, Maximum Expiratory Flow when 75%, 50% and 25% of FVC remains to be exhaled (MEF_{75}, MEF_{50}, MEF_{25}), and Peak Expiratory Flow (PEF).

Exposure data and disinfection

Personal inhalable dust and personal ammonia samples were taken during one work shift in summer and one in winter. Measurements took on average 8.3 hours (standard deviation 0.6, range 5.2-10.4 hours). Ammonia exposure was assessed in duplicate using a passive monitoring method[17]. Basically, GF-A glass fibre filters (diameter 2.4 cm) were coated with a saturated solution of tartaric acid in diethylether and placed in a badge. For entrance membrane a Teflon filter was used. After sampling, 20 ml demineralized water was added to the glass fibre filters which were placed in an ultrasonic bath for 15 minutes. Samples were further analyzed according to a modified indophenol detection method[18], and concentration was assessed by spectrophotometry. The exposure concentration was expressed as time-weighted average exposure. For 163 farmers exposure could be assessed during two days. For them, the log of the geometric mean of both exposure concentrations was used as measure of exposure in epidemiological analyses.

The procedures for dust exposure measurements and analysis of dust samples on endotoxins have been described elsewhere[19,20]. A mathematical modelling technique using regression equations reported elsewhere[21], and data on farm characteristics and time spent on activities in pig farming during two full weeks, was used to estimate long-term average exposure to dust and endotoxins. For 164 farmers data were available on long-term average dust and endotoxin exposure.

Pig farms in the study comprised on average 19 compartments. Compartments in the farrowing and finishing section, and in the section with weaned piglets are small. After emptying a compartment, when animals are brought to another section, compartments are cleaned. Most farmers regularly disinfect after cleaning. Information on use, type and quantity of disinfectant was obtained during a visit to the farm. All visits were done by the same trained interviewer. Type of disinfectant was classified as chloramine-T, quaternary ammonium compounds, quaternary ammonium compounds combined with aldehydes (glutaraldehyde, glyoxal, formaldehyde), a combination of these compounds, and other compounds. 154 out of the 168 farmers using disinfectants gave additional information in an interview by telephone on working practices concerning disinfecting. Information was obtained on: frequency of disinfection on the farm, frequency of disinfection by the farmer, means of application, used pressure, duration of procedure, time until re-entrance of compartments after disinfection, and the use of personal protection equipment during mixing and application of disinfectants.

Data analysis

Associations between respiratory health and determinants of exposure were evaluated in multiple linear regression analysis (PROC REG) for lung function and multiple logistic regression analysis (PROC LOGISTIC) for chronic respiratory symptoms. Associations with lung function were adjusted for age, standing height and smoking habits (pack years). Associations with respiratory symptoms were adjusted for age and smoking habits (current smoking).

In all models, log-transformed values were used for exposure to endotoxins, dust and ammonia. Statistical analyses were performed with SAS/PC version 6.04.

Results

Characteristics of the entire population and of the groups with one or more and without chronic respiratory symptoms (chronic cough, chronic phlegm, ever wheezing, frequent wheezing, shortness of breath, and/or chest tightness (asthma)) are given in Table 5.1. In the entire population, average FVC and PEF exceeded predicted values as given by Quanjer[16], FEV_1 was about equal to the reference value, and other lung function parameters were below reference values. The largest differences with reference values were observed for MEF_{25} and MMEF, with 71% and 82% of predicted, respectively. All lung function parameters expressed as percentage of predicted were lower in symptomatic than in asymptomatic farmers (t-test, $p < 0.01$). The largest differences were approximately 20% and were observed for MMEF, MEF_{75}, MEF_{50} and MEF_{25}. Average age, years in pig farming and prevalence of current smoking was higher among cases. Differences in lung function between symptomatic and asymptomatic farmers remained statistically significant after adjusting for smoking habits in multiple linear regression analysis.

Table 5.1 Characteristics of entire population of pig farmers, and of farmers without and with chronic respiratory symptoms*.

	all (n=198)	asymptomatic (n=100)	symptomatic (n=98)
age (years (SD))	38 (10)	36 (9)	40 (10)
years in pig farming (SD)	15 (8)	13 (8)	16 (8)
standing height (cm (SD))	177 (6)	178 (6)	177 (6)
smoking habits (%)			
current smoker	32	18	45
ex-smoker	32	33	30
never-smoker	36	49	23
base-line lung function (% predicted † (SD))			
FVC (L)	108 (13)	111 (13)	105 (13) ‡
FEV_1 (L)	101 (17)	106 (15)	95 (17) ‡
PEF (L/s)	107 (24)	113 (23)	101 (24) ‡
MMEF (L/s)	82 (31)	91 (29)	72 (29) ‡
MEF_{75} (L/s)	96 (28)	105 (26)	86 (27) ‡
MEF_{50} (L/s)	89 (33)	99 (31)	79 (31) ‡
MEF_{25} (L/s)	71 (33)	79 (31)	62 (32) ‡

* chronic cough, chronic phlegm, ever wheezing, frequent wheezing, shortness of breath, and chest tightness (asthma): no vs. one or more

† according to Quanjer[16]

‡ lower than in asymptomatic farmers (t-test, $p < 0.01$)

Table 5.2 Disinfection procedures in entire population of pig farmers, and in farmers without and with chronic respiratory symptoms*.

	all (n=154)	asymptomatic (n=74)	symptomatic (n=80)
	n (%)	n (%)	n (%)
frequency on farm †			
2 times/week	45 (29.2)	21 (28.4)	24 (30.0)
1 time/week	61 (39.6)	29 (39.2)	32 (40.0)
1 time/2 weeks	25 (16.2)	9 (12.2)	16 (20.0)
less often	22 (14.3)	14 (18.9)	8 (10.0)
pressure			
no pressure	21 (13.6)	13 (17.6)	8 (10.0)
low (< 20 bar)	102 (66.2)	52 (70.3)	50 (62.5)
medium (20-50 bar)	21 (13.6)	8 (10.8)	13 (16.3)
high (> 50 bar)	10 (6.5)	1 (1.4)	9 (11.3)
type of application †			
pressure washer	120 (77.9)	53 (71.6)	67 (83.7)
foam washer	16 (10.4)	10 (13.5)	6 (7.5)
watering can	10 (6.5)	6 (8.1)	4 (5.0)
spray washer	7 (4.5)	4 (5.4)	3 (3.7)
time to reentry after disinfection †			
< half a day	38 (24.7)	22 (29.7)	16 (20.0)
half a day	26 (16.9)	11 (14.9)	15 (18.8)
one day	88 (57.1)	41 (55.4)	47 (58.7)
protection during disinfection †			
no	84 (54.5)	41 (55.4)	43 (53.7)
yes	69 (44.8)	33 (44.6)	36 (45.0)
protection during mixing of disinfectants †			
no	106 (68.8)	51 (68.9)	55 (68.7)
yes	46 (29.9)	22 (29.7)	24 (30.0)
duration of disinfection procedure			
< 5 min	70 (45.5)	38 (51.4)	32 (40.0)
5-10 min	60 (39.0)	30 (40.5)	30 (37.5)
> 10 min	24 (15.6)	6 (8.1)	18 (22.5)

* chronic cough, chronic phlegm, ever wheezing, frequent wheezing, shortness of breath, and chest tightness (asthma): no vs. one or more

† total not equal to 100%, some observations not classifiable

Of the 154 farmers interviewed on disinfection procedures, only one asymptomatic farmer (1.4%) versus 9 symptomatic farmers (11.3%) used high pressure during disinfection (Table 5.2). Six (8.1%) asymptomatic farmers spent each time more than 10 minutes on disinfection, compared to 18 (22.5%) symptomatic farmers. For other aspects of disinfection the distribution was similar in both groups. Average exposure to dust was 2.7 mg/m^3, to endotoxins 111 ng/m^3, and to ammonia 1.7 mg/m^3 (Table 5.3). Exposure levels did not differ between symptomatic and asymptomatic farmers.

Table 5.3 Exposure to endotoxins and ammonia in entire population of pig farmers, and in farmers without and with chronic respiratory symptoms*.

	n	arithmetic mean	geometric mean	geometric standard deviation	range
dust (mg/m^3)					
all	164	2.7	2.6	1.3	0.9 - 5.9
asymptomatic	83	2.7	2.7	1.3	1.5 - 4.5
symptomatic	81	2.6	2.5	1.3	0.9 - 5.9
endotoxins (ng/m^3)					
all	164	111	104	1.5	36.3 - 316
asymptomatic	83	111	104	1.4	41.4 - 216
symptomatic	81	112	103	1.5	36.3 - 316
ammonia (mg/m^3)					
all	163	1.7	1.5	1.6	0.3 - 4.2
asymptomatic	79	1.8	1.6	1.7	0.4 - 4.2
symptomatic	84	1.6	1.5	1.6	0.3 - 3.7

* chronic cough, chronic phlegm, ever wheezing, frequent wheezing, shortness of breath, and chest tightness (asthma): no vs. one or more

In multiple logistic regression analysis, medium or high pressure, and a disinfection procedure lasting more than 10 minutes were statistically significantly and positively associated with respiratory symptoms (Table 5.4). Medium and high pressure were combined because only one asymptomatic farmer used high pressure. In the analysis, no difference was observed between disinfection for less than 5 minutes and 5-10 minutes. These two categories were taken together in the analysis. Exposure level to dust, endotoxins and ammonia, quantity of disinfectants used in one year, and type of disinfectant were not associated with respiratory symptoms. These variables did not affect effect estimates of other variables, and were therefore not included in the model.

Table 5.4 Multiple logistic regression analysis on chronic respiratory symptoms* and disinfection procedure among 154 pig farmers.

	odds ratio	95 % confidence interval	p-value
age (per 10 years)	1.6	(1.1 - 2.3)	0.02
current smoking (yes/no)	3.4	(1.6 - 7.3)	< 0.01
pressure in procedure			
low vs. no	2.2	(0.7 - 6.3)	0.16
medium/high vs. no	7.0	(1.9 - 26.2)	< 0.01
duration of procedure			
> 10 min vs. < 10 min	3.9	(1.3 - 11.6)	0.02

* chronic cough, chronic phlegm, ever wheezing, frequent wheezing, shortness of breath, and chest tightness (asthma): no vs. one or more

For 109 farmers a complete data set was available to test associations between base-line lung function, and exposure to endotoxins and ammonia and disinfection procedures. Log-transformed endotoxin and ammonia exposure was used in the analysis, which means that one unit change stands for an increase in exposure with a factor of 2.72. Such an increase in endotoxin exposure level was weakly associated with a decrease in FVC and FEV_1 of approximately 250 ml in the entire group (Table 5.5, $p < 0.10$). Over the range of exposure levels in our population (5-95 percentile: 54 to 196 ng/m^3), the estimated decrease is about 320 ml. The exposure-response relationships were much stronger in asymptomatic farmers, with estimated decreases of more than 900 ml and 800 ml, respectively, over the range of exposure levels in our population. Exposure levels to ammonia were strongly and inversely related to all lung function parameters, except FVC. For example, an increase in ammonia exposure level with a factor of 2.72 was related to a decrease of 270 ml in FEV_1. The decrease in FEV_1 over the exposure levels in our population (5-95 percentile: 0.6 to 3.2 mg/m^3) was 450 ml.

Table 5.5 Multiple linear regression analysis of lung function on endotoxin exposure, ammonia exposure and disinfection procedure among 109 pig farmers. Adjusted for age, standing height, and smoking habits (pack years).

		regression coefficient	standard error	p-value*
FVC (L)	no vs. low/medium pressure	0.04	0.18	0.413
	high vs. low/medium pressure	- 0.26	0.26	0.165
	> 10 vs. < 10 minutes	- 0.48	0.18	0.004
	endotoxin exposure †	- 0.28	0.17	0.053
	ammonia exposure †	- 0.04	0.13	0.371
FEV_1 (L)	no vs. low/medium pressure	- 0.09	0.18	0.315
	high vs. low/medium pressure	- 0.31	0.26	0.125
	> 10 vs. < 10 minutes	- 0.44	0.18	0.007
	endotoxin exposure †	- 0.23	0.17	0.086
	ammonia exposure †	- 0.27	0.13	0.020
PEF (L/s)	no vs. low/medium pressure	0.36	0.61	0.275
	high vs. low/medium pressure	- 0.81	0.89	0.181
	> 10 vs. < 10 minutes	- 1.09	0.59	0.034
	endotoxin exposure †	- 0.17	0.57	0.380
	ammonia exposure †	- 0.74	0.43	0.044
MMEF (L/s)	no vs. low/medium pressure	- 0.40	0.32	0.102
	high vs. low/medium pressure	- 0.53	0.46	0.125
	> 10 vs. < 10 minutes	- 0.52	0.31	0.047
	endotoxin exposure †	- 0.25	0.30	0.197
	ammonia exposure †	- 0.69	0.22	0.001
MEF_{75} (L/s)	no vs. low/medium pressure	- 0.23	0.57	0.343
	high vs. low/medium pressure	- 1.44	0.83	0.042
	> 10 vs. < 10 minutes	- 1.31	0.55	0.009
	endotoxin exposure †	- 0.16	0.53	0.384
	ammonia exposure †	- 1.12	0.40	0.003
MEF_{50} (L/s)	no vs. low/medium pressure	- 0.55	0.40	0.085
	high vs. low/medium pressure	- 0.94	0.58	0.053
	> 10 vs. < 10 minutes	- 0.67	0.38	0.041
	endotoxin exposure †	- 0.36	0.37	0.165
	ammonia exposure †	- 0.82	0.28	0.002
MEF_{25} (L/s)	no vs. low/medium pressure	- 0.25	0.18	0.082
	high vs. low/medium pressure	- 0.06	0.26	0.405
	> 10 vs. < 10 minutes	- 0.22	0.17	0.108
	endotoxin exposure †	- 0.19	0.17	0.132
	ammonia exposure †	- 0.32	0.13	0.007

* tested one sided

† log-transformed exposure concentration

Relationships between lung function and ammonia were for different parameters 25% to 40% steeper in the group symptomatic farmers than in the entire group. In the group asymptomatic farmers negative but statistically not significant associations were observed.

A disinfection procedure lasting more than 10 minutes was statistically significantly associated with lower values for most lung function variables (Table 5.5).

For example, FVC and FEV_1 were estimated to be 480 and 440 ml less, respectively, in farmers disinfecting more than 10 minutes. Associations with disinfection procedure were not statistically significant in symptomatic farmers.

Overall, the best lung function parameters were observed if low or medium pressure was applied and the worst when using high pressure. Low and medium pressure were taken together in the analysis since their association with lung function was similar. In the entire population, the association with MEF_{75} and MEF_{50} reached (borderline) statistical significance ($p < 0.05$ and $p < 0.10$, respectively). A statistically significant decrease, of about 20%, was only observed for MMEF, MEF_{50} and MEF_{25} among asymptomatic workers. Dust exposure and type of disinfectant were not related to lung function, and did not influence other exposure-response estimates and were not included in the model. In similar models as presented in Table 5.5, with quantity of specific disinfectant used in one year added as explanatory variable, quantity was inversely but not statistically significantly associated with lung function in the entire group. In contrast, the associations with MMEF, MEF_{75}, MEF_{50} and PEF were highly statistically significant among asymptomatic farmers. This effect was independent of duration and frequency of procedure. For each type of disinfectant, the use of median quantity was associated with a similar decrease in lung function, which ranged on average from about 30% for PEF, to 40% for MMEF.

The associations between respiratory symptoms and lung function and determinants of exposure were adjusted for all other variables in the models presented in Table 5.4 and Table 5.5. The different determinants of exposure were independently from each other associated with respiratory health. The association between lung function or chronic respiratory symptoms and other aspects of disinfection, given in Table 5.2, were also evaluated. Of those, only type of application was more or less consistently related to lung function, but this effect is probably the same as observed with pressure since both variables are correlated.

Discussion

In our population of 1,432 male pig farmers, including 299 working daily less than five hours in pig farming, 32.9% reported at least one chronic respiratory symptom. The prevalence rate of these symptoms has been shown to be invariably high in other studies among pig farmers in countries in Europe and Northern America as well[1-6,22]. In our study a duration of disinfection procedure of 10 minutes or more was much more prevalent among farmers with chronic respiratory symptoms than among asymptomatic farmers. The use of higher pressure levels (20 bar or more) during disinfection was positively related to chronic respiratory symptoms as well. Associations with duration and pressure were independent from each other. We are not aware of any other study that reported the use of disinfectants as potential respiratory health hazard for pig farmers.

Exposure levels to ammonia, inhalable dust and endotoxin exposure were not associated with chronic respiratory symptoms.

Characteristics of the disinfection procedure (longer duration, no or high pressure, larger quantity of disinfectants) and exposure level to endotoxins and ammonia were associated with lower base-line lung function in pig farmers. Associations were most clearly present in asymptomatic farmers for disinfection procedure and endotoxin exposure. In this group the estimated effect of increase in endotoxin exposure level with a factor of 2.72 and a disinfection procedure of more than 10 minutes were each associated with a decrease of about 15% in FVC and parameters in the first part of the spirogramme. The magnitude of the statistically significant relationships with ammonia exposure differed, and ranged from approximately 5% for FEV_1 to 18% for MMEF in the entire population, but associations were 25% to 40% steeper in symptomatic farmers.

Symptomatic farmers were oversampled in our study. Data on lung function as presented for the entire population are therefore not representative for the base population. Exposure-response relationships for a representative sample of the base population are expected to be closer to those reported for asymptomatic than for symptomatic farmers. When the results for our asymptomatic farmers are being compared with other studies we can make the following observations. Similar patters for level of lung function parameters were found, with FVC and parameters in the primary part of the spirogramme (FEV_1, PEF) in the same range as reference values, and flow-volume parameters MMEF and MEF_x lower than reference values[1-5,7,23]. A decrease in parameters of the final part of the spirogramme is regarded as the earliest change associated with flow limitation, even if the initial part of the spirogramme is unaffected[24].

Duration of the disinfection procedure was consistently associated with lung function and respiratory symptoms. Multiple regression analyses showed that this could not be attributed to the use of larger quantities of disinfectant in one year, type of disinfectant, other evaluated aspects of the disinfection procedure, or differences in exposure levels to dust, endotoxins or ammonia. Potentially, this relationship found for duration of disinfection reflects an association with the level to ambient exposure concentration and/or critical time of exposure.

Most commonly used disinfectants contain quaternary ammonium compounds with or without aldehydes (glutaraldehyde, glyoxal, formaldehyde), or chloramine-T. In the literature, several effects following exposure to chloramine-T have been reported. These include respiratory symptoms such as cough, wheezing and dyspnoea, and immediate and late asthmatic reactions. Effects on eyes and the upper respiratory system are reported either together with or without symptoms of the lower respiratory system[8,9,11,25]. Recently, two cases have been reported with occupational asthma due to exposure to disinfectants containing quaternary ammonium compounds[13,14]. Glutaraldehyde has been shown to be able to induce occupational asthma by means of serial PEF measurements[10,12]. Norbäck et al.[26] performed a cross-sectional study among 39 workers in medical services exposed to glutaraldehyde levels below the Swedish occupational exposure limits and 68 unexposed controls. They reported differences in symptom prevalence of the upper respiratory tract, but not of symptom prevalence of the lower respiratory tract. To our knowledge this is the only study reporting prevalence of respiratory health effects in an occupational group exposed to quaternary ammonium compounds, glutaraldehyde, or chloramine-T.

Respiratory effects of exposure to formaldehyde, mostly from sources other than disinfectants, have frequently been reported. These include occupational asthma, effects on base-line lung function and associations with respiratory symptoms in some occupational groups, although other studies fail to show such associations[27-30].

In the entire group, the estimated decrease in FVC and FEV_1 of about 250 ml with an increase in endotoxin exposure level with a factor of 2.72 was borderline statistically significant. In asymptomatic farmers the associations were statistically significant, and estimated decreases in lung function were 2.5 - 3 times larger (approximately 14% of median of the lung function). The decrease in the entire population was similar to that reported by Heederik et al.[6] in a group of 62 farmers. Concentrations of endotoxins in swine confinement buildings and interindividual variation in exposure in that study were in the same range as that of personal exposure in our study. They reported a decrease in FVC of 263 ml ($p < 0.10$) and in FEV_1 of 208 ml ($p < 0.05$) per 100 ng/m^3 increase in endotoxin concentration. Such an increase is similar to the difference between the 10 and 90 percentile of the exposure distribution in our study, which corresponds with an increase of nearly a factor of 2.72. However, taking oversampling of symptomatic farmers into account, the exposure-response relationship is expected to be about a factor two steeper in our study. Zejda et al.[7] reported in a group of 46 pig farmers a borderline statistically significant decrease in FVC of 145 ml at an increase in endotoxin concentration level in swine buildings with a factor of 2.72, about 40% smaller than in our study. In their study FEV_1 was not related to endotoxin concentration. They reported an endotoxin exposure level that was five times higher than in our study, despite similar dust exposure levels. Endotoxin concentration multiplied by number of hours working in swine buildings was strongly related to FVC ($p=0.02$), and weakly related to FEV_1 ($p=0.06$) in their study. However, it is not clear whether this finding can be attributed to number of hours alone, which might be related to any type of exposure.

No detailed comparison can be made between our study and that of Heederik et al.[6] and Zejda et al.[7], since exposure was not based on personal exposure measurements in their studies. Generally there is poor correlation between exposure assessed by ambient air sampling and personal sampling[19,31]. It is unlikely that static measurements induced only systematic bias, since exposure levels largely depend on the activity of pigs caused by disturbance by the farmer, and by the own activity of the farmer[21,32].

The inverse association between lung function and exposure to ammonia was not expected, since ammonia is hygroscopic and therefore dose is generally expected to be low. In free form it will be captured in the upper part of the respiratory tract. However, it has been suggested that dust particles can act as carriers that bring ammonia into smaller airways[33,34], which may explain our results. In the epidemiological studies by Heederik et al.[6] and Zejda et al.[7], reported exposure levels were three to seven times higher than in our study. Associations adjusted for confounding factors between ammonia exposure levels and either base-line lung function or chronic respiratory symptoms were not observed or reported in these and other studies. Reports on effects of chronic exposure to ammonia at any exposure level are sparse. Holness et al.[35] studied 58 workers exposed to on average 9.2 mg/m^3 ammonia in the soda ash industry, but did not find differences in symptoms and lung function between exposure categories and neither with control workers.

Exposure-response relationships differed for respiratory symptoms and lung function. Also other studies in pig farming and the animal feed industry, using the same questionnaire and lung function tests, showed this phenomenon[6,36]. In addition, associations with lung function differed between symptomatic and asymptomatic farmers in our study. These inconsistencies may have different reasons. First, different biological mechanisms may be underlying these discrepancies. Secondly, symptomatic farmers may adjust their working practice in order to limit inhaled dose of pollutants which they associate with symptoms. This may be done by avoiding activities with high (dust related) exposure, using dust masks, and more subtle behavioral changes. In addition, symptomatic farmers may be more sensitive to normal ammonia exposure levels encountered in pig farming, whereas at these exposure levels ammonia does not have to cause respiratory symptoms in symptom free farmers.

Lung function tests were taken between 8.30 a.m. and 15.15 p.m., but no indications were found that discrepancies in relationships between exposure and lung function could be attributed to acute effects of exposure prior to lung function testing in either asymptomatic or symptomatic farmers.

In conclusion, our results suggest that the etiology of chronic respiratory health effects in pig farmers is multifactorial. Disinfectants were until this study not regarded as potential health hazards. In our study, exposure to disinfectants was only based on qualitative information and used quantity. The relationships need to be substantiated by actual exposure measurements.

Associations with endotoxin exposure levels support the hypothesis of endotoxins to be causally related to chronic respiratory health effects. However, inconsistency between relationships in reported symptoms and tested lung function, and in lung function between symptomatic and asymptomatic farmers requires more attention. It is expected that cross-sectional studies can add little more to the insight into the etiology of respiratory symptoms of pig farmers, than already obtained from our study. Our study involved very labour intensive and costly fieldwork campaigns. However, small interindividual variation in exposure in the presence of relatively large intraindividual variation, changing levels of exposure over a longer period of time, the multifactorial etiology of symptoms and combined exposures, require preferably prospective study designs, with an even more detailed quantification of exposure and investigation of health effects.

Acknowledgements

The study was supported by the Dutch Prevention fund (grant no. 2821440), and the NCB Pig Farmers Association in the Southern Netherlands. The authors wish to thank Dr. Stijn Gielen for lung function testing and employees of the Animal Health Service in the Southern Netherlands, and participating students, for their assistance in data collection.

References

1. Bongers P, Houthuijs D, Remijn B, Brouwer R, Biersteker K. Lung function and respiratory symptoms in pig farmers. Br J Ind Med 1987;44:819-23.
2. Holness DL, O'Blenis EL, Sass-Kortsak A, Pilger C, Nethercott JR. Respiratory effects and dust exposures in hog confinement farming. Am J Ind Med 1987;11:571-80.
3. Dosman JA, Graham BL, Hall D, Pahwa P, McDuffie HH, Lucewicz M, To T. Respiratory symptoms and alterations in pulmonary function tests in swine producers in Saskatchewan: Results of a survey of farmers. J Occup Med 1988;30:715-20.
4. Donham K, Haglind P, Peterson Y, Rylander R, Belin L. Environmental and health studies of farm workers in Swedish swine confinement buildings. Br J Ind Med 1989;46:31-7.
5. Zejda JE, Hurst TS, Rhodes CS, Barber EM, McDuffie HH, Dosman JA. Respiratory health of swine producers. Focus on young workers. Chest 1993;103:702-9.
6. Heederik D, Brouwer R, Biersteker K, Boleij JSM. Relationship of airborne endotoxin and bacteria levels in pig farms with the lung function and respiratory symptoms in farmers. Int Arch Occup Environ Health 1991;62:595-601.
7. Zejda JE, Barber E, Dosman JA, Olenchock SA, McDuffie HH, Rhodes C, Hurst T. Respiratory health status in swine producers relates to endotoxin exposure in the presence of low dust levels. J Occup Med 1994;36:49-56.
8. Bourne MS, Flindt MLH, Miles Walker J. Asthma due to industrial use of chloramine. Br Med J 1979;2:10-2.
9. Dijkman JH, Vooren PH, Kramps JA. Occupational asthma due to inhalation of chloramine-T. I. Clinical observations and inhalation-provocation studies. Int Arch Allergy Appl Immunol 1981;64:422-7.
10. Benson WG. Case Report. Exposure to glutaraldehyde. J Soc Occup Med 1984;34: 63-4.
11. Blomqvist AM, Axelsson IGK, Danielsson D, Kiviloog J, Ulander A, Zetterström O. Atopic allergy to chloramine-T and the demonstration of specific IgE antibodies by the radioallergosorbent test. Int Arch Occup Environ Health 1991;63:363-5.
12. Chan-Yeung M, McMurren T, Catonio-Begley C, Lam S. Occupational asthma in a technologist exposed to glutaraldehyde. J Allergy Clin Immunol 1993;91:974-8.
13. Burge PS, Richardson MN. Occupational asthma due to indirect exposure to lauryl dimethyl benzyl ammonium chloride used in a floor cleaner. Thorax 1994;49:842-3.
14. Bernstein JA, Stauder T, Bernstein DI, Bernstein IL. A combined respiratory and cutaneous hypersensitivity syndrome induced by work exposure to quaternary amines. J Allergy Clin Immunol 1994;94:257-9.

15. Biersteker K, Dijk WH van, Eissens JBMF, Geuns HA van. Ervaringen met geneeskundig onderzoek op CARA bij gemeentepersoneel te Rotterdam, 1970-1971. Tijdschr Soc Geneeskd 1974;52:158-62.
16. Quanjer PhH. Standardized lung function testing. Report of the Working Party "Standardization of Lung Function Tests". Bull Eur Physiopathol Respir 1983;19 (suppl 5):1-95.
17. Willems JJH, Hofschreuder P. A passive monitor for measuring ammonia. In: Allegrini I, Febo A, Perrino C, editors. Proceedings of the workshop on Field intercomparison exercise on ammonia and ammonium measurement, April 29 to May 4, 1990, Rome. Commission of the European Communities, Directorate-General for Science, Research and Development. Air Pollution Research Report 37;113-8.
18. Nederlands Normalisatie Instituut 1983. Fotometrische bepaling van het gehalte aan ammonium. NEN-6472.
19. Smid T, Heederik D, Mensink G, Houba R, Boleij JSM. Exposure to dust, endotoxins, and fungi in the animal feed industry. Am Ind Hyg Ass J 1992;53:362-8.
20. Hollander A, Heederik D, Versloot P, Douwes J. Inhibition and enhancement in the analysis of airborne endotoxin levels in various occupational environments. Am Ind Hyg Assoc J 1993;54:647-53.
21. Preller L, Heederik D, Kromhout H, Boleij JSM, Tielen MJM. Determinants of dust and endotoxin exposure of pig farmers: development of a control strategy using empirical modelling. Ann Occup Hyg 1995. Accepted for publication.
22. Choudat D, Goehen M, Korobaeff M, Boulet A, Dewitte JD, Martin MH. Respiratory symptoms and bronchial reactivity among pig and dairy farmers. Scan J Work Environ Health 1994;20:48-54.
23. Zhou C, Hurst TS, Cockcroft DW, Dosman JA. Increased airways responsiveness in swine farmers. Chest 1991;99:941-4.
24. American Thoracic Society: Lung function testing: selection of reference values and interpretative strategies. Am Rev Respir Dis 1991;144:1202-18.
25. Wass U, Belin L, Eriksson NE. Immunological specifity of chloramine-T-induced IgE antibodies in serum from a sensitized worker. Clin Exp Allergy 1989;19:463-71.
26. Norbäck D. Skin and respiratory symptoms from exposure to alkaline glutaraldehyde in medical services. Scand J Work Environ Health 1988;14:366-71.
27. Burge PS, Harries MG, Lam WK, O'Brien IM, Patchett PA. Occupational asthma due to formaldehyde. Thorax 1985;40:255-60.
28. Nordman H, Keskinen H, Tuppurainen-M. Formaldehyde asthma--rare or overlooked? J Allergy Clin Immunol 1985;75:91-9.
29. Horvath EP, Anderson H, Pierce WE, Hanrahan L, Wendlick JD. Effects of formaldehyde on the mucous membranes and lungs. A study of an industrial population. JAMA 1988;259:701-7.
30. Malaka T, Kodama AM. Respiratory health of plywood workers occupationally exposed to formaldehyde. Arch Environ Health 1990;45:288-94.
31. Cinkotai, FF, Gibbs, ACC Sharpe TC. A comparison of exposure to airborne dust in cotton processing plants estimated from personal and workzone samples. Ann Occup Hyg 1984;28:347-52.
32. Zeitler-Feicht MH, Praml GJ, Riedel H. Die zeitliche Variation der Feinstaubbelastung der Luft im Mastschweinestall. Tierhygienische und arbeitsmedizinische Aspekte. Berl Münch Tierärztl Wschr 1991;104:84-9.
33. Donham KJ, Scallon LJ, Popendorf W, Treuhaft MW, Roberts RC. Characterization of dusts collected from swine confinement buildings. Am Ind Hyg Assoc J 1986;47:404-10.

34. Schwartz DA, Landas SK, Lassise DL, Burmeister LF, Hunninghake GW, Merchant JA. Airway injury in swine confinement workers. Ann Internal Med 1992;116:630-5.
35. Holness DL, Purdham JT, Nethercott JR. Acute and chronic respiratory effects of occupational exposure to ammonia. Am Ind Hyg Assoc J 1989;50:646-50.
36. Smid T, Heederik D, Houba R, Quanjer PH. Dust- and endotoxin-related respiratory effects in the animal feed industry. Am Rev Respir Dis 1992;146:1471-9.

6. Exposure to disinfectants and endotoxins in relation to atopic sensitization and symptoms consistent with asthma

Liesbeth Preller, Gert Doekes, Dick Heederik, Roel Vermeulen,
Peter F.J. Vogelzang, Jan S.M. Boleij

Abstract

In a population of 198 Dutch pig farmers we evaluated associations between chronic respiratory symptoms, specific and total serum IgE levels, use of disinfectants, and endotoxin exposure levels. In the total population and a population restricted according to mild bronchial responsiveness to histamine (n=126: symptomatic and PC_{10} $\leq$ 16 mg/ml, asymptomatic and PC_{10} > 16 mg/ml), the combination of IgE to common allergens (house dust mite, grass pollen, birch pollen) and use of any disinfectant containing quaternary ammonium compounds was more prevalent among symptomatics than asymptomatics (OR 4.3, 95%-CI 1.3-14.1, and OR 8.7, 95%-CI 1.7-44.3, respectively). A combination of atopic sensitization and high endotoxin exposure level (> 101 ng/m^3) was associated with respiratory symptoms in the restricted population only (OR 6.3, 95%-CI 1.0-37.9). Atopy, use of quaternary ammonium compounds, and endotoxin exposure level were independently not associated with respiratory symptoms. IgE to common allergens was associated with use of disinfectants containing only quaternary ammonium compounds (OR 7.3, 95%-CI 1.3-42.5). ORs for other disinfectants ranged from 2.2 to 4.2 ($p > 0.05$). Atopic sensitization was not associated with the endotoxin exposure level. Our results suggest that occupational exposure to non-allergenic agents may induce IgE sensitization to common aeroallergens (disinfectants only), and that the combination of atopy and exposure to these agents (disinfectants and endotoxin) is an important risk factor for development of symptoms consistent with asthma.

Submitted in revised form to American Journal of Respiratory and Critical Care Medicine

Introduction

Many people suffer from allergic respiratory disorders, among which asthma. These allergic disorders are characterized by an increased production of allergen-specific IgE antibodies. Sensitization, and subsequent development of allergic respiratory symptoms, depend on several intrinsic and extrinsic factors. The inherited propensity to produce specific IgE antibodies in response to environmental allergens is of primary importance, and allergen exposure has been shown to be a relevant risk factor for both atopic sensitization as well as the induction of respiratory symptoms in sensitized individuals[1,2]. However, not all genetically predisposed and allergen exposed subjects become sensitized, and not all sensitized and exposed individuals develop allergic asthma. Thus there must be other co-factors that determine the risk of atopic sensitization and development of asthma[2,3]. Results of human experimental and epidemiological studies indicate that exposure to non-allergenic air pollutants might be such a co-factor in the onset of allergic disease or in exacerbation of symptoms[4-6]. It has also been suggested that air pollutants may act as a co-factor in atopic sensitization. This latter hypothesis is mainly based on results of experimental animal studies. In these studies, exposure to ozone, diesel-exhaust particles, and SO_2, combined with exposure to airborne allergens, resulted in increased risk of atopic sensitization[7-9]. Combined exposures to allergens and non-allergenic air pollutants are very common in indoor, outdoor and occupational environments. However, despite increased interest in the role of air pollutants as co-factors that modify the response to allergens, so far no studies have been reported that exposure to such pollutants in these environments was associated with an increase in atopic sensitization.

In this report we describe epidemiological findings in a group of pig farmers. This occupational group is exposed to a variety of airway irritants as manure gases like NH_3 and H_2S, chemicals used for disinfection, bacterial endotoxins from manure and animal feed, and to potential allergens originating from animals and feed. The prevalence of work-related respiratory symptoms is known to be high among pig farmers, but the etiology is uncovered to a limited extent[10]. This study was undertaken to investigate whether non-allergenic occupational exposures and atopic sensitization were risk factors for chronic respiratory symptoms in general and for symptoms consistent with asthma in particular. In addition, the association between IgE sensitization and non-allergenic occupational exposures was studied.

Methods

Population and health data

The population existed of 198 pig farmers, living in the two southeastern provinces of the Netherlands. The population was recruited from a group of 1,133 male pig-farm owners who worked at least five hours per day in pig farming. Selection was based on chronic respiratory symptoms reported in the Dutch version of a shortened questionnaire on respiratory symptoms of the British Medical Research Council[11]. This was part of a self administered questionnaire, in which farmers reported also allergies to pets, birds and house dust mite, food allergy, hay fever, and allergies to other agents which they had to specify. 100 farmers were selected without chronic respiratory symptoms, and 98 were selected with one or more symptoms of: chronic cough, chronic phlegm, ever wheezing, frequent wheezing, shortness of breath, and chest tightness (asthma). Presence or absence of the symptoms was checked in a medical survey held in winter 1990/1991. During this survey venous blood samples were taken for analysis on IgE antibodies. Non-Specific Bronchial Responsiveness (NSBR) was tested by histamine provocation among 195 of the 198 farmers according to a modified procedure of the method described by Cockcroft et al.[12]. Histamine concentration ranged from 0.03 mg/ml to 16 mg/ml.

IgE measurements

Sera were stored at -20°C until IgE analysis. Total serum IgE was determined with a sandwich enzyme immunoassay, in which diluted serum samples (routinely 1/10, 1/20 and 1/40) were incubated in microwells coated with monoclonal mouse anti-human IgE (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (CLB), Amsterdam), and bound IgE was quantified with a peroxidase conjugate prepared from the same monoclonal anti-IgE (CLB), and ortho-phenylenediamine (OPD) as the peroxidase substrate. The assay was calibrated by including in each assay serial dilutions of IgE reference preparations containing 1,000 IU/ml (Kabi Pharmacia). The assay has a sensitivity of approximately 1 IU/ml, and intra- and interassay CV-values of less than 15% (Doekes et al., unpublished observations).

Specific IgE to house dust mite (*Dermatophagoides pteronyssinus*), grass pollen (1 : 1 mixture of *Lolium perenne* and *Phleum pratense*), birch pollen (*Betula verrucosa*) and cat allergen was assessed with a modification of a previously described EIA[13]. Microwells were coated at 0.025 mg/ml with commercially available lyophilized extracts (ALK Benelux, Houten, the Netherlands) of the allergens. Sera were incubated at a 1/10 dilution, and bound IgE was measured by subsequent incubations with monoclonal mouse anti-human IgE (1/16,000; CLB), biotinylated rabbit IgG anti-mouse Ig (1/5000; Dakopatts, Copenhagen, Denmark), avidin-peroxidase (1/2000; DAKO) and OPD. This assay correlates very well with commercially available test kits for specific serum IgE and has a similar sensitivity and specificity with regard to skin prick tests (Doekes et al., unpublished observations). In each plate positive sera containing IgE to the tested allergens were included as positive controls.

All serum-allergen combinations giving an OD value exceeding the OD+3 SD of the reagent blank (no serum control) were retested, all on the same day, together with an equal number of randomly selected negative sera. In the second assay, the OD+3 SD was also used as the cut-off value. In this way a small number of sera with a weakly positive reaction in the first test were eventually classified as negative, while all of the retested negative serum-allergen combinations remained negative in the second test.

IgE reacting with chloramine-T was assessed in microwells coated with human serum albumin and treated with chloramine-T and $Na_2S_2O_3$[14]. The binding of specific IgE was quantified as described above. IgE with a specificity for quaternary ammonium compounds (QAC) was assessed using the Phadezym RAST method (Kabi Pharmacia; Uppsala, Sweden) with discs containing suxamethonium. The test was done in a selected group of 40 pig farmers including all 19 using disinfectants containing QAC only, and 21 using QAC in combination with other active compounds.

Exposure data

Most farmers use disinfectants when cleaning animal housing, and they generally use disinfectants about once a week during less than 15 minutes. Information on use and type of disinfectant was obtained by a visit to the farm. All visits were done by the same trained interviewer. Disinfectants were categorized according to active ingredients. Most commonly used disinfectants contain QAC with or without aldehydes (glutaraldehyde, glyoxal, formaldehyde), or chloramine-T.

Personal dust samples were taken twice and analyzed on endotoxin according to procedures described by Hollander et al.[15]. A mathematical modelling technique similar to that used by Eisen et al.[16] and data on farm characteristics and time spent on activities in pig farming during two full weeks, was used to estimate long-term average exposure to endotoxins. In this way, for 164 farmers data on endotoxin exposure was available.

Data analysis

Associations between chronic respiratory symptoms, IgE sensitization or self-reported allergies as outcome variables, and risk factors were evaluated by means of a multiple logistic regression analysis. Associations between respiratory symptoms and risk factors were studied in the entire population and in a population restricted according to mild bronchial responsiveness to histamine. Mild bronchial responsiveness was defined as a decrease in FEV_1 of at least 10% at a histamine concentration $\leq$ 16 mg/ml (PC_{10} $\leq$ 16 mg/ml)[17]. This selection procedure can be described as followed:

included: asymptomatic, no bronchial responsiveness (n=83);
symptomatic, mild bronchial responsiveness (n=43);
excluded: asymptomatic, bronchial responsiveness (n=17);
symptomatic, no bronchial responsiveness (n=55).

IgE sensitization to common allergens was defined as a positive reaction to one or more common allergens. Total IgE levels were dichotomized, with 100 IU/ml taken as cut-off level. Exposure to endotoxins was dichotomized by taking the median exposure level of 101 ng/m^3 observed in this population as cut-off point.

Statistical analyses were performed with SAS/PC version 6.04[18] using PROC LOGISTIC.

Results

Population

Table 6.1 gives an overview of the study population, for the entire population of 198 farmers and the restricted population of 126 farmers comprising the 43 with chronic respiratory symptoms and mild bronchial responsiveness (PC_{10} $\leq$ 16 mg/ml), and the 83 farmers without chronic respiratory symptoms and negative bronchial responsiveness. The cases in the restricted population had predominantly reported symptoms indicative of variable airflow obstruction (ever wheezing, frequent wheezing, shortness of breath, and/or chest tightness (asthma)). Relatively few farmers reporting only bronchitis-like symptoms (chronic cough and/or chronic phlegm) were present in the restricted case group.

Table 6.1 Characteristics of population of 198 Dutch pig farmers and population restricted according to mild bronchial responsiveness to histamine.

	entire population		restricted population	
	no symptoms*	symptoms*	no symptoms, no NSBR †	symptoms, NSBR †
	(n=100)	(n=98)	(n=83)	(n=43)
age (years, mean [SD])	36 [9]	40 [10]	35 [8]	43 [10]
smoking habits (n (%))				
current smoker	18 (18)	45 (46)	14 (17)	23 (53)
ex-smoker	32 (32)	30 (31)	27 (33)	11 (26)
never-smoker	50 (50)	23 (23)	42 (51)	9 (21)
$PC_{10} \leq 16$ mg histamine/ml (n (%))	17 (17)	43/95 (45)	0 (0)	43 (100)
IgE to common allergens (n (%))				
1 or more	13 (13)	20 (20)	12 (15)	8 (19)
house dust mite	7 (7)	14 (14)	6 (7)	7 (16)
grass pollen	6 (6)	6 (6)	5 (6)	2 (5)
birch pollen	3 (3)	2 (2)	3 (4)	0 (0)
cat allergens	0 (0)	0 (0)	0 (0)	0 (0)
total IgE > 100 IU/ml (n (%))	22 (22)	31 (32)	19 (23)	16 (37)
total IgE (IU/ml, median)	28	49	28	61
self-reported allergies (n (%))	2/96 (2)	22/86 (26)	1/80 (1)	12/37 (32)
disinfectant (n (%))				
none	19 (19)	11 (11)	19 (23)	5 (12)
chloramine-T	25 (25)	29 (30)	21 (25)	13 (30)
QAC ‡	11 (11)	8 (8)	8 (10)	6 (14)
QAC + aldehydes	25 (25)	27 (28)	19 (23)	10 (23)
QAC + aldehydes + chloramine-T	7 (7)	10 (10)	6 (7)	5 (12)
other	13 (13)	13 (13)	10 (12)	4 (9)
endotoxin exposure (ng/m^3, median)	102	101	100	109

* chronic cough, chronic phlegm, ever wheezing, frequent wheezing, shortness of breath, and/or chest tightness (asthma)

† NSBR: non-specific bronchial responsiveness. No: $PC_{10} > 16$ mg histamine/ml, yes: $PC_{10} \leq 16$ mg/ml

‡ quaternary ammonium compounds

In the entire population, 33 farmers had detectable IgE levels to one or more of the common allergens, but none showed sensitization to cat allergens. 53

farmers had a total IgE level exceeding 100 IU/ml. 182 farmers had valid observations on self-reported allergies. A vast majority reporting allergies, fell into the category of farmers with chronic respiratory symptoms. None of the 198 farmers had detectable specific IgE to chloramine-T, and two out of 40 farmers using QAC reacted positively to QAC, RAST class 1 and 2, respectively (not shown in table).

Respiratory symptoms and risk factors

The crude data in Table 6.1 show that IgE sensitization to house dust mite and a high total serum IgE level was more prevalent among cases than among controls. More cases than controls used disinfectants. Additionally, cases were older and more were smoking. In subsequent multiple logistic regression analyses, associations between respiratory symptoms, IgE sensitization and exposure were adjusted for smoking habits (current smoking yes/no) and age. The results for the entire and restricted population are shown in Table 6.2. IgE sensitization to one or more common allergens was strongly and positively related to respiratory symptoms, and seemed to be a strong predictor within the population restricted according to mild bronchial responsiveness (asymptomatic and $PC_{10} > 16$ mg/ml, or symptomatic and $PC_{10} \leq 16$ mg/ml). The associations between chronic respiratory symptoms and high total serum IgE level were positive but statistically not significant. The use of disinfectants tended to be positively related to respiratory symptoms, but none of the associations were statistically significant. The highest Odds Ratio (OR), being 3.3 (95%-CI 0.6 - 18.2), was estimated for the use of QAC in the restricted population. No differences existed in endotoxin exposure between cases and controls.

Interaction between specific IgE sensitization and the use of disinfectants was tested by grouping individuals according to sensitization and the use of any disinfectant containing QAC. The combination of the use of disinfectants containing QAC, together with specific IgE to common allergens was significantly more prevalent among symptomatics than asymptomatics, whereas atopic sensitization and the use of QAC were independently not associated with respiratory symptoms, with ORs close to 1 (Table 6.3, model 1). The association between symptoms, and the combination of specific IgE sensitization with the use of QAC was strongest within the population restricted according to mild bronchial responsiveness, the OR being 8.7 (95%-CI 1.7 - 44.3).

Table 6.2 Multiple logistic regression analysis on associations between chronic respiratory symptoms and risk factors.

	entire population		restricted population*	
	OR	(95%-CI)	OR	(95%-CI)
MODEL 1; specific IgE				
against common allergens †	2.3	(1.0 - 5.3)	4.1	(1.1 - 14.6)
age (per 10 years)	1.7	(1.2 - 2.3)	3.2	(1.9 - 5.5)
current smoking (yes/no)	3.9	(2.0 - 7.5)	5.6	(2.2 - 14.5)
MODEL 2; total IgE ‡				
> 100 vs. ≤ 100 IU/ml	1.8	(0.9 - 3.6)	2.5	(0.9 - 6.7)
MODEL 3; disinfectants ‡				
none	1.0		1.0	
chloramine-T	1.7	(0.6 - 4.4)	1.9	(0.5 - 7.6)
QAC §	1.3	(0.4 - 4.5)	3.3	(0.6 - 18.2)
QAC + aldehydes	1.7	(0.7 - 4.6)	1.5	(0.3 - 6.2)
QAC + aldehydes + chloramine-T	1.8	(0.5 - 6.6)	1.5	(0.2 - 9.3)
other	1.6	(0.5 - 5.0)	1.2	(0.2 - 6.7)
MODEL 4; endotoxin exposure ‡				
> 101 vs. ≤ 101 ng/m^3	0.8	(0.4 - 1.5)	1.7	(0.7 - 4.4)

* chronic respiratory symptoms and PC_{10} ≤ 16 mg histamine/ml, no chronic respiratory symptoms and PC_{10} > 16 mg/ml

† IgE to 1 or more common aeroallergens: house dust mite, grass pollen, birch pollen, cat allergens

‡ adjusted for age and smoking habits

§ quaternary ammonium compounds

Interaction between specific IgE to common allergens and endotoxin exposure was tested in a similar way. In the restricted population, a high exposure level and specific IgE sensitization were independently only moderately associated with respiratory symptoms, but the combination of both risk factors was strongly associated with respiratory symptoms, the estimated OR being 6.3 (95%-CI 1.0 - 37.9, Table 6.3, model 2). The combination of risk factors was not significantly associated with respiratory symptoms in the entire population.

Similar analyses with dichotomized total IgE level showed comparable but weaker trends than presented in Table 6.3. The use of QAC, a high endotoxin exposure level, and a high total IgE level (> 100 IU/ml) were independently not more prevalent among symptomatics than asymptomatics. In the restricted population, the combinations of high total IgE level with either the use of QAC or high endotoxin exposure level, were significantly associated with respiratory symptoms. The estimated OR was 4.9 (95%-CI 1.0 - 23.7) for the combination with the use of QAC, and 4.4 (95%-CI 1.0 - 19.1) for the combination with high endotoxin exposure. In the entire population respiratory symptoms were statistically significantly associated with the combination of a high total IgE level and the use of QAC only (OR=4.0, 95%-CI 1.2 - 12.9). The results of the analyses based on total IgE level agree with results found for specific IgE sensitization.

Table 6.3 Multiple logistic regression analysis on associations between respiratory symptoms, atopy* and exposure to QAC or endotoxins. Adjusted for age and smoking habits.

	entire population			restricted population †		
	n	OR	(95%-CI)	n	OR	(95%-CI)
MODEL 1; QAC ‡						
no QAC, no atopy	96	1.0		63	1.0	
QAC, no atopy	69	0.8	(0.4 - 1.6)	43	0.8	(0.3 - 2.3)
no QAC, atopy	14	1.0	(0.3 - 3.3)	9	1.4	(0.2 - 9.3)
QAC, atopy	19	4.3	(1.3 - 14.1)	11	8.7	(1.7 - 44.3)
MODEL 2; endotoxin exposure §						
low, no atopy	67	1.0		42	1.0	
high, no atopy	69	0.7	(0.4 - 1.5)	44	1.5	(0.5 - 4.4)
low, atopy	15	1.5	(0.4 - 4.9)	9	2.1	(0.3 - 15.6)
high, atopy	13	1.9	(0.5 - 6.8)	9	6.3	(1.0 - 37.9)

* IgE to ≥ 1 common allergens

† chronic respiratory symptoms and $PC_{10} \leq 16$ mg histamine/ml, no chronic respiratory symptoms and $PC_{10} > 16$ mg/ml

‡ QAC= quaternary ammonium compounds only and QAC in combination with aldehydes or with aldehydes and chloramine-T

§ low: ≤ 101 ng endotoxin/m^3, high: > 101 ng/m^3

IgE and exposure

To study if the interaction between IgE sensitization and QAC or endotoxin exposure was caused by NSBR of atopics or an adjuvant effect, associations between IgE sensitization and these exposures were evaluated in the entire population of 198 farmers (Table 6.4). Associations were adjusted for age and current smoking. The estimated ORs for associations between specific or total IgE and current smoking ranged between 1.1 and 1.4 in the separate models, but did not reach statistical significance. IgE sensitization to common allergens was significantly and positively associated with the use of disinfectants containing QAC only, with an estimated OR of 7.3 (95%-CI 1.3 - 42.5). The association with disinfectants containing QAC in combination with aldehydes was borderline statistically significant (OR 4.2, 95%-CI 0.8 - 20.7). The ORs for the use of other disinfectants were all larger than two but these associations did not reach statistical significance. Regrouping farmers according to the use of any disinfectant containing QAC showed a positive association with specific IgE sensitization that was borderline statistically significant (OR=2.0, 95%-CI 0.9-4.3). In contrast, no clear positive association was observed between specific IgE sensitization and level of endotoxin exposure (higher or lower than median value of 101 ng/m^3).

There were no indications that total IgE level was associated with either the use of (specific) disinfectants or the level of endotoxin exposure.

Table 6.4 Multiple logistic regression analysis on association between atopy* and use of disinfectants (n=198) and endotoxin exposure (n=164).

	n	OR	(95%-CI)
MODEL 1; disinfectants			
no disinfectants	30	1.0	
chloramine-T	54	2.8	(0.5 - 14.3)
QAC †	19	7.3	(1.3 - 42.5)
QAC + aldehydes	52	4.2	(0.8 - 20.7)
QAC + aldehydes + chloramine-T	17	2.2	(0.3 - 18.0)
other	26	2.6	(0.4 - 15.7)
age (per 10 years)		0.6	(0.4 - 0.9)
current smoking		1.1	(0.5 - 2.5)
MODEL 2; endotoxin exposure ‡			
> 101 vs. ≤ 101 ng/m^3		0.9	(0.4 - 2.1)

* IgE to ≥ 1 common allergens

† quaternary ammonium compounds

‡ adjusted for age and smoking habits

Self-reported allergies

The use of specific disinfectants was not statistically significantly related to self-reported allergies (Table 6.5). There was a strong and positive association between self-reported allergies and endotoxin concentration. The odds ratio was 5.5 (95%-CI 1.7-17.6). Adjustment for atopic sensitization to common allergens slightly increased the odds ratio to 6.9 (95%-CI 2.0-23.4). In the group of symptomatic farmers, the estimated odds ratios were larger (not shown).

Table 6.5 Multiple logistic regression analysis on association between self-reported allergies and use of disinfectants (n=182) and endotoxin exposure (n=153) (adjusted for age and smoking habits).

	n	OR	(95%-CI)
MODEL 1; disinfectants			
no disinfectants	27	1.0	
chloramine-T	52	1.9	(0.5 - 7.7)
QAC †	19	1.6	(0.3 - 8.9)
QAC + aldehydes	44	0.8	(0.2 - 4.1)
QAC + aldehydes + chloramine-T	15	3.3	(0.6 - 18.7)
other	25	-*	-
MODEL 2; endotoxin exposure			
> 101 vs. ≤ 101 ng/m^3		5.5	(1.7 - 17.6)

* no farmers with self-reported allergies

† quaternary ammonium compounds

Discussion

The combination of atopic sensitization to common allergens and the use of disinfectants containing QAC was significantly and positively associated with respiratory symptoms of pig farmers. The association was strongest within the population restricted according to mild bronchial responsiveness to histamine (symptomatic and $PC_{10} \leq 16$ mg/ml, or asymptomatic and $PC_{10} > 16$ mg/ml). The ORs for the association between either of both risk factors and respiratory symptoms did not statistically significantly differ from 1. This suggests a strong interaction between atopy and QAC exposure in our population that substantially enlarges the risk of development of symptoms consistent with asthma. In addition, we found that IgE sensitization to common allergens was significantly associated with the use of disinfectants containing only QAC, which suggests an adjuvant effect of these disinfectants. Specific IgE antibodies studied were directed against allergens of house dust mite, grass and birch pollen, and cat allergens, although no farmer had detectable IgE antibodies against the latter. This panel of four allergens was expected to identify the majority of the individuals with atopic sensitization to common allergens[19]. It might be speculated that specific IgE against house dust mite may reflect sensitization to storage mites, and thus in fact be sensitization to occupational allergens. In our population, 20 farmers had positive IgE against storage mites. However, excluding those with both IgE against storage mites and house dust mite yielded similar associations between specific sensitization and the use of disinfectants. Hence, it seems possible that occupational exposure to QAC can enhance atopic sensitization to non-occupational allergens.

Positive associations between specific IgE sensitization and the use of any of the other disinfectants seemed also to exist, but these associations did not reach statistical significance at the 5% level because of small numbers in some cells. However, it is possible that the observed trends, with estimated ORs exceeding two, are also the result of an adjuvant effect of those disinfectants.

In the population restricted according to mild bronchial responsiveness, a strong association was observed between respiratory symptoms and the combination of specific IgE sensitization and high endotoxin exposure (> 101 ng/m^3), but not with both factors independently. This also suggest an interaction between these two risk factors. The large confidence intervals for both the interaction between atopy and the use of QAC, and atopy and endotoxin exposure separately, limits drawing conclusions on the strength of the interaction effects. The observed interactions could not be attributed to stronger atopic sensitization of the sensitized group with exposure to QAC or high endotoxin levels, compared to the sensitized group without exposure to QAC or with low endotoxin exposure levels. The results may reflect biological interactions. Increase in bronchial responsiveness to histamine of asthmatics after exposure to endotoxins[20] or QAC[21] may in part explain these interactions. Also, it is possible that atopics develop respiratory symptoms at lower exposure intensities of several non-allergenic agents than non-atopics. A larger airway sensitivity in atopics has recently been suggested by Jacobs et al.[22]. They showed that previously unexposed individuals with mild atopy and without a history of asthma, had a larger decrease in FEV_1 than non-atopics after experimental exposure to 1 mg/m^3 of cotton dust.

Respiratory health effects have been reported for disinfectant compounds QAC, chloramine-T, glutaraldehyde and formaldehyde[14,23-25]. QAC specific antibodies could not be demonstrated in an individual with occupational asthma following exposure to QAC[23], but have been found in individuals with muscle relaxants allergy[26]. Chloramine-T specific IgE antibodies have been demonstrated in individuals with chloramine-T induced asthma[14]. It is unclear whether aldehydes cause IgE-mediated asthmatic symptoms, although formaldehyde specific IgE antibodies have been demonstrated to be associated with skin problems, and discomfort in the upper airways[27,28]. No farmers in our study had specific IgE to chloramine-T and only two out of 40 using QAC had specific IgE to QAC. This finding does not support a role of disinfectant specific IgE-mediated mechanisms in observed associations.

The associations reported come from a case-control design within a particular group, using prevalent cases. Since the time sequence of events - exposure to disinfectants, leading to atopic sensitization, leading to symptoms and bronchial responsiveness - can only be studied by following a group of farmers over time, our findings need further confirmation in a prospective cohort study. However, after evaluation of several forms of bias, an adjuvant effect of QAC remains the most plausible explanation for its observed association with specific IgE sensitization. Known potential predictors of positive IgE titres as childhood or family history of allergic diseases did not differ between farmers with and those without QAC exposure. Although we did not have data on exposure to common allergens, it is unlikely that large differences existed between the two groups in exposure to these allergens.

Selection bias seems unlikely to explain the results. In our study, farmers were not selected on exposure to disinfectants. Selection of the population of 198 farmers was based on respiratory symptoms, with a larger chance on selection for symptomatic farmers. This potentially oversampled farmers with the combination of atopic sensitization and the use of QAC (Table 6.3). However, this did not influence our results since similar associations between atopic sensitization and the use of QAC existed among symptomatic and asymptomatic farmers.

Misclassification of disinfectants is unlikely to have introduced major bias, despite the cross-sectional study design in which type of disinfectants was not assessed prior to collection of serum for determination of IgE levels. All information on disinfectants was obtained by the same trained interviewer who visited all farms and personally checked disinfectants present at that time. He was unaware of respiratory symptoms of the farmers. Information on disinfectant use among a subgroup of 86 pig farmers, acquired by means of an interview by phone 1.5 year after initial data collection, showed that change of type of disinfectant was rare, and independent of atopy, type of disinfectant and respiratory symptoms.

We also evaluated a potential adjuvant role of endotoxin exposure. The level of endotoxin exposure is high among pig farmers, and is thought to be an important etiological factor in the development of respiratory effects[10]. In the present study no enhancing effect of endotoxin exposure on specific or total IgE sensitization was found. In fact, in an analysis with log-transformed endotoxin exposure level, a statistically negative association with specific IgE sensitization was present. The OR was 0.3 at an increase in exposure level of a factor 2.7 (95%-CI 0.1 - 1.0). A potential adjuvant effect of endotoxin seems therefore unlikely to exist among pig farmers. However, self-reported allergies were strongly and positively related to endotoxin exposure concentration. The inverse association between IgE sensitization and endotoxin exposure concentration suggests that the positive association with self-reported allergies is not a result of increased IgE sensitization. Norn et al.[29] reported that endotoxins can enhance histamine release from human leucocytes by IgE and non-IgE-mediated mechanisms. In an *in vitro* study, they reported a markedly increased release from cells from patients allergic to various common allergens when specific antigens were presented to the cells after pretreatment with lipopolysaccharides. Such a mechanism can play a role in the increased risk of chronic respiratory symptoms for the combination of IgE sensitization to common allergens and high endotoxin exposure, and for the higher prevalence of self-reported allergies in the presence of higher endotoxin exposure concentrations.

Several authors suggested that increased atopic sensitization due to exposure to non-allergenic air pollutants may be one of the mechanisms underlying the observed increase in prevalence of IgE-mediated asthma[30,31]. This suggestion is mainly based on experimental animal studies, in which exposure to air pollutants preceding or concurrent with allergen exposure resulted in an increase in atopic sensitization[7-9]. So far, only active smoking has been found to enhance atopic sensitization in humans. These observations were made in occupational epidemiological studies, of which the prospective study of Venables et al.[32] showed this effect most convincingly. In that study, smokers had a four to five fold increased risk to develop a positive skin prick test against platinum salts. In population based studies, usually higher total IgE levels were found among smokers, but the association between specific IgE sensitization and smoking habits is less obvious[33]. In our study we found that specific and total IgE was only moderately and positively related to current smoking. The potential enhancing effect of non-allergenic agents was in our study restricted to the use of QAC on IgE sensitization to common allergens. No clear relationship was observed between the use of disinfectants and total IgE sensitization. Such a discrepancy in associations between specific and total IgE with exogenous factors was also shown for smoking in the recently conducted population based study by Omenaas et al.[33].

Exposure to disinfectants takes place about once a week, when disinfectants are dispersed, usually with a pressure washer. This activity takes generally less then 15 minutes. This implies that even occasional short-term exposure may affect IgE sensitization. Several mechanisms have been suggested for the adjuvant effect of non-allergenic air pollutants. Adsorption of allergens to the adjuvant particles[8] is unlikely because of the non-concurrent exposure to disinfectants and the allergens against which specific IgE antibodies were tested. Increased permeability of the bronchial epithelium as suggested by Hulbert et al.[34] can not be ruled out. Holt[35] mentioned interaction with, and toxic effects on pulmonary alveolar macrophages, which would disturb the immunoregulatory role of these cells in the IgE response, as potential mechanism. In our study the use of disinfectants containing only QAC was strongest associated with specific IgE sensitization. The QAC dimethyl dioctadecyl ammonium bromide has been shown to affect the humoral response and the activity of macrophages in mice and *in vitro*[36]. This favours some role of the latter mechanism.

Associations between respiratory symptoms and risk factors have been presented for the entire population as well as the population in which the group of symptomatics was restricted to those with mild bronchial responsiveness to histamine ($PC_{10} \leq 16$ mg/ml), and the asymptomatics to those without bronchial responsiveness ($PC_{10} > 16$ mg/ml). Associations between respiratory symptoms and risk factors were strongest within the restricted population. Restriction was applied to realize a maximum contrast between those with and without symptoms consistent with asthma. Since bronchial hyperresponsiveness is regarded as a hallmark of asthma, but in itself is not a specific method to define asthma, we used the outcome of the histamine provocation test additionally to reported chronic respiratory symptoms. Thoelle et al.[37] proposed a similar procedure to define asthma in epidemiology, although their criteria differed from ours. The criterium of bronchial responsiveness of $PC_{10} \leq 16$ mg/ml used in our study could be subject of discussion, but a more severe criterium would have limited the possibility to perform epidemiological analyses.

In conclusion, this study showed an increased risk of IgE sensitization to common aeroallergens among farmers using disinfectants containing QAC, which is potentially the result of an adjuvant effect. In addition, atopic sensitization in combination with the use of QAC or with exposure to high endotoxin levels, seems to increase the risk of developing symptoms consistent with asthma. We thus have probably identified a co-factor for the risk of atopic sensitization and for the development of symptoms consistent with asthma. It is possible that similar effects exist for exposure to other non-allergenic agents in the occupational or general indoor or outdoor environment. To our knowledge, no studies have been reported that were designed to test this hypothesis. Although endotoxins were not related to an increase in IgE sensitization, our data suggest that they enhance allergic reactions in a way that has not been studied yet in detail. Since allergies occurred predominantly among farmers with chronic respiratory symptoms, endotoxins may be a risk factor for these symptoms through these mechanisms. The role of exposure to non-allergenic compounds in asthma and other chronic respiratory symptoms may therefore be largely underestimated. Occupational groups are particularly useful to study these concepts, since high exposure levels to non-allergenic agents as well as allergens occur in numerous occupational settings.

Acknowledgements

We would like to thank Prof. Dirkje S. Postma, MD, Dept. of Pulmonology, University of Groningen, and Gea de Meer, Dept. of Epidemiology and Public Health, Wageningen Agricultural University, MD, for critically reading the manuscript; Prof. Martin MJ Tielen, PhD, of the Animal Health Service in the southern Netherlands for his contributions to this study, and Hans van Gisteren and employees of the Animal Health Service for their participation in data collection.

The study was part of the European Union concerted action 'Epidemiology of occupational allergic asthma and exposure to bioaerosols', contract No. BMH1-CT94-1446

References

1. O'Connor GT, Sparrow D, Weiss ST. The role of allergy and nonspecific airway hyperresponsiveness in the pathogenesis of chronic obstructive pulmonary disease. Am Rev Respir Dis 1989;140:225-52.
2. Björkstén B. Risk factors in early childhood for the development of atopic diseases. Allergy 1994;49:400-7.
3. Cullinan P, Newman Taylor AJ. Asthma in children: Environmental factors. Br Med J 1994;308:1585-6.
4. Ishizaki T, Koizumi K, Ikemori R, Ishiyama Y, Kushibiki E. Studies of prevalence of Japanese cedar pollinosis among the residents in a densely cultivated area. Ann Allergy 1987;58:265-70.
5. Molfino NA, Wright SC, Katz I, Tarlo S, Silverman F, McClean PA, Szalai JP, Raizenne M, Slutsky AS, Zamel N. Effect of low concentrations of ozone on inhaled allergen responses in asthmatic subjects. Lancet 1991;338:199-203.
6. Wjst M, Reitmeir P, Dold S, Wulff A, Nicolai T, Von Loeffelholz-Colberg E, Von Mutius E. Road traffic and adverse effects on respiratory health in children. Br Med J 1993;307:596-600.
7. Biagini RE, Moorman WJ, Lewis TR, Bernstein IL. Ozone enhancement of platinum asthma in a primate model. Am Rev Respir Dis 1986;134:719-25.
8. Muranaka M, Suzuki S, Koizumi K, Takafuji S, Miyamoto T, Ikemori R, Tokiwa H. Adjuvant activity of diesel-exhaust particulates for the production of IgE antibody in mice. J Allergy Clin Immunol 1986;77:616-23.
9. Riedel F, Krämer M, Scheibenbogen C, Rieger CHL. Effects of SO_2 exposure on allergic sensitization in the guinea pig. J Allergy Clin Immunol 1988;82:527-34.
10. Rylander R, Donham KJ, Hjort C, Brouwer R, Heederik D. Effects of exposure to dust in swine confinement buildings - a working group report. Scand J Work Environ Health 1989;15:309-12.
11. Biersteker K, Dijk WH van, Eissens JBMF, Geuns HA van. Ervaringen met geneeskundig onderzoek op CARA bij gemeentepersoneel te Rotterdam, 1970-1971. Tijdschr Soc Geneeskd 1974;52:158-62.
12. Cockcroft D, Killian D, Mellon J, Hargreave F. Bronchial reactivity to inhaled histamine; a method and clinical survey. Clin Allergy 1977;7:235-43.
13. Wessels MW, Doekes G, Van Ieperen-Van Dijk AG, Koers WJ, Young E. IgE antibodies to *pityrosporum ovale* in atopic dermatitis. Br J Dermatol 1991;125:227-32.
14. Kramps JA, Toorenenbergen AW van, Vooren PH van, Dijkman JH. Occupational asthma due to inhalation of chloramine-T. Int Arch Allergy Appl Immunol 1981;64:428-38.
15. Hollander A, Heederik D, Versloot P, Douwes J. Inhibition and enhancement in the analysis of airborne endotoxin levels in various occupational environments. Am Ind Hyg Assoc J 1993;54:647-53.
16. Eisen EA, Smith TJ, Wegman DH, Louis TA, Froines J. Estimation of Long Term Dust Exposures in the Vermont Granite Sheds. Am Ind Hyg Assoc J 1984;45:89-94.
17. Rijcken B, Schouten JP, Weiss ST, Speizer FE, Lende R van der. The relationship of nonspecific bronchial responsiveness to respiratory symptoms in a random population sample. Am Rev Respir Dis 1987;136:62-8.
18. SAS/STAT guide for personal computers, Version 6 Edition. SAS Institute Inc. Cary, NC, 1985.

19. Kuehr J, Karmaus W, Forster J, Frisher, T, Hendel-Kramer A, Moseler M Urbanek SR, Weiss K. Sensitization to four common inhalant allergens within 302 nuclear families. Clin Exp Allergy 1993;23:600-5.
20. Michel O, Duchateau J, Sergysels R. Effects of inhaled endotoxin on bronchial reactivity in asthmatic and normal subjects. J Appl Physiol 1989;66:1059-64
21. Zhang YG, Wright WJ, Tam WK, Nguyen-Dang TH, Salome CM, Woolcock AJ. Effect of inhaled preservatives on asthmatic subjects. II. Benzalkonium chloride. Am Rev Respir Dis 1990;141:1405-8.
22. Jacobs RR, Boehlecke B, Hage-Hamsten M van, Rylander R. Bronchial reactivity, atopy, and airway response to cotton dust. Am Rev Respir Dis 1993;148:19-24.
23. Bernstein JA, Stauder T, Bernstein DI, Bernstein IL. A combined respiratory and cutaneous hypersensitivity syndrome induced by work exposure to quaternary amines. J Allergy Clin Immunol 1994;94:257-9.
24. Chan-Yeung M, McMurren T, Catonio-Begley C, Lam S. Occupational asthma in a technologist exposed to glutaraldehyde. J Allergy Clin Immunol 1993;91:974-8.
25. Bardana EJ, Montanaro A. Formaldehyde: an analysis of its respiratory, cutaneous, and immunologic effects. Ann Allergy 1991;66:441-52.
26. Didier A, Cador D, Bongrand P, Futoss R, Fourneron P, Senft M, Philip-Joet F, Charpin D, Charpin J, Vervloet D. Role of the quaternary ammonium ion determinants in allergy to muscle relaxants. J Allergy Clin Immunol 1987;79:578-84.
27. Bernstein RS, Stayner LT, Elliott LJ, Kimbrough R, Falk H, Blade L. Inhalation exposure to formaldehyde: an overview of its toxicology, epidemiology, monitoring, and control. Am Ind Hyg Assoc J 1984;45:778-85.
28. Wilhelmsson B, Holmstrom M. Possible mechanisms of formaldehyde-induced discomfort in the upper airways. Scand J Work Environ Health 1992;18:403-7.
29. Norn S, Baek L, Jensen C, Stahl Skov P, Permin H, Jarlov O, Koch C. Influence of bacterial endotoxins on basophil histamine release. Allergy 1986;41:125-30.
30. Holt PG. Environmental pollutants as co-factors in IgE production. Current Opinion Immunol 1989;1:643-6.
31. Molfino NA, Slutsky AS, Zamel N. The effects of air pollution on allergic bronchial responsiveness. Clin Exp Allergy 1992;22:667-72.
32. Venables KM, Dally MB, Nunn AJ, Stevens JF, Stephens R, Farrer N, Hunter JV, Stewart M, Hughes EG, Newman Taylor AJ. Smoking and occupational allergy in workers in a platinum refinery. Br Med J 299;1989:939-42.
33. Omenaas E, Bakke P, Elsayed S, Hanoa R, Gulsvik A. Total and specific serum IgE levels in adults: relationship to sex, age and environmental factors. Clin Exp Allergy 1994;24:530-9.
34. Hulbert WC, Walker DC, Jackson A, Hogg JC. Airway permeability to horseradish peroxidase in guinea pigs: the repair phase after injury by cigarette smoke. Am Rev Respir Dis 1981;123:320-6.
35. Holt PG. Inflammation in organic dust-induced lung disease: New approaches for research into underlying mechanisms. Am J Ind Med 1990;17:47-54.
36. Bloksma N, Reuver MJ de, Willers JM. Impaired macrophage functions as a possible basis of immunomodification by microbial agents, tilorone and dimethyldioctadecylammonium bromide. Antonie Van Leeuwenhoek 1983;49:13-22.
37. Thoelle BG, Peat JK, Salome CM, Mellis CM, Woolcock AJ. Toward a definition of asthma for epidemiology. Am Rev Respir Dis 1992;146:633-7.

7. IgG_4 response to occupational aeroallergens in relation to respiratory symptoms and bronchial hyperresponsiveness in pig farmers

Liesbeth Preller, Gert Doekes, Dick Heederik

Abstract

Associations between chronic respiratory symptoms and Non-Specific Bronchial Responsiveness (NSBR) on the one hand and IgG_4 against pig urinary proteins on the other hand were evaluated in 191 Dutch pig farmers. In multiple logistic regression analysis, IgG_4 titres were inversely related to bronchial responsiveness to histamine as outcome variable ($PC_{10} \leq 16$ mg/ml and $PC_{20} \leq 8$ mg/ml). In addition, the combination of chronic respiratory symptoms and $PC_{10} \leq 16$ mg/ml (both absent versus both present) was strongly and inversely related to specific IgG_4 titres. The odds ratio for titres from 6-50 compared to ≤ 5 was 0.33 (95%-CI 0.11-0.98) and for titres > 50 compared to ≤ 5 was 0.16 (95%-CI 0.04-0.66). Associations between chronic respiratory, irrespectively of presence or absence of NSBR, and IgG_4 titres were weak. Although type I allergy to pig-derived proteins is generally not regarded as the predominant mechanism explaining the high prevalence of respiratory symptoms in pig farmers, our study suggests that specific IgG_4 response to these occupational allergens may have a pronounced protective effect against respiratory allergy.

Submitted to Allergy

Introduction

It has been suggested that high levels of specific IgG_4 antibodies can have a protective role in atopic respiratory disease[1]. The hypothesis of a protective effect was originally based on the frequently observed association of successful immunotherapy with an allergen-specific IgG_4 response. In pig farmers, the prevalence rate of chronic respiratory symptoms is high, but no clear risk factors have been identified[2]. IgE sensitization to work-related allergens was found to be rare and could therefore not explain the high prevalence rate of respiratory symptoms[3]. Presence of IgG and IgG_4 antibodies against pig urinary protein have been shown in pig farmers[4,5]. The present study was undertaken to evaluate the relation between IgG_4 sensitization to pig urinary proteins and respiratory health effects in pig farmers.

Material and methods

A cross-sectional study was performed among 191 Dutch pig farmers. The population was selected based on results of a self administered questionnaire on respiratory symptoms of the British Medical Research Council[6]. 96 farmers were asymptomatic, and 95 had reported one or more of the following symptoms: chronic cough, chronic phlegm, ever wheezing, frequent wheezing, shortness of breath, or chest tightness.

Serology was performed, including analysis of IgG_4 and IgE antibodies against pig urinary proteins and IgE against common allergens (house dust mite, grass pollen, birch pollen and cat allergens). Anti-urine IgG_4 levels were assessed by titration in ELISA plates coated with pig urinary proteins, essentially as described by Hollander et al.[7]. For each serum, the titre was defined as the serum dilution giving an OD_{492} of 0.25. Titres below 1 were set to 1. Specific IgG_4 levels were also assessed in 45 controls working in bakeries, laboratory animal centres, in the potato starch industry, or in the animal feed industry. Assessment of specific IgE will be described elsewhere (Vermeulen et al., in preparation). Positive IgE against common allergens was defined as a positive reaction to one or more of the tested allergens.

Bronchial responsiveness was tested in 186 farmers by histamine provocation according to a modified procedure of the method described by Cockcroft et al.[8].

Histamine concentration ranged from 0.03 mg/ml to 16 mg/ml. Positive Non-Specific Bronchial Responsiveness (NSBR) was defined in two ways:

- $NSBR_{20/8}$: $PC_{20} \leq 8$ mg/ml ($\geq$ 20% decrease in FEV_1 at a concentration of $\leq$ 8 mg/ml). Of 2 farmers, $NSBR_{20/8}$ could not be assessed;
- $NSBR_{10/16}$: $PC_{10} \leq 16$ mg/ml.

Associations between chronic respiratory symptoms and both definitions of NSBR as outcome variable and IgG_4 titre as explanatory variable were evaluated. In addition, the association for the combination of chronic respiratory symptoms and $NSBR_{10/16}$ was assessed (symptomatic farmers with positive $NSBR_{10/16}$ (n=40) versus asymptomatic farmers with negative $NSBR_{10/16}$ (n=79)).

Associations between exposure group (pig farmers versus controls) or anti-pig urine IgE sensitization, and (non-transformed) IgG_4 levels were tested with a nonparametric test (PROC NPAR1WAY SAVAGE). Associations between health outcome as dependent variable and categorized levels of anti-urine IgG_4 were tested using multiple logistic regression analysis and expressed as odds ratios (OR) (PROC LOGISTIC). These associations were adjusted for smoking habits, age and IgE sensitization to one or more common allergens. Statistical analyses were performed with SAS/PC version 6.04.

Results and discussion

Pig farmers had higher anti-pig urine IgG_4 levels than controls (Figure 7.1) (Savage test, $p < 0.05$). The 50 and 75 percentiles of the IgG_4 titre in pig farmers were 8 and 130, respectively, and in controls 4 and 6, respectively. The difference in titres as shown in Figure 7.1 most probably indicates a difference in antigenic exposure. It might be speculated that the titres within the group of pig farmers are also related to exposure levels. The only study reporting on antigenic exposure in relation to IgG sensitization in pig farmers showed a relationship between exposure to urinary proteins and the anti-urine IgG titre[4]. However, no distinction was made between IgG subclasses.

Associations between health outcomes and specific IgG_4 levels within the group of pig farmers are presented in Table 7.1. An inverse trend was found for the relation between chronic respiratory symptoms and IgG_4 levels, but the associations were not statistically significant. However, a statistically significant strong inverse association was observed between IgG_4 levels and $NSBR_{20/8}$.

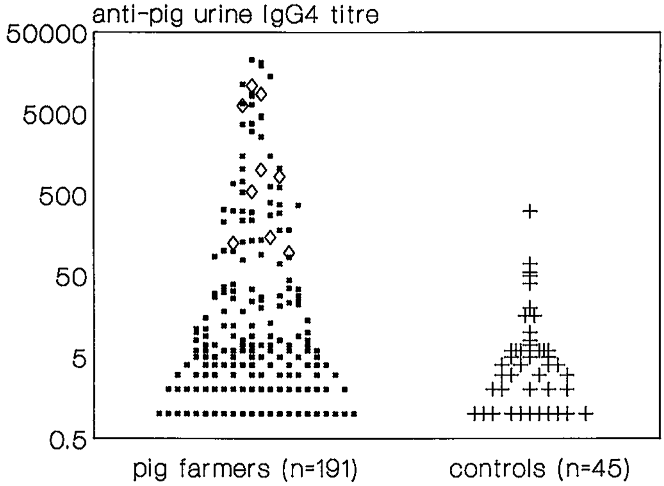

Figure 7.1 Anti-pig urine IgG$_4$ titres in pig farmers and controls. ■ pig farmers with negative IgE against pig urinary proteins; ◊ pig farmers with positive IgE against pig urinary proteins; + controls.

NSBR$_{20/8}$ is the parameter with which bronchial hyperresponsiveness is often defined in clinical studies. In this analysis the two highest categories of IgG$_4$ titres were taken together because of limited number of farmers with NSBR and high anti-urine IgG$_4$ titres. Because of the low number of pig farmers with positive NSBR$_{20/8}$, we also evaluated the association between NSBR$_{10/16}$ and IgG$_4$ levels. Using this definition, the association between NSBR by 3 categories of IgG$_4$ levels showed that higher anti-urine IgG$_4$ levels appeared to be associated with negative NSBR$_{10/16}$, although only the comparison of the highest titres (> 50) with the lowest titres ($\leq$ 5) resulted in a statistically significant association.

In our study, evaluated chronic respiratory symptoms comprised chronic bronchitis-like symptoms as well as asthma-like symptoms. Bronchial hyperresponsiveness is a hallmark of asthma, and since a strong association existed between NSBR and IgG$_4$ titre, we also evaluated the associations between respiratory symptoms combined with NSBR and IgG$_4$ titres. Thus, the difference in IgG$_4$ levels between asymptomatic farmers with negative NSBR$_{10/16}$ and symptomatic farmers with positive NSBR$_{10/16}$ was assessed. This enlarged the contrast in asthma-like symptoms compared to the first definition of chronic respiratory symptoms: 67% of the farmers reporting chest tightness also had positive NSBR$_{10/16}$, compared to only 32% of the farmers reporting chronic phlegm. The combination of symptoms and NSBR$_{10/16}$ was strongly and inversely related to anti-pig urine IgG$_4$ levels, with a small OR of 0.16 for the highest IgG$_4$ levels compared to the lowest IgG$_4$ levels. The differences in associations between chronic respiratory symptoms without and with combination with NSBR$_{10/16}$ indicates a stronger association with asthma-like symptoms than with bronchitis-like symptoms.

Table 7.1 Distribution of anti-pig urine IgG$_4$ titres (number of subjects) and associations between respiratory health effects and IgG$_4$ titres in multiple logistic regression analysis (adjusted for age, current smoking and IgE against common allergens*).

	IgG$_4$ titres				
	≤5	6-50		> 50	
chronic respiratory symptoms					
no symptoms	28	33		35	
symptoms	46	27		22	
OR (95%-CI)	1	0.53	(0.25-1.14)	0.56	(0.26 - 1.23)
NSBR$_{20/8}$					
negative	61	55		52	
positive	12	2		2	
OR (95%-CI)	1	0.24	(0.06-0.90) †		
NSBR$_{10/16}$					
negative	42	42		45	
positive	32	16		9	
OR (95%-CI)	1	0.56	(0.25-1.22)	0.36	(0.14-0.91)
chronic respiratory symptoms & NSBR$_{10/16}$					
both absent	22	27		30	
both present	26	10		4	
OR (95%-CI)	1	0.33	(0.11-0.98)	0.16	(0.04-0.66)

* 1 or more positive to house dust mite, grass pollen, birch pollen, or cat allergens

† categories of 6-50 and > 50 taken together

In the population of 191 farmers, 9 farmers showed IgE against pig urinary proteins. Anti-pig urine IgE and IgG_4 sensitization were positively correlated (Savage test, $p < 0.0001$). In Figure 7.1, IgE positive and IgE negative farmers have been indicated separately. Results of multiple logistic regression analyses of chronic respiratory symptoms, NSBR or the combination of both, on IgG_4 titre were essentially identical if the farmers with positive IgE against pig urinary proteins were excluded.

We conclude that the observed associations potentially reflect a protective effect of IgG_4 against respiratory health effects with a component of NSBR. Since bronchial hyperresponsiveness is a hallmark of asthma, high anti-pig urine IgG_4 levels may be particularly protective against asthma-like symptoms in pig farmers. To our knowledge, this is the first study suggesting such a beneficial effect of a specific IgG_4 response towards inhaled allergens outside therapeutical settings. Specific IgG_4 responses against inhalation allergens have been studied previously in e.g. bakers[9], but no association was reported with rhinitis. The general conclusion in comparable studies is that levels of specific IgG or IgG_4 reflect exposure, but that the role of these antibodies in inhalation allergy is uncertain. An association between exposure and IgG_4 level is also likely in our study. However, unlike other studies IgG_4 antibodies were strongly and inversely related to respiratory health effects. We are not aware of selection processes or factors not accounted for in the analyses that could have biased the associations between respiratory health and IgG_4 levels to an extent that could explain our findings.

In hyposensitization therapy, the induction of an allergen-specific IgG_4 response is thought to be responsible for the therapeutic effect, through the competition of these 'blocking' IgG_4 antibodies with specific IgE for the same allergen molecules. This effect can, however, not explain the beneficial effects of all hyposensitization therapies. A well established example of 'naturally' occurring IgG_4 with a protective effect against atopy is reported in beekeepers, in whom bee venom specific IgG_4, produced after prolonged exposure, competes with specific IgE[10].

An apparent controversy in our findings is that until now, IgE against pig urinary proteins has been regarded as relatively insignificant for the presence of respiratory symptoms in pig farmers[3,5], although two cases of asthma induced by pig urinary proteins have been described; one in a student in agricultural sciences and one in a pig butcher[11,12]. This would mean that the here reported effect of specific IgG_4 indicates that anti-pig urine IgE is in fact important, but that the effects of specific IgE *in vivo* are usually prevented by competing IgG_4, and that the excess of IgG_4 antibodies also precludes the detection of specific IgE *in vitro*. An alternative explanation might be, that the difference in IgG_4 titres reflects a more general type of protective response against the respiratory health effects of this specific occupational exposure, through an as yet unknown mechanism.

In conclusion, IgG_4 against pig urinary proteins seems to protect against chronic respiratory symptoms in pig farmers. A strong protective effect was present only for the combination of these symptoms with NSBR, which can potentially be attributed to a large extent to the strong association between NSBR and IgG_4 levels. To our knowledge, there are no studies in specific occupational settings or in the general population that studied the relationship between bronchial hyperresponsiveness and specific IgG_4. A protective role of specific IgG_4 sensitization may therefore be largely underestimated in respiratory allergy.

Acknowledgements

We thank Marcel Willemsen, Roel Vermeulen, Siegfried de Wind, Albert Hollander and Jan Paul Zock of the Department of Epidemiology and Public Health of the Wageningen Agricultural University and Peter F.J. Vogelzang of the Department of Occupational Medicine of the University of Nijmegen for their assistance in data supply and stimulating ideas on the topic.

References

1. Aalberse RC, Milligen F van, Tan KY, Stapel SO. Allergen-specific IgG_4 in atopic disease. Allergy 1993; 48: 559-69.
2. Donham KJ. Swine confinement buildings. In: Rylander R, Jacobs RR, eds. Organic dusts. Exposure, effects an prevention. Lewis Publishers, CRC Press, Boca Raton, 1994.
3. Iversen M, Dahl R. Specific antigens in dust from swine confinement buildings. Am J Ind Med 1994; 25: 49-51.
4. Virtanen T, Kalliokoski P, Vilhunen P, Taivainen A, Mäntyjärvi R. Concentrations of specific dusts in swineries and the humoral response of swinery workers. Allergy 1990; 45: 1-9.
5. Brouwer R, Biersteker K, Bongers P, Remijn B, Houthuijs D. Respiratory symptoms, lung function, and IgG_4 levels against pig antigens in a sample of Dutch pig farmers. Am J Ind Med 1986; 10: 283-5.
6. Biersteker K, Dijk WH van, Eissens JBMF, Geuns HA van. Ervaringen met geneeskundig onderzoek op CARA bij gemeentepersoneel te Rotterdam, 1970-1971. Tijdschr Soc Geneeskd 1974; 52: 158-62.

7. Hollander A, Heederik D. Pothuis J. Quantification of antigenic aerosol levels in the potato starch producing industry. Ann Occup Hyg 1994; 38 (suppl 1): 911-8.
8. Cockcroft D, Killian D, Mellon J, Hargreave F. Bronchial reactivity to inhaled histamine; a method and clinical survey. Clin Allergy 1977; 7: 235-43.
9. Tiikkainen U, Klockars M. Clinical significance of IgG subclass antibodies to wheat flour antigens in bakers. Allergy 1990; 45: 497-504.
10. Aalberse RC, Gaag R van der, Leeuwen J van. Serologic aspects of IgG_4 antibodies. I Prolonged immunization results in an IgG_4-restricted response. J Immunol 1983; 130: 722-26.
11. Harries MG, Cromwell O. Occupational asthma caused by allergy to pigs' urine. Br Med J 1982; 284: 867.
12. Brennan NJ. Pig Butcher's Asthma - case report and review of the literature. Irish Med J 1985;78:321-2.

8. General discussion

General Findings

Exposure variability

The evidence from the literature on potential causes of respiratory health effects among pig farmers is weak, and presents a quite confusing picture. High endotoxin exposure levels have been proposed as the major etiological factor in these effects. In our study, endotoxins were assessed in all dust samples that were taken during one day in summer and winter for each individual pig farmer. It was estimated that, given the exposure variability present, 42 endotoxin exposure measurements would be required to estimate a relationship between baseline lung function and endotoxin exposure which is underestimated to a limited extent only. With the two measurements per individual, it was unlikely that a statistically significant relationship would be detected. In one other study among pig farmers, exposure variability has been addressed[1]. In this study, personal exposure to endotoxins was estimated based on static measurements in differing types of compartments in two seasons. Exposure concentrations were weighted according to the time spent in these compartments. In this study, 33 exposure measurements would theoretically be required to obtain a regression coefficient which is underestimated by only 10%. This estimate of required sampling effort does not take into account the generally poor correlation between exposures assessed by personal and static sampling. One way to reduce the sampling effort in order to estimate exposure-response relationships more accurately would be the use of a control group with a much lower exposure level. This control group should resemble the target group on all risk factors aside from exposure. This seems a particularly difficult condition to enforce among pig farmers, given the reported variation in prevalence of symptoms relative to control populations as presented in Chapter 2. The number of exposure measurements required to estimate associations between chronic respiratory health effects and exposure to endotoxins was not met in any epidemiological study among pig farmers. In studies where individual exposure estimates were related to baseline lung function, the maximum number of repeated exposure estimates was two[1], as in our study. The resulting poor quantification of long-term average exposure can be a first reason why, even in the presence of high endotoxin exposure concentrations, relationships with chronic respiratory health effects could not be established. A much more favourable variability in endotoxin exposure may be the reason that these associations could be established in some other occupational settings.

As in previous studies, in our study the number of exposure measurements per individual was insufficient for use in epidemiological analyses. Simple correction for attenuation to obtain unbiased estimates of exposure-response relationships was not feasible, since correction for purely random error in exposure assessment is complex in multiple regression analysis[2] and is of no value if associations are weak.

An alternative approach in epidemiological studies to arrive at more valid exposure assessment with the same sampling effort is application of grouping strategies. In our study, however, small interindividual variation in exposure and the *a priori* absence of information that would allow grouping made a grouping strategy unfeasible. Therefore, we modelled long-term average exposure for each individual farmer, using data on outdoor temperature, farm characteristics and activities performed over a period of 14 days. Modelling was carried out independently of performance in exposure-response relationships. Stronger exposure-response relationships, and the higher correlation between modelled exposure and multiple exposure measurements in a small validation study among six farmers suggest that modelled exposure is a more valid measure of long-term average exposure than the average of measured exposure.

Epidemiological findings

Associations with health parameters were evaluated for exposure to dust, endotoxins, ammonia and disinfectants. Dust exposure was modelled similarly to endotoxin exposure. For ammonia, the average of two measurements during a period of approximately 8 hours were taken. Exposure to disinfectants was characterized by registration of type and amount of disinfectant and by application procedures. The following associations were observed in our study.

Endotoxins

- An inverse association existed between endotoxin exposure and baseline lung function in farmers without chronic respiratory symptoms and, less strongly, in farmers who smoke;

- The combination of chronic respiratory symptoms and bronchial responsiveness was positively associated with endotoxin exposure, only in farmers with serum-IgE against common allergens;
- A positive association was observed between endotoxin exposure and self-reported allergies.

There was no difference in exposure to endotoxins between farmers with and those without chronic respiratory symptoms, and there were no indications of a positive association between endotoxin exposure and specific or total serum IgE.

Disinfectants

- Baseline lung function and chronic respiratory symptoms were associated with characteristics of the application procedure such as pressure used in disinfection and duration of the procedure. This was independent of type of disinfectant;
- IgE sensitization to common allergens was positively related to the use of disinfectants containing Quaternary Ammonium Compounds (QAC);
- Chronic respiratory symptoms with and without the combination with bronchial responsiveness were positively associated with use of disinfectants containing QAC, only in farmers with serum-IgE against common allergens;
- Serum-IgE antibodies against chloramine-T were not detected in our population;
- In 2 out of 40 sera from farmers using QAC, IgE antibodies against QAC were detected.

Ammonia

- Baseline lung function parameters indicative of airways obstruction were inversely related to ammonia exposure, with stronger and steeper relationships in symptomatic farmers.

There was no relationship between ammonia exposure and chronic respiratory symptoms.

Dust

In our population, no associations were observed between dust exposure and chronic respiratory symptoms or baseline lung function.

Additional immunological findings

- In 12 sera (6%), IgE antibodies against pig urinary proteins were detected;
- In 10 sera (5%), IgE antibodies against pig stable dust were detected. All 10 sera contained IgE against pig urinary proteins as well. Pig urinary protein appeared to be the predominant IgE binding allergen in stable dust;
- If both sensitization to storage mites (16% positive) and pig urinary proteins is considered to be work-related, then IgE against work-related allergens was strongly and positively related to chronic respiratory symptoms;
- IgG_4 against pig urinary proteins was inversely related to respiratory health effects with a component of non-specific bronchial responsiveness.

Other epidemiological studies among pig farmers were primarily focused on associations between respiratory health effects and exposure to dust, endotoxins or, to a lesser extent, ammonia. In our study, however, the effect of exposure to disinfectants seemed to be very strong. If a strong risk factor is not considered when studying the etiology of a health endpoint, then there is a considerable risk that many health effects remain unexplained. In addition, insufficient correction for variables as covariates in multiple regression analysis yields larger confidence intervals for the other studied risk factors, limiting the chance that a primary risk factor is recognized as potential causal factor. This may be another important reason for the unexplained etiology in earlier studies.

Our study was an extension of the study initiated by the NCB Pig Farmers Association[3], in which farmers were selected based on symptoms of chronic nonspecific lung disease (CNSLD). That design was based on the hypothesis that the symptoms are all the expression of the same underlying disease entity. This concept is known as the Dutch hypothesis[4,5]. Evidence is still lacking to either refute or support this hypothesis[6]. According to this hypothesis, symptoms can become prevalent in genetically predisposed persons, and expression of various symptoms of CNSLD, at a certain point of time, depends on environmental factors. In pig farming, several types of exposure occur in relatively high concentrations. It could be argued that the various types of exposure are related to different expressions of CNSLD. The difference in exposure-response relationships between the entire population and the population that was restricted according to Non Specific Bronchial Responsiveness (NSBR) suggests that distinct respiratory effects do occur in the presence of various environmental and host factors. Analysis of the data without a discriminative definition of health effects may therefore obscure existing exposure-response relationships.

In other studies, etiological factors of chronic respiratory health effects have been studied by means of simple concepts which were generally focused on the variation in base-line lung function. Lung function is an objective, but aspecific measure of lung injury. Findings in our study suggest that explanatory models used so far may have been too simple to explain the high prevalence rate of chronic respiratory symptoms, being another reason of limited evidence on etiological factors.

Further, in our population several other noteworthy observations were made which have not been reported in other studies before. The first, a positive association between IgE sensitization to common aeroallergens and disinfectants containing QAC, suggests an adjuvant effect of QAC on this IgE response. Secondly, atopic sensitization to common allergens may interact with occupational exposure to endotoxins or quaternary ammonium compounds in the development of chronic respiratory symptoms. The strong positive association between endotoxin exposure and self-reported allergies was the third observation, and may reflect a causal relationship. Lastly, results suggested that specific IgG_4 antibodies protect against respiratory health effects. The immunological system seems thus to be involved in a complex fashion which is not yet completely understood. Until now, the role of specific IgG_4 or IgE sensitization in relation to respiratory health effects has been studied at the population level to a limited extent, and only in simple models. In addition, immunomodulating effects of non-allergenic exposures, and interactions between non-allergenic exposures and atopic sensitization have not been considered in studies in pig farmers. The absence of studies on immunological factors other than direct sensitization to work-related allergens could be a fourth reason why epidemiological studies showed little progress on finding etiological factors.

Bias

The selection procedure, with over-representation of farmers with (more severe) chronic respiratory symptoms, hampered assessing unbiased associations between exposure and baseline lung function in the base population, since associations differed between symptomatic and asymptomatic farmers. The base population comprised farmers working daily more than five hours in pig farming. On the other hand, over-representation of symptomatic farmers offered a better possibility to study interactions between atopic sensitization and exposure in relation to chronic respiratory symptoms, and to study factors that enhance the progress of respiratory health effects in already symptomatic farmers.

Selection bias

Only farmers responding after a request to participate in the study were known to us. No data of the base population was made available because of privacy regulations. It could therefore not be evaluated whether self selection introduced bias in exposure-response relationships by differences between the base population and the study population. In the next selection step, farmers working at least five hours a day in pig farming were selected. This led to a bias towards farmers with larger farms. Subsequent selection was done on the basis of symptomatology in two stages. Indications of selection bias in these stages can be obtained by comparing the distribution of risk factors as reported in the first stage questionnaire in included and excluded farmers, in both the symptomatic and asymptomatic group. These risk factors comprise exposure as well as personal characteristics such as smoking habits and atopic disease in childhood. We found no indications that selection had occurred which could have falsified our findings. Selection of cases was related only to the relative frequency of disinfection: more selected cases disinfected after each cycle than non-selected symptomatic farmers. This difference did not exist in asymptomatic farmers. Indications that disinfection was related to respiratory health, were already found in the first stage questionnaire data (Vogelzang et al., unpublished results), and were also observed for base-line lung function in asymptomatic farmers. This makes it plausible that the observed associations between health effects and disinfection reflect true exposure-response relationships, and that they are no artifacts as a result of the selection process. There were otherwise no differences in farm characteristics that might have been surrogates for occupational exposure, and neither in atopic disease in childhood and smoking habits between included and excluded farmers. In this study, however, several endpoints have been studied independently of the original selection procedure on respiratory symptoms, whereas these endpoints were present in both the symptomatic and the asymptomatic group. In these cases analyses stratified by symptomatic status have been performed. For base-line lung function, different exposure-response relationships were found in cases and controls, and no unbiased estimate could be made for the base population. For self-reported allergies, IgE sensitization to common allergens, and bronchial responsiveness to histamine as endpoints, the associations seemed to be independent of chronic respiratory symptoms. Therefore, the selection procedure is expected to bias the exposure-response relationships that could be anticipated in the base population to a limited extent only.

Because of economical reasons, it is unlikely that a healthy worker effect, in the sense that farmers quit their job after development of symptoms, plays a major role. Thelin et al.[7] studied this effect in a large group of 1,113 Swedish farmers, of whom specializations were not mentioned. They found that farmers changed occupation in a period of 18 years 2 times less often than 1,441 non-farming controls, and 3 times less than 174 farm workers who were no owners. For farmers, allergy was a relatively important reason to change occupation compared to control workers: 10 farmers versus 2 control workers per 1,000. Considering that not specifically the first years of employment were evaluated, change of job due to allergies could have been larger in starting farmers[8]. In our study, self-reported allergies were strongly related to chronic respiratory symptoms, particularly in combination with NSBR. In turn, in our population self-reported allergies were strongly and positively related to endotoxin exposure (Chapter 6). Selection as a result of allergy could therefore have biased the association between endotoxin exposure and chronic respiratory symptoms. Also, the role of allergies in chronic respiratory symptoms could have been underestimated in our population.

Information bias

Errors in exposure assessment may be nondifferential (independent of health outcome) and differential. Nondifferential misclassification generally leads to underestimation of the effect, although after categorization of exposure also overestimation or conversion of the true relationship can occur[9]. Differential misclassification can cause bias in any direction.

In our study, errors in exposure assessment may occur first by errors in current average exposure, which already have been discussed. In modelled exposure, the influence of intraindividual variation was reduced and errors by incomplete assessment of all sources of exposure was accounted for. However, this measure of exposure was also subject to error which was introduced by errors in variables used to estimate exposure and limitations of the models. It is unlikely that the errors in modelled exposure were differential, since no differences existed in residuals between measured exposure and modelled exposure during sampling between symptomatic and asymptomatic farmers (t-test, $p > 0.1$).

Secondly, inherent in a cross-sectional study design, exposures responsible for health effects may have altered after the onset of these effects. In the presence of still considerable exposures, this need not lead to reversion of the health effects. Misclassification of exposure by the time frame is possible by, for example, changes in farm characteristics or by task allocation. Given the small interindividual variation in exposure to endotoxins, dust and ammonia, a little exposure misclassification can result into largely biased, most likely underestimated, exposure-response relationships. Although associations between the same exposures and chronic respiratory symptoms have neither been reported in other occupational settings, it can not be ruled out that they exist but that exposure misclassification obscured the relationships. This can only be overcome adequately in prospective studies.

Confounding

By definition, a confounder is an independent risk factor of the studied health outcome, which is also related to the risk factor under study. A confounder is not an intermediate factor in the chain of events leading to disease. The relationship between the studied risk factor and disease may be biased in any direction, including conversion of the effect. Confounding of associations between risk factors and end points (Chapters 5, 6 and 7) by other types of exposure, age, smoking habits and atopic sensitization and atopic disease in childhood has been evaluated and can be ruled out as confounder with reasonable certainty. No adjustment was made for bronchial responsiveness, since this is likely not an independent risk factor but an intermediate factor or even an independent endpoint.

Association or causation

In this thesis the associations between respiratory health effects and exposure to dust, endotoxins, ammonia and disinfectants were evaluated. For discussing the possibility that these agents are causally related to respiratory health effects, several of the relevant criteria given by Hill[10] will be used. These criteria include the strength of associations, consistency with results in other circumstances, the biological gradient, plausibility, coherence with known facts, and analogy with other agents.

Before discussing causality for either of the agents, it should be stated that none of the exposures that were associated with health effects, were strongly related to one of the other evaluated exposures. In addition, in 60 samples taken on 10 farms, only a moderate correlation (R=0.53) existed between endotoxin level and level of (1→3)-ß-D-glucans (Douwes et al., unpublished results). Further, associations between health effects and endotoxin exposure were given for modelled exposure. Modelled exposure depended partly on time spent on activities, which had a large effect on modelled dust exposure levels as well. In none of the presented associations between endotoxin exposure and health outcome, dust exposure was (close to being) statistically significantly related to these outcomes. Therefore, the chance that modelled endotoxin exposure was only a surrogate for a true causal exposure which was also related to time spent in pig farming, or that one of the exposures was only a surrogate for any of the others, is not likely.

Endotoxins

The associations between endotoxin exposure concentration and base-line FVC and FEV_1 which were observed in asymptomatic farmers is consistent with results of cross-sectional studies in pig farming, cotton industry and grain workers[1,11-13]. The absence of an association between endotoxin exposure and chronic respiratory symptoms is also consistent with results of the study among workers in the Dutch animal feed industry[13]. It is not known what weight should be given to the consistency with results of other studies among pig farmers. As discussed, it is likely that exposure measured in those studies poorly reflect long-term average exposure, while this is not compensated by a larger interindividual variation in exposure.

To our knowledge, neither the interaction between endotoxin exposure and atopic sensitization as determinant of chronic respiratory symptoms, nor specific associations between self-reported allergies and endotoxin exposure have been observed in previous observational studies. One longitudinal study, however, suggests a positive association between exposure to endotoxin-containing grain dust and the risk of atopic sensitization to common allergens as measured by a skin prick test[14]. Although this is not in agreement with the absence of a positive association between endotoxin exposure and IgE sensitization in our study, the underlying mechanisms of both positive associations may be similar. An experimental study reported an enhancing effect of lipopolysaccharides (LPS) on the reaction to house dust mite (HDM) allergens in a skin prick test in 20 patients with HDM-allergy[15]. Earlier, Norn et al.[16] had reported that in the *in vitro* basophil histamine release test, pretreatment of the cells with LPS markedly enhanced the response to specific allergens. A similar effect may be responsible for both types of association found in our study.

The observation that endotoxin exposure seemed to be more strongly related to base-line lung function in asymptomatic workers than in symptomatic workers is not supported by other studies. In various experimental studies with human subjects, stronger acute obstructive reactions to exposure were found in asthmatics, patients with symptoms of CNSLD in combination with bronchial hyperresponsiveness, and rhinitis patients[17-19]. This apparent contradiction could be caused by potential misclassification of exposure in our study, limited comparability between acute and chronic effects, and the difference in response between individuals who are and who are not chronicly exposed to high levels of endotoxins.

A stronger effect of endotoxin exposure on base-line lung function was observed in ever smokers than in nonsmokers, although the association in ever smokers was only borderline statistically significant. A stronger effect in smokers is consistent with other studies reporting on associations between base-line lung function and exposure to endotoxins or to organic dust containing endotoxins[20-23].

Results of *in vivo* human and animal experimental studies and of *in vitro* studies strengthen the potential causal role of endotoxins/LPS in airways pathology (Chapter 2).

Ammonia

The association between lung function and ammonia exposure was strongest in symptomatic farmers. In analogy with, for example, the effect of inhaled sulphuric acid on pulmonary function in asthmatics[24], a stronger association in symptomatic farmers is plausible.

Similar associations between exposure concentrations at levels encountered in pig farming and respiratory health effects have not been reported in other occupational settings[25,26]. Evidence from other studies in pig farmers is very weak. However, it can not be excluded that ammonia is only a marker of a variety of gases in the animal buildings affecting the respiratory system.

Since ammonia is hygroscopic, it is unlikely that ammonia in itself will have an effect on the lower respiratory system, but its suggested indirect effect by binding to small particles that can be inhaled deeper into the respiratory system[27,28] may be an explanation for the found association with lung function. This hypothesis requires additional study.

Disinfectants

In other studies, chemical components present in disinfectants (Quaternary Ammonium Compounds (QAC), aldehydes, and chloramine-T) have been suggested to cause respiratory health effects (Chapter 2). The potential interaction of QAC with atopic sensitization in respiratory disease etiology is supported by previous reports of bronchoconstriction and increased bronchial responsiveness to histamine in asthmatics due to QAC used as preservatives in asthma drugs[29].

The trends in the associations between either lung function or chronic respiratory symptoms with pressure in and duration of the disinfection procedure, as found in our study, strengthens the possibility of a causal relationship.

IgE sensitization to chloramine-T was not observed in our population, whereas in other studies cases suffering from occupational asthma due to chloramine-T have been reported to show positive skin prick tests or IgE against chloramine-T[30-32]. QAC-specific IgE was detected in only 2 out of 40 farmers using QAC. IgE-mediated allergy has been shown in patients allergic to QAC used as muscle relaxants[33], but not in a case suffering from asthma induced by inhalation of QAC-containing disinfectants[34]. An interaction of exposure to QAC or similar chemicals and atopic sensitization, with chronic respiratory symptoms as endpoint, has not been reported in other studies. Also, adjuvant activity of QAC has not been reported before. The trend in the OR and strength of the association when comparing disinfectants containing QAC only and QAC in combination with aldehydes may reflect a dose-response effect, supporting the hypothesis of an adjuvant effect. QAC have been shown to be potent adjuvants in laboratory animals[35]. This further strengthens the possibility of an adjuvant effect of QAC in pig farmers, although effects on the IgE response have not been reported. An adjuvant effect is not known for other comparable inhaled chemical agents, but has been suggested for cigarette smoke, diesel exhaust particles and ozone[36-38]. Results also suggested positive associations between IgE sensitization to common allergens and the use of any other disinfectant, with ORs exceeding

2, but these associations did not reach statistical significance at the 5% level possibly because of small numbers in some cells.

Dust

Dust as such is not likely to be causally related to respiratory health effects. Consistent with our results, in experimental and observational studies on organic dust exposure, associations indicating an exposure-response relationship were not observed, or associations were weaker than with endotoxins present in the dust[13,39].

Work-related allergens

The prevalence rate of IgE sensitization to allergens in pig urine and pig stable dust was low with 6% and 5%, respectively, whereas 10% was sensitized to storage mites. Lower prevalence rates or prevalence rates in the same range were found in other studies[40-42]. In our study, the allergenic activity of pig farm dust could be attributed to pig urinary proteins. In another study, allergenic activity of feed particles was suggested[43]. The relatively strong allergenic activity of urinary proteins is in analogy with observations in workers with work-related allergy in laboratory animal centres[44].

At a population level, no other study among pig farmers has reported strong associations between sensitization to work-related allergens, including both storage mites and pig urinary proteins, and chronic respiratory symptoms, as found in our study. This positive association could not be attributed to potential cross reactivity between house dust mite and storage mite allergens[45], since associations remained strong after excluding farmers who showed sensitization to both types of mites at the same time.

Other organic dust components

Several studies indicated that endotoxins are not the only components in organic dust which are responsible for an inflammatory process in the respiratory system. Larsson et al.[46] compared the results of an experimental study on exposure to pig farm dust and BAL findings in previously unexposed subjects, with another study using LPS[47]. Swine dust had a larger influence than LPS on the increase in alveolar macrophages, fibronectin and albumin, whereas the change in lymphocytes and neutrophils was comparable. In an *in vitro* experiment by the same group, human lung carcinoma cells were exposed to LPS, grain dust, glucans and swine dust[48]. Swine dust was more potent than pure LPS to increase interleukin-8 production. Von Essen et al.[49] studied *in vitro* responses of human cells to grain dusts. They found that endotoxins alone were not responsible for inflammatory processes of grain dust, which is also present in pig farms. Direct neutrophil chemotactic activity and activation of complement was not reduced after grain sorghum was depleted from endotoxins, but the capacity to release chemotactic activity by alveolar macrophages was decreased. The nature of other organic dust components related to studied effects is not known. Recently, (1→3)-ß-D-glucans (present in fungi and plants) and peptidoglycans (components of gram-positive bacteria) have been mentioned as a potential respiratory health hazard[50,51]. These constituents have not been measured in our population for epidemiological purposes. The rather constant ratio between the concentration of total bacteria and endotoxin-containing gram-negative bacteria in pig farms (Chapter 2), could lead to a significant correlation between exposure to endotoxins and peptidoglycans. Although evidence in the literature on a causal role of endotoxins in respiratory disease is much stronger than for peptidoglycans, on the basis of our study it can not be excluded that peptidoglycans are to some extent responsible for the observed relationships between respiratory health and endotoxin exposure.

Control measures

Evidence exists that exposure to disinfectants and endotoxins are causally related to chronic respiratory health effects, the strongest effects being estimated for exposure to disinfectants. Based on this knowledge, control measures should be directed on reducing the exposure levels to or inhaled dose of disinfectants in the first place and endotoxins in the second place. Depending on the agent, these measures can include in the following order: substitution, reduction of source emission, isolation of the source, and use of personal protective equipment.

Under current housing systems, disinfection remains necessary to control animal disease. Studies in the veterinary discipline could be initiated to study in the first place whether housing systems which require less disinfection, are possible alternatives and secondly, whether the current use of disinfectants could be reduced without an increase in animal disease. At the occupational hygiene level, development of systems which do not require presence of the farmer during disinfection can be considered. In addition, substitution of the specific disinfectant or adjustment of the procedure are options to reduce health hazards, depending on the individual situation. When atopic sensitization to the applied disinfectant is shown, substitution by other disinfectants may reduce the health hazard. In our study the strongest associations with health risks were observed for QAC-use. Although our results, in combination with observations on QAC in other studies, make a causal relationship plausible, more evidence is needed prior to considering total substitution of QAC. Our results further suggest that, independent of the type of disinfectant, a shorter disinfection procedure and avoidance of high pressure may reduce health hazards. Hygiene studies on exposure levels and size distribution of particles to support these findings have not been performed. Such studies are needed to investigate the background of the reported associations and to recommend detailed control measures.

In Chapter 4, associations between farm characteristics and activities on the one hand and endotoxin exposure concentrations on the other hand were evaluated. The empirical models in which these were quantified, in combination with common working conditions, suggested that adjustments in several flooring characteristics and some feeding characteristics could yield considerable reductions in exposure concentrations. The use of dry feeding was associated with lower endotoxin exposure levels and may therefore be preferred above wet feeding. Not all types of flooring can be applied in each compartment. However, the data suggest for example that a convex floor can limit the exposure of farmers to endotoxins, and that the use of floor heating should be kept to the minimum required for the animal health. The use of personal protective equipment during specific activities can be considered to limit effective exposure. At the population level, the largest effect on long-term average exposure is expected when used during feeding, but for individual farmers this may be different. The models can, however, not predict actual reductions because of limited explained variance of the model and false inferences on causal associations by correlation between independent variables. Before taking control measures which require large investments, studies are needed to corroborate the findings in practical situations. In general, evaluation of the actual effect of control measures by measurements is an essential step in the total process of designing and implementation of these measures[52]. These studies need a good theoretical basis with a sufficient number of samples for proper evaluation[53].

Concluding remarks and future studies

The picture on the etiology of respiratory health effects in pig farmers emerging from this study is more complex than has been suggested so far. This study strongly suggests that different agents as well as a variety of immunological factors, with potentially a strong hereditary component, are important in the etiology of chronic respiratory health effects. It seems that respiratory health effects in pig farmers are not confined to one single disease entity. Models used hitherto to identify causal factors seem to have oversimplified the actual exposure-disease pathways. In addition, characterization of organic dust related exposure seemed insufficient to establish exposure-response relationships. Poor characterization was caused by an unfavourable exposure variability and most likely also by the complex logistics of field work and large investments for exposure assessment per individual compared to many other occupational settings.

Work-related allergies appear to account for only part of the chronic respiratory symptoms present in pig farmers. On the other hand, atopic sensitization to common allergens seems to be an important condition for development of chronic respiratory symptoms particularly those in combination with NSBR. Atopic sensitization to common allergens may be enhanced by non-allergenic occupational exposures.

In earlier studies, emphasis has been put on symptoms consistent with chronic bronchitis. No potential etiological factors have been found that were specifically related to this type of symptoms. However, exposure to disinfectants and IgE sensitization to work-related allergens may have contributed to the development of bronchitis-like symptoms, since reported positive associations with chronic respiratory symptoms were not confined to symptoms that were consistent with asthma. Also, measured as well as unmeasured agents may be responsible for these symptoms. A potential association between measured agents and bronchitis-like symptoms could have been obscured by strong exposure misclassification.

The number of observations used in our epidemiological analyses varied. Next to intentional restriction and data lacking on health outcomes because farmers refused participation, this was most often caused by incomplete exposure assessment and related aspects. Without substantial increase in study means, this is difficult to avoid in this occupational setting.

Future studies on etiological factors

Animal models could be used to support findings on the potential role of disinfectants in respiratory disease etiology. Hypotheses on adjuvant activity of components such as diesel or ozone, are also primarily based on animal models.

Studies using invasive techniques such as bronchoalveolar lavage may help to understand mechanisms leading to impaired respiratory health. This should be studied in pig farmers, since large differences were observed between pig farmers and previously unexposed subjects.

Future epidemiological studies should be designed to investigate the role of a variety of exposures and immunological processes in relation to incidence of respiratory health effects and sensitization. The design and the factors to be studied are determined by the potential complex etiology with a so far unknown sequence of events, the unfavourable variability of several exposures, and by potential differential exposure misclassification in cross-sectional studies. A prospective study is needed with a population of considerable size. It should exceed the largest studies carried out so far, which incorporated exposure assessment, by a magnitude. To optimize study efficiency a population should be selected which varies with respect to atopy, smoking habits and exposure. For the latter, knowledge on associations between working conditions and exposure levels as described in this study could be used. An international multicentre study might have the additional advantage of a larger interindividual exposure variability than a national cohort, although earlier studies suggested that this may not be true for exposure to dust, endotoxins and ammonia. Exposure characterization should be performed repeatedly. Models such as presented in Chapter 3 could be helpful to limit costs of exposure assessment. These models used information that can be obtained with relative low costs. They enable in the first place an increase in the study population and thus an increase in power without a proportional increase in sampling effort. In the second place they allow registration of changes in exposure over time by changes in working conditions of farmers.

References

1. Zejda JE, Barber E, Dosman JA, Olenshock SA, McDuffie HH, Rhodes C, Hurst T. Respiratory health status in swine producers relates to endotoxin exposure in the presence of low dust levels. J Occup Med 1994;36:49-56.
2. Willett W. An overview of issues related to the correction of non-differential exposure measurement error in epidemiologic studies. Stat in Med 1989;8:1031-40.

3. Preller L, Vogelzang P. Gezondheid varkenshouder aan risico's blootgesteld. Gezondheidsdienst voor Dieren, rapport nr. 93.001, 1993.
4. Orie NGM, Sluiter HJ, Vries K de, Tammeling GJ, Witkop J. The host factor in bronchitis. In: Bronchitis. NGM Orie, HJ Sluiter, eds, Royal Vangorcum, Assen, 1961:43-59.
5. Orie NGM. Appendix on terminology of bronchitis. In: Bronchitis II. NGM Orie, HJ Sluiter, eds, Royal Vangorcum, Assen, 1964:398.
6. Sluiter HJ, Koëter GH, Monchy JGR de, Postma DS, Vries K de, Orie NGM. The Dutch Hypothesis (chronic non-specific lung disease) revisited. Eur Respir J 1991;4:479-89.
7. Thelin A, Höglund S. Change of occupation and retirement among Swedish farmers and farm workers in relation to those in other occupations. Soc Sci Med 1994;38:147-51.
8. James AL, Zimmerman MJ, Ee H, Ryan G, Musk AW. Exposure to grain dust and changes in lung function. Br J Ind Med 1990;47:466-72.
9. Flegal KM, Keyl PM, Nieto FJ. Differential misclassification arising from nondifferential errors in exposure measurement. Am J Epidemiol 1991;134:1233-44.
10. Hill AB. The environment and disease: association or causation? Proc R Soc Med 1965:295-300.
11. Kennedy SM, Christiani DC, Eisen EA, Wegman DH, Greaves IA, Olenshock SA, Ye TT, Lu PL. Cotton dust and endotoxin exposure-response relationships in cotton textile workers. Am Rev Respir Dis 1987;135:194-200.
12. Heederik D, Brouwer R, Biersteker K, Boleij JSM. Relationship of airborne endotoxin and bacteria levels in pig farms with the lung function and respiratory symptoms of farmers. Int Arch Occup Environ Health 1991;62:595-601.
13. Smid T, Heederik D, Houba R, Quanjer PH. Dust- and endotoxin-related respiratory effects in the animal feed industry. Am Rev Respir Dis 1992;146:1471-9.
14. Lin FJ, Kennedy SM, Chan-Yeung M. Effect of grain exposure and smoking on the longitudinal changes in immediate skin reactivity. Clin Exp Allergy 1994;24:949-54.
15. Michel O, Ginanni R, Bon B Le. Duchateau J. Effect of endotoxin contamination on the antigenic skin test response. Ann Allergy 1991;66:39-42.
16. Norn S, Baek L, Jensen C, Stahl Skov P, Permin H, Jarlov O, Koch C. Influence of bacterial endotoxins on basophil histamine release. Allergy 1986;41:125-30.
17. Michel O, Duchateau J, Sergysels R. Effects of inhaled endotoxin on bronchial reactivity in asthmatic and normal subjects. J Appl Physiol 1989;66:1059-64.
18. Michel O, Ginanni R, Bon Le B, Content J, Duchateau J, Sergeysels R. Inflammatory response to acute inhalation of endotoxin in asthmatic patients. Am Rev Respir Dis 1992;146:352-7.
19. Zwan JC van der, Orie GM, Kauffman HF, Wiers PWJ, Vries K de. Bronchial obstructive reactions after inhalation with endotoxin and precipitinogens of *Haemophilus influenzae* in patients with chronic non-specific lung disease. Clin Allergy 1982;12:547-59.
20. Sheridan D, Deutscher C, Tan L, Maybank J, Gerrard J, Horne S et al. The relationship between exposure to cereal grain dust and pulmonary function in grain workers. In: Dosman JA, Cotton DJ, eds. Occupational pulmonary disease: focus on grain dust and health. New York: Academic Press 1979:196-213.
21. Cotton DJ, Graham BL, Li KYR, Froh F, Barnett GD, Dosman JA. Effects of smoking and occupational exposure on peripheral airway function in young cereal grain workers. Am Rev Respir Dis 1982;126:660-5.
22. Pahwa P, Anthilselvan A, McDuffie HH, Dosman JA. Longitudinal estimates of pulmonary function decline in grain workers. Am J Respir Crit Care Med 1994;150:656-62.

23. Glindmeyer HW, Lefante JJ, Jones RN, Rando RJ, Weill H. Cotton dust and across-shift change in FEV1 as predictors of annual change in FEV1. Am J Respir Crit Care Med 1994;149:584-90.
24. Koenig JQ, Pierson EW, Horike M. The effects of inhaled sulphuric acid on pulmonary function in adolescent asthmatics. Am Rev Respir Dis 1983;128:221-5.
25. Holness DL, Purdham JT, Nethercott JR. Acute and chronic respiratory effects of occupational exposure to ammonia. Am Ind Hyg Assoc J 1989;50:646-50.
26. Swotinsky RB, Chase KH. Health effects of exposure to ammonia: scant information. Am J Ind Med 1990;17:515-21.
27. Donham KJ, Scallon LJ, Popendorf W, Treuhaft MW, Roberts RC. Characterization of dusts collected from swine confinement buildings. Am Ind Hyg Assoc J 1986;47:404-10.
28. Schwartz DA, Landas SK, Lassise DL, Burmeister LF, Hunninghake GW, Merchant JA. Airway injury in swine confinement workers. Ann Internal Med 1992;116:630-5.
29. Zhang YG, Wright WJ, Tam WK, Nguyen-Dang TH, Salome CM, Woolcock AJ. Effect of inhaled preservatives on asthmatic subjects. II. Benzalkonium chloride. Am Rev Respir Dis 1990;141:1405-8.
30. Kramps JA, Toorenenbergen AW van, Vooren PH van, Dijkman JH. Occupational asthma due to inhalation of chloramine-T. Int Arch Allergy Appl Immunol 1981;64:428-38.
31. Wass U, Belin L, Eriksson NE. Immunological specifity of chloramine-T-induced IgE antibodies in serum from a sensitized worker. Clin Exp Allergy 1989;19:463-71.
32. Blomqvist AM, Axelsson IGK, Danielsson D, Kiviloog J, Ulander A, Zetterström O. Atopic allergy to chloramine-T and the demonstration of specific IgE antibodies by the radioallergosorbent test. Int Arch Occup Environ Health 1991;63:363-5.
33. Didier A, Cador D, Bongrand P, Futoss R, Fourneron P, Senft M, Philip-Joet F, Charpin D, Charpin J, Vervloet D. Role of the quaternary ammonium ion determinants in allergy to muscle relaxants. J Allergy Clin Immunol 1987;79:578-84.
34. Bernstein JA, Stauder T, Bernstein DI, Bernstein IL. A combined respiratory and cutaneous hypersensitivity syndrome induced by work exposure to quaternary amines. J Allergy Clin Immunol 1994;94:257-9.
35. Bloksma N, Reuver MJ de, Willers JM. Impaired macrophage functions as a possible basis of immunomodification by microbial agents, tilorone and dimethyldioctadecyl-ammonium bromide. Antonie Van Leeuwenhoek 1983;49:13-22.
36. Venables KM, Dally MB, Nunn AJ, Stevens JF, Stephens R, Farrer N, Hunter JV, Stewart M, Hughes EG, Newman Taylor AJ. Smoking and occupational allergy in workers in a platinum refinery. Br Med J 1989;299:939-42.
37. Muranaka M, Suzuki S, Koizumi K, Takafuji S, Miyamoto T, Ikemori R, Tokiwa H. Adjuvant activity of diesel-exhaust particulates for the production of IgE antibody in mice. J Allergy Clin Immunol 1986;77:616-23.
38. Biagini RE, Moorman WJ, Lewis TR, Bernstein IL. Ozone enhancement of platinum asthma in a primate model. Am Rev Respir Dis 1986;134:719-25.
39. Rylander, R., Haglind, P. and Lundholm, M. Endotoxin in cotton dust and respiratory function decrement among cotton workers in an experimental cardroom. Am Rev Respir Dis 1985;131:209-15.
40. Brouwer R, Biersteker K, Bongers P, Remijn B, Houthuijs D. Respiratory symptoms, lung function, and IgG_4 levels against pig antigens in a sample of Dutch pig farmers. Am J Ind Med 1986;10:283-5.
41. Brouwer R, Heederik D, Swieten P van. IgG_4 antibodies against pig-derived antigens. Am J Ind Med 1990;17:96-8.

42. Iversen M, Dahl R. Specific antigens in dust from swine confinement buildings. Am J Ind Med 1994;25:49-51.
43. Crook B, Robertson JF, Travers Glass SA, Botheroyd EM, Lacey J, Topping MD. Airborne dust, ammonia, microorganisms, and antigens in pig confinement houses and the respiratory health of exposed farm workers. Am Ind Hyg Assoc 1991;52:271-9.
44. Newman Taylor AJ, Gordon S. Laboratory animal and insect allergy. In: Bernstein IL, Chan-Yeung M, Malo JL, Bernstein DI, eds. Asthma in the workplace. Marcel Dekker, New York, 1993:399-414.
45. Johansson E, Borga A, Johansson SGO, Hage-Hamsten M van. Immunoblot multi-allergen inhibition studies of allergenic cross-reactivity of the dust mites *Lepidoglyphus destructor* and *Dermatophagoides pteronyssinus*. J Immunol 1991;147:972-9.
46. Larsson, KA, Eklund, AG, Hansson, LO, Isaksson, BN, Malmberg, PO. Swine dust causes intense airways inflammation in healthy subjects. Am J Respir Crit Care Med 1994;150:973-7.
47. Sandström T, Bjermer L, Rylander R. Lipopolysaccharide (LPS) inhalation in healthy subjects increases neutrophils, lymphocytosis, and fibronectin levels in bronchoalveolar lavage fluid. Eur Respir J 1992;5:992-6.
48. Palmberg L, Isaksson B-M, Larsson K, Malmberg P. Swine dust is more potent than LPS to induce interleukin-8 production in a pulmonary epithelial cell line. Eur Respir J 1994;7 (suppl 18):73s.
49. Essen SG von, Robbins RA, Thompson AB, Ertl RF, Linder J, Rennard S. Mechanisms of neutrophil recruitment to the lung by grain dust exposure. Am Rev Respir Dis 1988;138:921-7.
50. Fogelmark B, Sjöstrand M, Rylander R. Pulmonary inflammation induced by repeated inhalations of ß(1,3)-D-glucan and endotoxin. Int J Exp Pathol 1994;75:85-90.
51. Cinco M, Perticarari S, Presani G, Dobrina A, Liut F. Biological activity of a peptidoglycan extracted from *Leptospira interrogans*: in vitro studies. J Gen Microbiol 1993;139:2959-64.
52. Buringh E, Noy D, Pouwels H, Swuste P. A systematic implementation of control measures for airborne contaminants in the workplace air. Staub - Reinhalt. Luft 1992;52:347-51.
53. Goldenhar LM, Schulte PA. Intervention research in occupational health and safety. J Occup Med 1994;33:763-75.

Summary

In the Netherlands, some 13,000 people work 20 or more hours per week on pig farms (Chapter 1). Most are involved in typical family farms, each having one or only a few workers. Studies carried out during the past two decades have shown that working in pig farming, even for just a few hours per day, is associated with a high prevalence rate of respiratory symptoms. However, evidence from the literature on the potential causes of respiratory health effects is weak, and gives a confusing picture. This study specifically focused on chronic respiratory health effects.

In Chapter 2, a review of the literature is presented. A large number of studies, usually focused on the assessment of the presence of respiratory symptoms and on changes in respiratory ventilation, have been published. Only a few included exposure assessment for the study of exposure-response relationships or to identify determinants of exposure. In most studies, a large percentage of pig farmers reported chronic respiratory symptoms, mainly cough, phlegm, and chronic bronchitis. There were indications that exposure-related reductions in base-line FEV_1, FVC and FEV_1/FVC were present in pig farmers, while flow-volume parameters (MMEF and MEF_x) seemed to be more strongly reduced. Work-related allergies could not explain the high prevalence rate of respiratory symptoms. Studies performed on non-specific bronchial responsiveness in pig farmers show inconsistent results. Endotoxins are frequently suggested to be a potential cause of respiratory health effects. In some studies associations between the level of endotoxin exposure and base-line lung function were observed, but in none of these studies was this association based on personal exposure assessment. In these studies, endotoxin exposure levels were not related to chronic respiratory symptoms. Exposure-response relationships between dust/ammonia and chronic respiratory symptoms/base-line lung function were generally not observed. The studies failed to uncover the etiology of the health effects, for which several explanations were suggested.

By intensive exposure assessment and by assessing a large number of health parameters, we studied potential etiological factors more extensively than previous studies. 200 pig-farm owners working more than five hours per day in pig farming were selected to participate in a cross-sectional case-control study.

100 farmers with and 100 farmers without chronic respiratory symptoms were selected, based on validated questionnaires and a medical survey.

Poor characterization of exposure was one of the reasons suggested for the limited evidence found concerning potential causes of chronic respiratory health effects. Compared to other studies, we substantially improved exposure assessment by measuring the exposure of the 200 pig farmers for two days, and by means of personal sampling. In Chapter 3, we evaluated the extent to which our sampling strategy for endotoxin exposure was useful for application in exposure-response relationships. It was shown that, even with improved exposure assessment, relationships between the average of both endotoxin exposure measurements and base-line lung function were still expected to be biased. Because of the large ratio between intraindividual and interindividual variation in exposure alone, theoretically 42 measurements per individual would be required to obtain an underestimation of those relationships by 10% or less. Other factors, such as rarely occurring exposures (during activities which are performed only once every few days) and large differences in exposure outside the periods of exposure measurement, further biased exposure-response relationships. Therefore, data on time activity patterns over a period of 14 days and on farm characteristics were used to estimate long-term average exposure to endotoxins by means of empirical modelling. Associations between base-line lung function and exposure were found to be stronger when modelled exposure was used, compared to individual average measured exposure. By modelling and using information concerning more days than those during which exposure was measured, first the influence of intraindividual variation in exposure was reduced and second, presumably a more valid measure of long-term average exposure was obtained. This was also substantiated by the results of a small-scale validation study.

In Chapter 4, we describe the identification of determinants of exposure. The results were used to reduce exposure to dust and endotoxins in order to minimize health hazards. The mean Time-Weighted Average (TWA) exposure to endotoxins was 129 ng/m^3 (arithmetic mean, range 6-1503 ng/m^3), and to dust 3.0 mg/m^3 (arithmetic mean, range 0.3-27 mg/m^3), averaged over all separate actual measurements. The suggested and accepted threshold values for occupational exposure were often exceeded for endotoxin exposure, and sometimes for dust exposure. A number of flooring and some feeding characteristics were found to be potential contributors to high endotoxin exposure levels, and aspects of hygiene and feeding to high dust exposure levels. In

addition, activities performed frequently and those involving the handling of active animals were positively associated with exposure to dust and endotoxins. The results suggest that in specific cases, large reductions in effective exposure can be obtained by modifying farm characteristics and introducing appropriate personal protection equipment during selected activities. However, additional studies are needed to assess the actual effect in practical situations.

Associations between chronic respiratory symptoms and base-line lung function on the one hand and various exposures on the other hand are evaluated in Chapter 5. The results show strong associations between the duration of and the pressure used during the disinfection procedure. For example, a procedure lasting more than 10 minutes was associated with chronic respiratory symptoms (odds ratio (OR)=3.9, 95% confidence interval (CI) = 1.3-11.6), and with a decrease in base-line lung function, e.g. 440 ml (se 180 ml) in FEV_1 in the entire population. Exposure to endotoxins (average individual exposure: 111 ng/m^3) was inversely related to base-line lung function. In the total population, the estimated decrease was approximately 315 ml for both FVC and FEV_1 ($p < 0.10$, tested one-sided) over the 5-95 percentile range of exposure levels. In asymptomatic farmers these associations were stronger in terms of statistical significance and magnitude. The average individual exposure to ammonia was 1.7 mg/m^3 (range 0.3-4.2 mg/m^3). The decrease in FEV_1 over the 5-95 percentile range of ammonia exposure levels was 470 ml ($p < 0.05$) in the total population, but stronger (statistical significance and magnitude) in symptomatic farmers. Exposure to dust (average over individuals 2.7 mg/m^3) was not related to base-line lung function. Exposure to dust, endotoxins and ammonia was not associated with chronic respiratory symptoms. The results suggest that the use of disinfectants may be an important etiological factor in chronic respiratory health effects on pig farmers. Evidence indicating exposure to endotoxins or ammonia playing a role in this was weaker. Because of the population selection procedure, the presented associations do not give unbiased estimates of these associations in the base population.

Atopic (IgE) sensitization to aeroallergens can be a cause of respiratory health impairment. We studied the association between chronic respiratory symptoms and IgE sensitization to common as well as work-related allergens in various ways. In Chapter 6, associations between chronic symptoms and atopic sensitization to common allergens in combination with exposure to specific disinfectants or endotoxins are evaluated. Laboratory analyses of serum-IgE antibodies against potential work-related allergens and associations with symptoms are described in Appendix 2. In addition, it was evaluated whether non-allergenic exposures (disinfectants and endotoxins) could have an effect on atopic sensitization to common allergens and self-reported allergies (Chapter 6). The results of the analyses suggested previously-unreported etiological pathways of respiratory impairment in pig farmers.

First, a clearly positive association between chronic respiratory symptoms and sensitization to potential work-related allergens (pig urinary proteins, pig-pen dust, storage mites) was present (OR=2.7, 95%-CI 1.1-6.4). This relationship was independent of sensitization to house dust mite. The observed association indicates that in some pig farmers chronic respiratory symptoms may develop as a result of IgE sensitization to work-related allergens.

Secondly, the combination of IgE to common allergens and the use of disinfectants containing quaternary ammonium compounds was strongly and positively related to respiratory symptoms. Compared to farmers without IgE sensitization to common allergens and who used no disinfectants or disinfectants without quaternary ammonium compounds, the odds ratio was 4.3 (95%-CI 1.3-14.1) for chronic respiratory symptoms and 8.7 (95%-CI 1.7-44.3) for asthma-like symptoms. Asthma-like symptoms were defined as a combination of chronic respiratory symptoms and non-specific bronchial responsiveness ($PC_{10} \leq 16$ mg of histamine/ml). A combination of sensitization to common allergens and high endotoxin exposure levels (> 101 ng/m^3) was strongly associated with asthma-like symptoms only (OR=6.3, 95%-CI 1.0-37.9). Atopic sensitization to common allergens and exposure to specific disinfectants or high endotoxin exposure levels were independently not related to respiratory symptoms. This suggests that an interaction may exist between non-allergenic exposures and atopic sensitization, resulting in an increased risk of respiratory symptoms.

Thirdly, IgE sensitization to common allergens appeared to be positively associated with the use of disinfectants containing only quaternary ammonium compounds (OR=7.3, 95%-CI 1.3-42.5). Odds ratios for other disinfectants or combinations of disinfectants ranged from 2.2 to 4.2, but were statistically not significant ($p > 0.05$). Atopic sensitization was not positively related to endotoxin exposure, but there was a strong positive association between self-reported allergies and high endotoxin exposure levels (> 101 ng/m^3; OR=5.5, 95%-CI 1.7-17.6). This indicates that non-allergenic occupational exposure could in itself enhance IgE sensitization to common allergens or affect the immuno-response in another way, with increased risk of respiratory symptoms. Associations which are to some extent comparable have been described before only in laboratory animal studies and in a few human epidemiological studies in incomparable settings. Since evidence from the literature is sparse and the sequence of events cannot be studied in a cross-sectional design, future research is needed to obtain more evidence on the studied effects. This should preferably be prospective in design.

A protective effect against IgE-mediated allergies has been suggested for some antigen-specific IgG_4 antibodies by their potential blocking properties. We studied such an effect for IgG_4 against pig urinary proteins (Chapter 7), although type I allergy to pig-derived proteins is generally not regarded to be common among pig farmers. In our population only 6% showed IgE sensitization to pig urinary proteins (Appendix 2). IgG_4 titres were inversely related to non-specific bronchial responsiveness to histamine ($PC_{10} \leq 16$ mg/ml and $PC_{20} \leq 8$ mg/ml) and to the combination of chronic respiratory symptoms and $PC_{10} \leq 16$ mg/ml (both absent versus both present). For the latter, the odds ratio for titres from 6 to 50 compared to lower than 6 was 0.33 (95%-CI 0.11-0.98) and the odds ratio for titres exceeding 50 compared to lower than 6 was 0.16 (95%-CI 0.04-0.66). Associations between chronic respiratory symptoms, irrespectively of non-specific bronchial responsiveness, and IgG_4 titres were weak. The observed inverse associations with respiratory health effects might be the result of the suggested protective effect of antigen-specific IgG_4.

The results of our study have been discussed in view of other studies, taking potential effects of bias into account (Chapter 8). The cross-sectional study design might have led to misclassification of exposure that was relevant to the studied health effects. Misclassification is possible in pig farming by, for example, changes in farm characteristics or by task allocation. Given the small interindividual variation in exposure to endotoxins, dust and ammonia, a little exposure misclassification can result in seriously biased (usually underestimated) exposure-response relationships.

The results of this study suggest that chronic respiratory health effects may be caused by different agents, including non-allergenic as well as potential allergenic agents. Some of the observed associations between non-allergenic exposures and health effects seem to be independent of specific immunological sensitization. The strength of the effects varied between groups classified according to symptomatology or smoking habits. Some respiratory symptoms could be caused by non-allergenic exposures that may act through mechanisms which involve specific immunological sensitization to non-work-related allergens. It is likely that such mechanisms are present at the level of an increase in IgE response as well as an enhanced reaction in type I allergy. Specific IgE and IgG_4

sensitization to allergens or antigens that are potentially work-related may increase the risk of or protect against respiratory impairment, respectively.

A priori no such complex etiology was expected. As a result, small groups were often used to study associations, with large confidence intervals for estimated exposure-response relationships. Despite this limitation and the drawback of the cross-sectional study design, the observed associations most likely reflect actual mechanisms. At the population level, especially prospective studies which are designed to study the postulated complex etiology are necessary. The potential multifactorial causes and the role of specific immunological sensitization with large interindividual variation require these studies to include a large number of farmers or to apply appropriate restriction. Given the unfavourable exposure-variability of several agents, exposure should be assessed on multiple occasions.

Samenvatting

In Nederland werken ongeveer 13.000 mensen minimaal 20 uur per week in de varkenshouderij. De meerderheid hiervan werkt op typische familiebedrijven, waar één of enkele mensen min of meer full-time werkzaam zijn. Eerder onderzoek wees uit dat werken in de varkenshouderij, ook al is dit slechts enkele uren per dag, samengaat met een hoge prevalentie van luchtwegklachten. In de literatuur kunnen echter geen duidelijke aanwijzingen gevonden worden wat de mogelijke oorzaken zijn van de effecten op longen en luchtwegen. In ons onderzoek hebben wij ons met name gericht op de chronische effecten.

Hoofdstuk 2 geeft een overzicht van de literatuur. In het verleden zijn er al veel studies gedaan in verschillende landen die betrekking hadden op luchtwegklachten van varkenshouders. Deze studies beschreven meestal het vóórkomen van klachten en van afwijkingen in de longfunctie. Er bestaan slechts enkele studies waarin blootstelling werd gemeten om de relatie met respiratoire effecten te kunnen onderzoeken, of waarin de relatie tussen blootstelling en werkomstandigheden werd onderzocht. In de meeste onderzoeken bleken veel varkenshouders chronische klachten te rapporteren, vooral van hoesten, slijm ophoesten, of chronische bronchitis. Er waren aanwijzingen dat het werken in de varkenshouderij een verlaging teweegbrengt van de belangrijkste parameters van de basis-longfunctie (FVC en FEV_1), maar negatieve effecten op de flow-volume parameters (MMEF and MEF_x) leken sterker te zijn. De hoge prevalentie van klachten kon niet verklaard worden door allergie tegen werk-gerelateerde allergenen. De studies waarin effecten op niet-specifieke bronchiale reactiviteit werden geëvalueerd, lieten uiteenlopende resultaten zien. Er werd vaak gesuggereerd dat blootstelling aan endotoxine een belangrijke oorzaak zou kunnen zijn van respiratoire effecten. In enkele studies kon een verband worden aangetoond tussen het niveau van de endotoxine-blootstelling en de basis-longfunctie, maar de blootstelling was in geen van deze studies persoonlijk gemeten. In dezelfde studies werd echter geen verband gevonden tussen het vóórkomen van chronische klachten en het blootstellingsniveau. In het algemeen konden evenmin associaties worden aangetoond tussen de blootstelling aan stof of ammoniak, en de longfunctie of chronische klachten. Uit eerdere onderzoeken is moeilijk af te leiden wat de oorzaken zijn van de effecten op longen en luchtwegen, en via welke mechanismen deze verlopen. Hiervoor werden verschillende redenen gegeven.

Wij onderzochten mogelijke oorzakelijke factoren gedetailleerder dan in andere studies door een groter aantal gezondheidsparameters te bepalen en uitgebreidere karakterisering van de blootstelling. Er werden 200 eigenaren van varkensbedrijven geselecteerd voor deelname aan een dwarsdoorsnede onderzoek met een case-controle opzet. Uit een groep varkenshouders die dagelijks 5 uur of meer in de varkenshouderij werkzaam is, werden er 100 met en 100 zonder CARA-klachten geselecteerd op basis van gevalideerde vragenlijsten en een gezondheidskundig onderzoek.

In hoofdstuk 2 werd de slechte karakterisering van de blootstelling genoemd als één van de mogelijke redenen waarom er nog weinig bekend is over oorzaken van chronische respiratoire effecten. In ons onderzoek was de karakterisering van de blootstelling sterk verbeterd ten opzichte van andere onderzoeken door de blootstelling aan stof, endotoxines en ammoniak gedurende twee dagen te meten voor de 200 varkenshouders en door dit te meten met persoonlijke monstername. In hoofdstuk 3 werd voor endotoxine-blootstelling beoordeeld in welke mate deze uitgebreidere karakterisering geschikt was om blootstellings-effect relaties te kunnen kwantificeren. Zelfs met deze uitgebreidere meetstrategie bleek het niet mogelijk een redelijke schatting te kunnen maken van de werkelijke relaties tussen de basis-longfunctie en de gemiddelde endotoxine-blootstelling. Door de relatief grote variatie in blootstelling binnen personen ten opzichte van die tussen personen zouden er theoretisch 42 metingen per individu nodig zijn om een onderschatting van ten hoogste 10 procent te bereiken. Andere factoren zorgden voor verdere vertekening van de blootstellings-effect relaties, zoals het incidenteel uitvoeren van bepaalde activiteiten met specifieke blootstelling en de grote verschillen in de blootstelling buiten de periodes waarin de blootstelling werd gemeten. We gebruikten daarom gegevens over werkzaamheden gedurende twee volledige weken en over bedrijfskenmerken om, met behulp van empirische modellen, de lange-termijn blootstelling aan endotoxine te kunnen schatten. De relaties tussen longfunctie en blootstelling waren sterker (in termen van grootte en statistische significantie) voor de gemodelleerde blootstelling dan voor de gemiddelde gemeten blootstelling. Door de nadelige invloed van de variatie in de blootstelling met modelleren te verkleinen en door gebruik te maken van informatie over werkzaamheden gedurende 14 dagen, is het aannemelijk dat er een betere maat werd verkregen voor de lange-termijn blootstelling. Dit idee werd ondersteund door de resultaten van een kleine validatie-studie.

In hoofdstuk 4 beschreven we hoe we determinanten van blootstelling hebben onderscheiden. De resultaten werden gebruikt om methoden aan te kunnen geven waardoor de effectieve blootstelling aan stof en endotoxinen verminderd kan worden, om daarmee het risico op respiratoire effecten te verkleinen. De gemeten Tijd Gewogen Gemiddelde blootstelling aan endotoxinen was 129 ng/m^3 (rekenkundig gemiddelde, range 6-1503 ng/m^3), en aan stof 3,0 mg/m^3 (rekenkundig gemiddelde, range 0,3-27 mg/m^3). Deze waarden zijn gemiddelden over alle metingen. De voorgestelde grenswaarde voor blootstelling aan endotoxine in de arbeidssituatie werd vaak overschreden, terwijl de vastgestelde waarde voor stof in een beperkt aantal gevallen werd overschreden. Vooral veel vloertypen en enkele voedermethoden waren gerelateerd aan de endotoxine-blootstelling, terwijl met name een aantal voedermethoden en aspecten van hygiëne gerelateerd bleken te zijn aan de stofblootstelling. Verder was er een positief verband tussen een aantal activiteiten die dagelijks werden uitgevoerd of waarbij erg actieve dieren betrokken waren, en het niveau van stof- en endotoxine-blootstelling. Deze resultaten suggereren dat er door aanpassingen in bedrijfskenmerken en het gebruik van goede persoonlijke adembescherming in specifieke gevallen een sterk verlaagde effectieve blootstelling gerealiseerd kan worden. Er zijn echter nog aanvullende studies nodig om het werkelijke effect te kunnen bepalen.

In hoofdstuk 5 werden de verbanden geëvalueerd tussen CARA-klachten en basis-longfunctie enerzijds en verscheidene typen blootstelling anderzijds. Er werden sterke associaties gevonden met de duur van de desinfectie-procedure en de druk die daarbij gebruikt werd. Een procedure van meer dan 10 minuten bleek gerelateerd te zijn aan voorkomen van CARA-klachten (odds ratio (OR)=3,9, 95%-betrouwbaarheidsinterval 1,3-11,6) en een slechtere basislongfunctie, bijvoorbeeld gemiddeld een 440 ml (se 180 ml) lagere FEV$_1$ in de gehele populatie (volume bij geforceerde uitademing in 1^e seconde). Blootstelling aan endotoxinen (gemiddelde individuele blootstelling: 111 ng/m^3) was negatief geassocieerd met de longfunctie. In de hele populatie was de geschatte daling in de FEV$_1$ en FVC (totaal volume bij geforceerde uitademing) ongeveer 315 ml over de 5-95 percentiel range ($p < 0,10$, eenzijdig getest), maar de associaties waren sterker in termen van grootte en statistische significantie in de groep varkenshouders zonder CARA-klachten. De gemiddelde individuele blootstelling aan ammoniak was 1,7 mg/m^3 (range 0,3-4,2 mg/m^3). De daling in

FEV_1 over de 5-95 percentiel range van de ammoniak blootstelling was 470 ml ($p < 0,05$) en sterker bij varkenshouders met CARA-klachten. Blootstelling aan stof (gemiddeld over individuen 2,7 mg/m^3) was niet gerelateerd aan de longfunctie. Er was geen verband tussen stof-, endotoxine of ammoniakblootstelling en CARA-klachten. De resultaten suggereren dat desinfectantia mogelijk belangrijk zijn bij het ontstaan van chronische respiratoire effecten bij varkenshouders. De resultaten van ons onderzoek die wijzen op een mogelijk causaal verband met blootstelling aan endotoxinen en ammoniak zijn zwakker. Vanwege de gehanteerde selectieprocedure zijn de gepresenteerde verbanden niet representatief voor de verbanden die in de basis-populatie verwacht mogen worden.

Atopische (IgE-) sensitisatie tegen aeroallergenen kan een oorzaak zijn van verslechterde respiratoire gezondheid. Wij bestudeerden op verschillende manieren het verband tussen CARA-klachten en IgE-sensitisatie tegen algemeen voorkomende (huisstofmijt, graspollen, berkepollen en kat) en werkgerelateerde allergenen. In hoofdstuk 6 zijn de relaties geëvalueerd met atopische sensitisatie tegen algemene allergenen in combinatie met blootstelling aan specifieke desinfectantia of aan endotoxine-blootstelling. In Appendix 2 zijn laboratorium analyses van IgE-antilichamen tegen mogelijk werkgerelateerde allergenen beschreven, en de relatie met klachten. Daarnaast werd gekeken of niet-allergene blootstelling gerelateerd was aan atopische sensitisatie en zelf gerapporteerde allergieën (hoofdstuk 6). De resultaten van deze analyses suggereren mogelijk oorzakelijke verbanden die nog niet eerder genoemd zijn.

In eerste instantie bleek er een positief verband te bestaan tussen CARA-klachten en sensitisatie tegen werkgerelateerde allergenen: varkensurine-eiwitten, stalstof en voorraadmijt, hoewel het niet zeker is dat sensitisatie tegen voorraadmijt veroorzaakt wordt door werken in de varkenshouderij. Voor dit verband was de odds ratio 2,7 (95%-bthi 1,1-6,4). Het verband was onafhankelijk van sensitisatie tegen huisstofmijt. Het lijkt dus aannemelijk dat een deel van de varkenshouders CARA-klachten kan hebben door specifieke sensitisatie tegen deze werkgerelateerde allergenen.

Ten tweede bleek er een sterk verband te bestaan tussen CARA-klachten en de combinatie van IgE tegen algemene allergenen en het gebruik van desinfectantia met quaternaire ammonium verbindingen. Ten opzichte van de varkenshouders zonder IgE-sensitisatie en die geen of andere desinfectantia gebruikten, was de odds ratio 4,3 (95%-bthi 1,3-14,1) voor alle CARA-klachten en 8,7 (95%-bthi 1,7-44,3) voor astma-achtige klachten. Astma-achtige klachten waren gedefinieerd als de combinatie van CARA-klachten met licht verhoogde niet-specifieke bronchiale reactiviteit ($PC_{10} \leq 16$ mg histamine per ml). De combinatie van IgE-sensitisatie tegen algemene allergenen en een hoge endotoxine-blootstelling (> 101 ng/m^3) was op een vergelijkbare manier sterk geassocieerd met astma-achtige klachten (OR=6,3, 95%-bthi 1,0-37,9). IgE-sensitisatie, het gebruik van desinfectantia met quaternaire ammonium verbindingen, en een hoge endotoxine-blootstelling bleken onafhankelijk van elkaar niet samen te hangen met de aanwezigheid van CARA-klachten in het algemeen of met astma-achtige klachten. Het gevonden verband wijst op een mogelijke interactie tussen IgE-sensitisatie en blootstelling aan niet-allergene stoffen, die leidt tot een verhoogd risico op het krijgen van verschillende CARA-klachten.

Ten derde bleek er een sterk positief verband te bestaan tussen IgE-sensitisatie tegen algemene allergenen en het gebruik van desinfectantia die alleen quaternaire ammonium verbindingen als actieve componenten bevatten (OR=7,3, 95%-bthi 1,3-42,5). Odds ratio's voor andere desinfectantia of combinaties van actieve stoffen lagen tussen 2,2 en 4,2, maar waren niet statistisch significant ($p > 0,05$). Atopische sensitisatie was niet positief gerelateerd aan de endotoxine-blootstelling, maar daarentegen bleek er een sterk positief verband te bestaan tussen zelf gerapporteerde allergieën en endotoxine-blootstelling (> 101 versus < 101 ng/m^3) met een odds ratio van 5,5 (95%-bthi 1,7-17,6). Deze verbanden suggereren dat niet-allergene blootstellingen specifieke IgE-sensitisatie kunnen bevorderen of dat ze het immuunsysteem op een andere manier beïnvloeden met een verhoogd risico op respiratoire symptomen. Deze typen associaties met niet-allergene inhalatie expositie zijn eerder alleen gevonden in proefdier-onderzoek en in enkele humaan epidemiologische studies, maar alleen in situaties die niet vergelijkbaar zijn met de varkenshouderij. Omdat er tot nu toe weinig vergelijkbare verbanden zijn gerapporteerd en omdat de volgorde van de effecten die uiteindelijk leiden tot een verhoogd risico op luchtwegklachten niet bestudeerd kan worden in een dwarsdoorsnede onderzoek, is op dit terrein aanvullend onderzoek nodig dat bij voorkeur prospectief van opzet is.

In de literatuur is gesuggereerd dat sommige antigeen-specifieke IgG_4-antilichamen mogelijk een beschermend effect hebben tegen IgE-gemedieerde allergieën, met name door competitie met IgE-antilichamen tegen dezelfde allergenen. Wij hebben een dergelijk effect bestudeerd voor IgG_4 tegen varkensurine-eiwitten (hoofdstuk 7), hoewel er in het algemeen wordt aangenomen dat een type-I allergie tegen varkens-allergenen geen belangrijke rol speelt bij het voorkomen van CARA-klachten bij varkenshouders. 6 procent van onze populatie varkenshouders bleek IgE-antilichamen te hebben tegen varkensurine-eiwitten (Appendix 2). Bij varkenshouders zonder bronchiale reactiviteit tegen histamine (zowel PC_{10} > 16 mg/ml als PC_{20} > 8 mg/ml) en die geen astma-achtige klachten hadden (geen CARA-klachten en PC_{10} > 16 mg/ml), werden er duidelijk vaker hoge IgG_4-titers gevonden dan bij varkenshouders met bronchiale reactiviteit of astma-achtige klachten. Voor het verband met astma-achtige klachten werd een odds ratio gevonden van 0,33 (95%-bthi 0,11-0,98) voor titers van 6 tot 50 vergeleken met 5 of lager, terwijl de odds ratio voor titers van 50 of hoger vergeleken met 5 of lager 0,16 was (95%-bthi 0,04-0,66). De verbanden met CARA-klachten in het algemeen waren wel negatief, maar deze waren niet sterk. De gevonden inverse verbanden met astma-achtige klachten en bronchiale reactiviteit zijn mogelijk het gevolg van de veronderstelde beschermende werking van antigeen-specifiek IgG_4.

De resultaten van ons onderzoek worden in hoofdstuk 8 bediscussieerd in het licht van resultaten van andere onderzoeken. Daarbij werd ook rekening gehouden met mogelijke vertekening van de uitkomsten, wat in dit type epidemiologisch onderzoek veroorzaakt kan worden door verschillende onderzoeks-gerelateerde factoren. Het is mogelijk dat er in ons onderzoek misclassificatie van de blootstelling optrad die de relaties tussen blootstelling en effecten wezenlijk vertekende. Deze misclassificatie is in de varkenshouderij bijvoorbeeld mogelijk door taakverdeling tussen mensen binnen hetzelfde bedrijf. Bij het geringe verschil in blootstelling aan stof, endotoxinen en ammoniak tussen de onderzochte varkenshouders, was het mogelijk dat geringe misclassificatie al tot gevolg kan hebben dat werkelijke effecten sterk onderschat of in het geheel niet gedetecteerd worden.

De resultaten van ons onderzoek suggereren dat chronische respiratoire effecten het gevolg kunnen zijn van blootstelling aan verschillende typen blootstelling, zowel allergene als niet-allergene stoffen. Sommige associaties met niet-allergene stoffen lijken onafhankelijk te zijn van specifieke immunologische sensitisatie. De sterkte van deze effecten varieerde tussen verschillende groepen, afhankelijk van aanwezigheid van klachten en rookgewoonten. Sommige respiratoire effecten door blootstelling aan niet-allergene stoffen zouden echter het gevolg kunnen zijn van mechanismen die verlopen via interactie met specifieke immunologische sensitisatie tegen niet-werkgerelateerde allergenen. Het is waarschijnlijk dat deze interacties aanwezig zijn op het niveau van versterkte IgE-sensitisatie en een versterkte reactie bij een type-I allergie. Daarnaast lijkt het mogelijk dat specifieke IgE- en IgG_4-sensitisatie tegen werkgerelateerde allergenen en antigenen het risico op effecten respectievelijk verhogen en verlagen.

Bij aanvang van het onderzoek was er geen rekening gehouden met zo'n grote verscheidenheid aan etiologische factoren en mechanismen. Hierdoor konden veel verbanden slechts beschreven worden voor kleine groepen, waardoor de betrouwbaarheidsintervallen groot waren. Ondanks deze beperking en het nadeel van de dwarsdoorsnede-opzet van het onderzoek, is het waarschijnlijk dat de beschreven effecten in werkelijkheid voorkomen. Om meer zekerheid te krijgen over de mechanismen en typen blootstelling die leiden tot chronische respiratoire effecten, zal vervolg-onderzoek vooral prospectief dienen te zijn. Door de vele factoren en mechanismen die onafhankelijk van elkaar mogelijk deze effecten veroorzaken, is het nodig dat dergelijk onderzoek wordt uitgevoerd in een grote groep varkenshouders, of dat door juiste restrictie de verstorende effecten van andere typen blootstelling en mechanismen worden vermeden. Omdat er binnen de groep varkenshouders relatief weinig variatie bestaat in gemiddelde blootstelling aan verschillende stoffen en juist relatief veel variatie in de tijd binnen één en dezelfde persoon, zal de blootstelling aan deze stoffen herhaald uitgevoerd moeten worden om werkelijke blootstellings-effect relaties beter te kunnen kwantificeren.

Appendix 1. Questionnaires on Chronic Respiratory Symptoms

Appendix 1.1

1. Heeft u gedurende de laatste 2 jaar wel 3 maanden vrijwel dagelijks gehoest?
2. Heeft u gedurende de laatste 2 jaar wel 3 maanden vrijwel dagelijks slijm opgehoest?
3. Heeft u wel eens last van kortademigheid als u met leeftijdsgenoten in normaal tempo op vlak terrein wandelt?
4. Hebt u ooit last van piepen op de borst gehad?
5. Zo ja, heeft u dit de laatste 2 jaar wel eens meer dan één week achtereen gehad?
6. Heeft u weleens aanvallen van benauwdheid (astma) gehad?

Appendix 1.2

1. Hoest u vrijwel dagelijks, wel 3 maanden per jaar?
2. Geeft u zo vrijwel dagelijks fluimen op, wel 3 maanden per jaar?
3. Heeft u in de afgelopen 3 jaren weleens een periode gehad van (een toename van) hoesten en opgeven van fluimen, die minstens 3 weken duurde?
4. Heeft u last van kortademigheid, wanneer u met andere mensen van uw leeftijd in normaal tempo op vlak terrein loopt?
5. Heeft u ooit last van 'piepen' op de borst' gehad?
6. Heeft u in de afgelopen 3 jaren vaker dan 1 keer een periode gehad van (een toename van) hoesten en opgeven van fluimen, die minstens 3 weken duurde?
7. Heeft u last van kortademigheid, wanneer u met andere mensen van uw leeftijd in normaal tempo op vlak terrein loopt?
8. Heeft u de meeste dagen of nachten last van 'piepen' op de borst'?
9. Heeft u weleens in rust aanvallen van benauwdheid met 'piepen' op de borst' gehad? (astma aanvallen)

Appendix 2

IgE antibodies against pig urinary proteins in pig farmers

Roel Vermeulen, Liesbeth Preller, Gert Doekes, Jack Spithoven, Dick Heederik

Abstract

Respiratory symptoms are common among pig farmers. Type I reactivity to pig stable allergens has been described in some pig farmers as a cause of occupational asthma. This study was performed (1) to estimate more accurately the prevalence of type I reactivity towards pig stable allergens; (2) to identify the IgE-binding allergens; and (3) to assess its contribution to the occurrence of respiratory health problems in this occupational group. Sera of 200 pig farmers were selected: 100 from subjects with chronic respiratory symptoms and 100 without any of the chronic respiratory symptoms. Enzyme immunoassays (EIA) were used to assess IgE reacting with pig stable dust extracts, or with pig urinary proteins (PUP) and inhibition EIA to determine cross-reactivity of PUP with pig stable dust extracts, pig serum proteins, and serum albumin. Immunoblotting was performed with pig stable dust extract and PUP to determine major IgE binding components. IgE to pig stable dust and PUP was found in 5% and 6%, respectively, of the total population. A strong correlation between the IgE reactions with PUP, pig stable dust and pig serum was observed, and these preparations showed almost complete mutual inhibition in the IgE EIA. Immunoblotting showed an almost identical IgE pattern for PUP and pig stable dust extracts, identifying the 58 kDa and 33 kDa band as major allergens. Almost no differences in reaction pattern between the individual sera were observed.

Respiratory symptoms had a non-significant tendency to occur more frequently among seropositive individuals. Serum IgE to PUP can be found in a small but substantial proportion of all pig farmers, and accounts for practically all of the IgE reactivity towards crude pig stable dust extracts, at least in our population. The major IgE binding components in PUP are serum-derived proteins of 58 kDa and 33 kDa. Although atopic sensitization to PUP seems to be no major risk factor, it might significantly contribute to the occurrence and severity of respiratory health problems in pig farmers.

Submitted to Journal of Allergy and Clinical Immunology

Introduction

Respiratory symptoms are common among pig farmers[1-4]. Epidemiological studies have demonstrated high prevalence rates of respiratory symptoms such as chronic cough, chronic phlegm, shortness of breath, wheezing and chest tightness. In a health survey among 172 Dutch pig farmers, more than 40% reported at least one chronic respiratory symptom[3]. Exposure to pig stable dust is presumed to be the major cause of these respiratory symptoms[5,6]. However, crude pig stable dust is a heterogenous mixture of moulds, bacteria, insect parts, pollen, pig feed, pig urinary proteins (PUP), dried pig faeces and microbial compounds like endotoxins[5,6]. Because of the complex composition of pig stable dust, respiratory symptoms might be caused by several inflammatory reaction mechanisms: non-specific reactions to irritants, toxic reactions and immediate or late phase allergic mechanisms.

Several studies in the general farming population have investigated the occurrence of type I allergy to work-related allergens, such as storage mites, animal feed components and mammalian derived allergens. In general, sensitization to storage mites had the highest prevalence, whereas the prevalence of sensitization to pig allergens appeared to be low[7-13]. In the majority of these studies the study population consisted of dairy farmers or a combination of farmers from the general farming population. Few studies have been performed exclusively among pig farmers, and in the available studies the populations were generally small. Moreover, conflicting results were reported with respect to the presence of IgE to pig allergens. Katila et al.[14], Brouwer et al.[12], Zhou et al.[15] and Larsson et al.[16] found no IgE sensitization to pig allergens. Matson et al.[17] found 1 out of 41 pig farmers to be sensitized to pig allergens, whereas Crook et al.[18], Cormier et al.[19] and Zuskin et al.[20] found much higher prevalences of sensitization to pig allergens, varying from 12.5% to 44.0%. However, only two cases have been described in which asthma was caused by the presence of IgE antibodies to pig-derived allergens[21,22]. The pig-derived antigens tested in these studies were epithelium, hair, urine and plasma. In studies among laboratory animal workers it has been shown that urine from rats, mice, rabbits, dogs, and guinea pigs are important allergens[24-26].

This study was performed (1) to estimate more accurately the prevalence of IgE antibodies reacting with pig stable dust components and/or PUP; (2) to identify the IgE-binding components in pig stable dust and PUP; and (3) to investigate the relation between the IgE response to pig stable dust and PUP, and the presence of self-reported respiratory symptoms in pig farmers.

Methods

Subjects

Sera were collected from 200 male owners of pig farms, who worked at least 5 hours a day in pig farming. These subjects were selected from a group of 1,504 pig farmers living in the southeast of the Netherlands, in which a large-scale health survey was performed using self-administered questionnaires[27,28]. Of all pig farmers, 33% reported one or more of the following respiratory symptoms: chronic cough, chronic phlegm, shortness of breath, ever wheezing, frequent wheezing or chest tightness. One hundred farmers with at least one, and 100 farmers without chronic respiratory symptoms were selected for further medical investigation and exposure measurements. Venous blood samples were taken, and collected serum was stored at -20°C. For the present study all sera were defrosted and divided into 6 tubes and stored at -20°C before use. For each test a new tube was used. Personal inhalable dust and personal ammonia samples were taken during one work shift in summer and in winter. The procedures for assessment of dust, ammonia and endotoxin exposure have been described elsewhere[29-31].

Allergens

Pig urine was collected from several sows and finishing pigs and centrifuged at 25,000 g at 4°C for 30 minutes. The supernatant was dialysed for 48 hours against 0.05 M NH_4HCO_3, 48 hours against distilled water, and lyophilized. Settled pig stable dust was collected from pipes in swine confinement buildings on eye-level and suspended in carbonate-buffered saline pH 7.2 containing 42 mM phenol to prevent the growth of fungi or bacteria. The slurry was shaken for 48 hours and centrifuged at 25,000 g. The supernatant was filtered through a 0.45 μm Millipore filter (Millex-HA), dialysed against distilled water for 48 hours, and lyophilized.

Preparations of common environmental allergens and storage mites were obtained as lyophilized extracts from ALK Benelux (Houten, The Netherlands): birch pollen (*Betula verrucosa*, product no. 02.04), cat hair (*Felis Catus*, 15.02), house dust mite (*Dermatophagoides pternoyssinus*, 10.01), rye grass pollen

(*Lolium perenne*, 01.03), timothy grass pollen (*Phleum pratense*, 01.07), and storage mites (mixture of *Lepidoglyphus destructor*, *Tyrophagus putrescentiae* and *Acarus siro*, 35.00).

Stock solutions of these allergens, prepared by dissolving lyophilized extracts at 1 mg/ml in fresh phosphate-buffered saline (PBS), were made weekly and stored in aliquots at -20°C. For the determination of anti-grass pollen IgE, a 1/1 mixture of rye grass and timothy pollen allergen solution was used.

Measurement of specific IgE

Specific IgE was measured by enzyme immunosorbent assay (EIA) as described previously[32]. All incubations were performed with 0.1 ml aliquots and each incubation was followed by extensive washing, 3 cycles on an automatic plate washer (LKB-Pharmacia) with PBS pH 7.4 containing 0.05% (v/v) Tween-20 (PBT) (Polysorbatum, Merck, München, Germany). Flat-bottom 96-wells polystyrene microtiter plates with high binding capacity (product no. 655061; Greiner, Nuertingen, Germany) were coated overnight at 4°C with allergen dilutions - pig stable dust and PUP at 50 μg/ml, and the common environmental allergens at 25 μg/ml in PBS. Sera diluted 1/10 in PBT containing 0.5% (w/v) gelatin (Merck, Darmstadt, Germany) (PBTG) were added to the wells, and incubated for 2 hrs at 37°C. Bound IgE was measured with a four-step procedure, consisting of three 1 hr incubations at 37°C with monoclonal mouse anti-human-IgE (1/16,000; product no. M 1294; Central Laboratory of the Red Cross Bloodtransfusion Service (CLB), Amsterdam, The Netherlands), biotinylated rabbit anti-mouse immunoglobulins (1/5,000; no. E 354; Dakopatts (DAKO), Copenhagen, Denmark), avidin-peroxidase (1/2,000; no. P 364; DAKO), and finally an incubation for 30 minutes with o-phenylenediamine (OPD), 2 mg/ml in 0.05 M citrate phosphate buffer pH 4.5 (no. P 1526; Sigma Chemicals, St. Louis, MO, USA) containing 0.015% (v/v) H_2O_2. The reaction was stopped after 30 minutes by the addition of 50 μl 2N HCl and the absorption was read at 492 nm. In the assays for IgE anti-PUP and pig stable allergens, 10% (v/v) normal pig serum was added to the biotinylated rabbit anti-mouse immunoglobulins to prevent binding of apparently cross-reacting antibodies in this conjugate to the pig urine and pig stable dust coating. The addition of normal pig serum decreased the OD_{492} of reagent blanks from 0.624 to 0.052 and from 0.594 to 0.165 for PUP and pig stable dust extract, respectively. All sera were tested in duplicate wells on the same microtiter plate. Each plate included a positive control serum tested in duplicate, and two reagent blanks (no-serum controls) for each allergen. For each allergen, the mean OD_{492} of the reagent blank during the whole testing period plus three times the standard deviation was calculated. Sera were considered positive if the mean OD_{492} was higher than this value. After completion of the first series of serologic screening, all sera with a positive serum-allergen combination and a subset of negative sera were re-tested at one microtiter plate on the same day.

Measurement of total IgE

Total IgE was measured with a sandwich EIA in which IgE was captured on a coating of mouse anti-human IgE (CLB) diluted 1/4,000 in PBS and bound IgE was detected with a two step detection method, consisting of a 1 hr incubation with peroxidase-labelled monoclonal mouse anti-human IgE (1/1,000) (product no. m1334;CLB) and an incubation of 30 minutes with OPD containing 0.015% (v/v) H_2O_2. The sera were incubated at dilutions 1/10, 1/20 and 1/40 in PBTG for 2 hrs at 37°C. Each microtiter plate included one positive control and two reagent blanks and a serial dilution of an IgE standard (Pharmacia) ranging from 0.78 IU/ml to 50 IU/ml, in duplicate. To calculate IgE concentrations, OD_{492} values were logit-transformed, and a log-logit calibration line was constructed. Correlation coefficients for this regression line always exceeded 0.99. Only the results obtained with serum dilutions giving an OD_{492} exceeding the mean + 3 SD of the reagents blank were used. If the results obtained, at different serum dilutions, differed more than 15%, the test was repeated at higher serum dilutions.

Inhibition

Serial dilutions of PUP, pig stable dust extract, normal pig serum or pig albumin (A1173; Sigma Chemicals, St. Louis, MO, USA) (50 μl) were added to wells coated with one of these allergens. Diluted sera were added (50 μl), mixed and the plates were incubated for 2 hrs at 37°C. Bound IgE was measured with the four-step procedure as described above. The percentage of inhibition was calculated as:

$$\%inhibition=\frac{OD_{(no\text{-}inh)}-OD_{(test)}}{OD_{(no\text{-}inh)}-OD_{(blank)}}*100 \tag{1}$$

in which $OD_{(no\text{-}inh)}$= OD_{492} of sera without inhibitor, $OD_{(test)}$= OD_{492} of sera with dilution of inhibitor and $OD_{(blank)}$= OD_{492} of reagent blank

SDS-polyacrylamide gel electrophoresis & immunoblotting

Lyophilized pig urine and pig stable dust proteins were dissolved in sample buffer (1 mg protein per ml) according to Laemmli[33], incubated for 10 minutes at 100°C, and applied in a 10*7.5*0.05 cm 13.5% polyacrylamidegel (30μl extract per cm), in a Mini-Protean II slab cell unit (Biorad, Richmond, CA USA). Electrophoresis was performed at 100 Volt for 10 minutes and 200 Volt at 50 minutes, successively. Low molecular weight markers (Biorad) were included in duplicate in separate lanes.

After SDS-PAGE, proteins were blotted onto a nitrocellulose sheet (Sleicher & Schuell;0.22 μm pore size), using a Mini-Trans blot transfer unit (Biorad). The transfer was performed at 3.33 mA/cm^2 for 1 hour at room temperature.

After blotting, the nitrocellulose sheet was cut into strips of 0.5 cm, washed three times (10 min each in PBS, containing 0.1% (v/v) Tween 20), incubated 1 hr with PBT containing 0.2% (w/v) gelatin (PBTG), and washed again. Strips were incubated overnight at room temperature with 140 μl undiluted individual sera from IgE sensitized farmers.

Bound IgE was detected by subsequent 1 hour incubations with 1/500 diluted monoclonal mouse anti-human-IgE, 1/500 diluted biotinylated rabbit anti-mouse immunoglobulins (Dakopatts) with the addition of 10% (v/v) normal pig serum, and 1/200 diluted avidin-peroxidase (Dakopatts). Bound peroxidase was detected by incubation for 30 minutes with stabilized TMB (Promega Corporation, Madison, WI USA)

Statistical analysis

Associations between self-reported respiratory symptoms and IgE sensitization were studied with multiple logistic regression analyses, using SAS software (SAS PC version 6.04; PROC LOGISTIC). All analyses were done comparing pig farmers with a specific respiratory symptom, or a cluster of one or more respiratory symptoms present with a control group of pig farmers without respiratory symptoms (n=100). Odds ratios were adjusted for smoking habits (yes/no) and age.

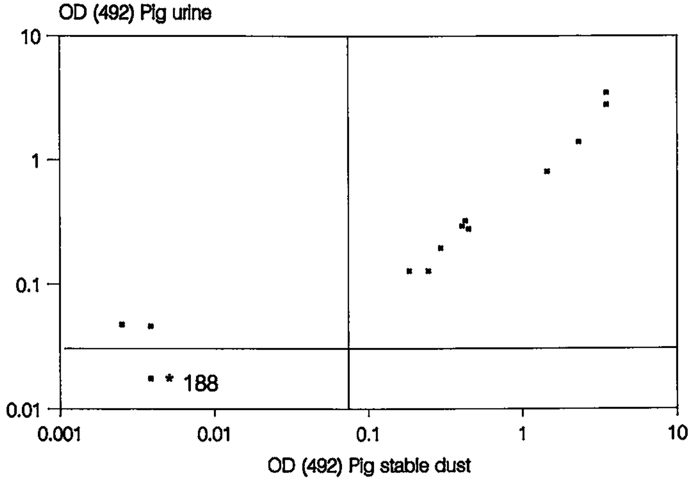

Figure A2.1 IgE to pig urine and pig stable dust in the sera of 200 pig farmers. Values on the X and Y-axis represent OD_{492} values, corrected for reagent blank, obtained with 1/10 diluted sera in wells coated with pig stable dust extract or pig urinary proteins, respectively. Drawn horizontal and vertical lines indicate the detection limits (3SD of the reagent blank for the whole series).

Results

Measurement of specific IgE to pig-derived allergens

The EIA appeared to be a reliable and highly sensitive method to detect and quantify IgE directed against pig stable dust or pig urine allergens. Strongly positive sera gave positive test results even at high dilutions (1/100-1/1000). Titration curves for these sera and for moderately positive sera revealed a linear relation between antibody concentration and resulting OD_{492} up to OD_{492} values of >1.5. In the first screening, sera were found positive on either pig stable dust or pig urine allergens: 11 sera on stable dust extract, 20 sera on PUP and 10 sera on both. Upon re-testing, these figures changed to 10, 12, and 10, respectively. Sera considered positive in the first series but negative in the second test, had shown very low OD_{492} values in the first test series. All re-tested negative sera from the first series were also considered negative in the second test.

The EIA results for all pig farmers are presented in Figure A2.1. IgE to pig stable dust and pig urine was found in 5% and 6% of the pig farmers, respectively. The IgE reactions to pig urine and pig stable dust showed a great similarity in the way that all farmers with IgE antibodies to pig stable dust had also IgE antibodies to pig urine and that, the resulting OD_{492} values were highly correlated. Only two pig farmers had detectable IgE antibodies to pig urine while no anti-pig stable dust IgE could be detected, presumably as a result of the difference in sensitivity for the two assays.

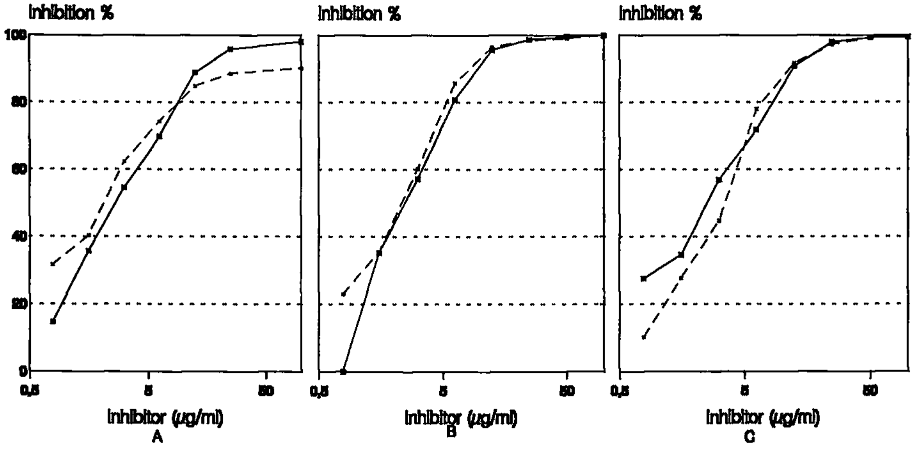

Figure A2.2 Cross-inhibition of the IgE reactions with coated pig stable dust extract and pig urinary proteins for three representative sera (A-C). Dotted lines: coating: PUP, fluid-phase inhibitor: pig stable dust. Solid lines: coating: pig stable dust extract, fluid-phase inhibitor: PUP.

Identification of IgE-binding components

The very strong correlation of the IgE reactions with PUP and pig stable dust allergens suggested a marked similarity of both preparations. This was confirmed by IgE inhibition EIA with all positive sera: in all cases complete mutual inhibition was observed, and inhibition curves for the two preparations were practically identical (Figure A2.2). To identify the nature of the PUP allergens all sera from farmers, with detectable IgE against pig urine or pig stable dust (n=12), were further investigated in EIA with coated pig serum and pig albumin.

Table A2.1 Atopic status and characteristics of farmers with and those without serum IgE to pig urine or pig stable dust allergens.

	pig farmers with serum IgE to PUP or pig stable dust												pig farmers without serum-IgE to PUP or pig stable dust	
	1	2	3	4	5	6	7	8	9	10	11	12	without respiratory symptoms (n=95)	with respiratory symptoms (n=93)
age (years)	28	46	27	30	44	27	50	32	27	28	28	31	36*	40*
pig farming (years)	8	18	10	8	20	7	22	20	5	8	6	12	13*	16*
chronic cough	+	+	-	-	-	-	+	-	-	+	-	+	-	64
chronic phlegm	+	+	-	-	+	-	+	-	-	-	-	+	-	52
shortness of breath	-	-	-	-	+	-	-	-	-	-	-	-	-	27
ever wheezing	-	+	-	+	-	-	-	-	-	+	-	-	-	73
frequent wheezing	-	-	-	+	-	-	-	-	-	+	-	-	-	38
chest tightness	-	-	-	-	-	-	-	-	-	-	-	-	-	29
≥ 1 respiratory symptoms	+	+	-	+	+	-	+	-	-	+	-	+	-	93
specific IgE														
common allergens †	-	-	-	-	-	+	-	-	-	-	-	-	12	20
pig urine ‡	0.07	0.07	0.20	0.28	0.34	0.42	0.45	0.52	1.77	2.71	4.04	4.36	-	-
pig stable dust ‡	-	-	0.20	0.25	0.43	0.38	0.44	0.44	2.19	2.12	4.28	4.62	-	-
pig serum ‡	0.03	0.02	0.15	0.22	1.04	0.21	0.13	0.35	3.66	2.77	4.51	4.65	nd §	nd §
pig albumin ‡	-	-	-	0.02	-	0.08	0.10	0.06	-	-	0.04	0.04	nd §	nd §
total serum IgE (IU/ml)	16	12	18	8	78	392	8	25	101	64	292	484	31 ¶	51 ¶

* arithmetic mean (AM)

† one or more IgE reactions to grass pollen, birch pollen, house dust mite and cat hair

‡ optical density (OD_{492}) in 1/10 serum dilution, corrected for OD_{492} reagent blank

§ not determined

¶ geometric mean

All sera with detectable IgE anti-pig stable dust showed serum IgE reactions to pig serum and 6 sera also had low levels of IgE reacting with pig albumin. The two farmers that had IgE to pig urine but not to pig stable dust showed low levels of IgE against pig serum but no reaction to pig albumin (Table A2.1). 20 Farmers were sensitized to storage mites. None of the farmers showed sensitization to both pig urinary proteins and storage mite allergens.

In the 12 sera the IgE reactivity to the several antigens was highly correlated (correlation coefficient ranging form 0.87 to 0.98), except for the IgE reactivity to pig albumin with a correlation coefficient ranging from 0.08 to 0.39. This suggested that the proteins in pig urine were serum derived, but not albumin. This was confirmed by IgE inhibition EIA with all positive sera. In IgE inhibition EIA with a coating of PUP, pig serum appeared to be a very potent inhibitor of the IgE reaction whereas for pig albumin proteins only at high concentrations inhibition was observed (Figure A2.3).

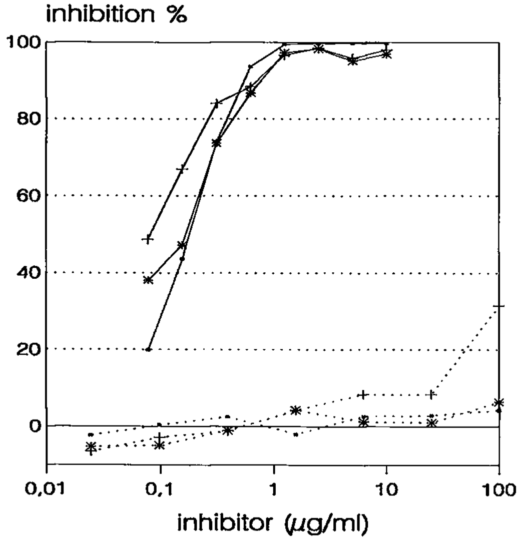

Figure A2.3
Cross inhibition of the IgE reactions with coated pig urinary proteins for three representative sera. Solid lines fluid-phase inhibitor: pig serum. Dotted lines fluid-phase inhibitor: pig albumin.

All positive sera gave an IgE reaction in the immunoblotting with PUP and pig stable dust extract. The similarity between the EIA test and immunoblotting was not only qualitative as well quantitative with subjects with a higher OD_{492} in the EIA test showing a more pronounced IgE-binding pattern in the immunoblotting (Figure A2.4 an A2.5).

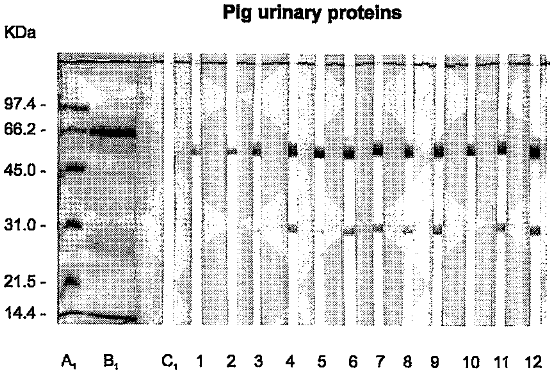
Pig urinary proteins

Figure A2.4
SDS-PAGE immunoblotting of PUP for 12 subjects with serum IgE to PUP. Number of subjects correspond with the subject numbers in Table A2.1. a_1: mol mass markers PUP immunoblotting, B_1: no serum control PUP immunoblotting and c_1: reagent blank.

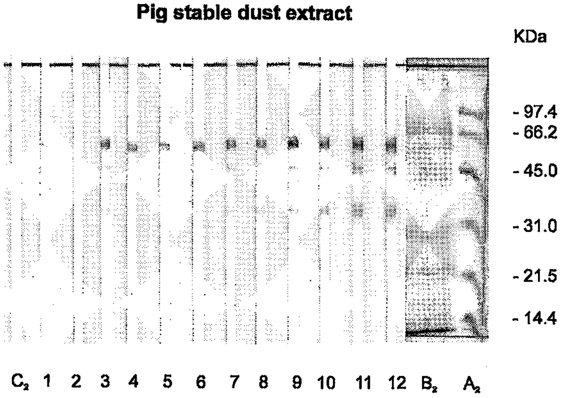
Pig stable dust extract

Figure A2.5
SDS-PAGE immunoblotting of pig stable dust extracts for 10 subjects with serum IgE to pig stable dust and 2 subjects with IgE to PUP. Number of subjects correspond with the subject numbers in Table A2.1. a_2: mol mass markers pig stable dust immunoblotting, B_2: no serum control pig stable dust immunoblotting and c_2: reagent blank.

Almost no interindividual variation was observed between the sensitized subjects. The immunoblotting experiments with PUP and pig stable dust extract showed a remarkable similarity in IgE binding pattern. Two major bands were observed at about 58 kDa and 33 kDa in both preparations. Some sera showed a weak reaction with a 65 kDa component, presumably pig albumin. In the pig stable dust extract an other major band at 46 kDa could be seen. Only for the two subjects who had detectable IgE to pig urine but not to pig stable dust, a different band pattern was observed in the immunoblotting experiments with only a reaction to the 58 kDa band. One subject also showed a reaction to the 25 kDa band in the PUP immunoblotting.

Atopy and respiratory symptoms

The atopic status, health status, and some other characteristics of the farmers with and those without serum IgE to pig urine or pig stable dust are given in Table A2.1. Pig farmers with serum IgE to pig urine or pig stable dust were on average five years younger than farmers without work-related IgE (t-test, p = 0.08). All distinct respiratory symptoms tended to occur more frequently among farmers with serum IgE to pig urine or pig stable dust (Table A2.2), with odds ratios ranging from 2.1 to 4.2. The association between chronic phlegm and IgE to pig urine was borderline statistically significant (OR=3.2, p=0.09). The odds ratio for one or more of the respiratory symptoms was estimated to be 2.7 (95%-CI 0.8-9.5). The odds ratios are higher then could be expected on the basis of the observed prevalences, but smoking and age acted as strong confounders.

If both IgE sensitization to storage mites and pig urine/pig stable dust are considered to be work related, then a total of 32 farmers were sensitized to work-related allergens. Sensitization to storage mites was independently not statistically significantly related to chronic respiratory symptoms.

Table A2.2 Relation between self-reported respiratory symptoms as response variable and specific IgE sensitization. Adjusted for smoking habits (yes/no) and age*.

	IgE sensitization †			
	PUP/SD		PUP/SD/SM	
	OR	(95%-CI)	OR	(95%-CI)
Chronic cough	3.2	(0.8 - 12.4)	2.7	(1.0 - 6.9) §
Chronic phlegm	3.2	(0.8 - 12.2) ‡	2.2	(0.8 - 5.9)
Shortness of breath	2.6	(0.2 - 29.2)	2.4	(0.6 - 8.9)
Ever wheezing	2.1	(0.5 - 9.9)	2.4	(0.9 - 6.5) ‡
Frequent wheezing	4.2	(0.7 - 26.4)	3.6	(1.2 - 10.9) §
Chest tightness	–		2.0	(0.6 - 6.7)
≥ 1 respiratory symptoms	2.7	(0.8 - 9.5)	2.7	(1.1 - 6.4) §

* All analysis were done comparing pig farmers with a specific respiratory symptom present or a cluster of one or more respiratory symptoms present with the control group of pig farmers without respiratory symptoms (n=100).

† PUP= pig urinary protein, SD= stable dust, SM= storage mites

‡ p < 0.10

§ p < 0.05

However, associations between sensitization to any type of potential work-related allergen, was positively and statistically significantly associated with chronic respiratory symptoms (Table A2.2). IgE-sensitization to house dust mite is generally considered to be a risk factor for chronic respiratory symptoms. Ten farmers had positive IgE against house dust mite as well as storage mites, which may be the result of cross sensitization. When these farmers were excluded from the analyses, the odds ratios increased and remained statistically significant at the 5% level for chronic respiratory symptoms.

With respect to measured exposure, positive farmers were exposed to higher concentrations of NH_3. The exposure, averaged over winter and summer measurements, was statistically significant higher, approximately 50%, for 9 seropositive farmers with two valid measurements, compared to 154 negative farmers with two valid measurements. The distribution of exposure to dust or endotoxin was similar in both groups.

There appeared to be a clear difference in time spent in pig farming: the positive farmers spent on average 6.9 hours a day on activities in pig farming, whereas negative farmers spent only 5.4 hours a day on these activities (t-test, $p < 0.01$).

No association was found between IgE to common allergens or elevated total-IgE levels and serum IgE to pig urine or pig stable dust, although the prevalence of IgE to common allergens was remarkably low.

Discussion

In this study among 200 Dutch pig farmers, serum IgE to pig stable dust and pig urine was found in 5% and 6% of the farmers, respectively. Because of the case-control selection criteria, these figures may not be regarded directly as prevalence rate in the population of all Dutch pig farmers. However, since the observed prevalence rate did not differ largely between the pig farmers with and those without respiratory symptoms, the prevalence rate in Dutch pig farmers in general will probably be very similar.

The prevalence rate found is on average higher than the prevalence rates found in other studies, in which similar immunochemical techniques were used[13,14,17,18]. Crook et al.[18], Matson et al.[17] and Katila et al.[14] studied small populations of 24, 41 and 20 pig farmers, respectively, and the reported prevalence rates varied largely, from 12.5% (RAST with pig skin and urine allergens), to 2.4% (RAST with pelt, urine and serum extract) and 0% (RIA with swine epithelium extract), respectively. Iversen et al.[13] found specific IgE antibodies to swine proteins in only one out of 247 farmers, but the study population was probably not a homogeneous group of pig farmers. The higher prevalence rate found in our study might thus be due to differences in the study population, the allergen extracts and immunochemical techniques used.

In two studies, high prevalence rates of positive skin-prick tests to pig-derived antigens were reported. Cormier et al.[19] reported 18.4% of 488 pig farmers having a positive skin-prick test to pig hair and dander, and Zuskin et al.[20] found 34% of 32 swine farmers to be sensitized to swine hair. However, in both studies a relatively high prevalence of positive reactions (9.9% and 17%, respectively) was found in non-exposed controls as well, which suggests that at least part of the observed *in vivo* reactivity was non-specific and/or due to non-IgE-mediated mechanisms. Nevertheless, even after correction for these apparently non-specific reactivity the prevalence rate of a positive SPT is remarkably higher than that of *in vitro* IgE tests. Thus, in this particular case, *in vivo* tests might be more sensitive than serologic measurement of specific IgE against pig urinary proteins (PUP). This would agree with our recent studies in laboratory animal workers, in which both SPT and an EIA for IgE against rat urinary proteins were performed (Doekes et al., submitted). With regard to the SPT, the IgE EIA appeared to have a sensitivity and specificity of 0.75 and 0.96, respectively. Since the IgE EIA was essentially the same as the assay used in the here presented study, the prevalence rate of IgE reactivity to pig allergens found with our IgE EIA is probably an underestimation of the prevalence of type I sensitization to pig allergens.

All pig farmers in our study who had IgE to pig stable dust also had an IgE reaction to pig urine, and the IgE inhibition experiments showed that the reaction to pig stable dust could be explained largely as a reaction to PUP. The dose-response curves even suggested that the proteins in pig stable dust extract mainly consisted of PUP. Immunoblotting experiments confirmed these results and preliminary identification of major allergens in pig stable dust extract and PUP showed two major binding components of 58 kDa and 33 kDa, while the immunoblotting with pig stable dust showed an additional band of 46 kDa (Figure A2.4 and A2.5). Thus most but not all of the allergenic activity of pig stable dust can be ascribed to PUP. This remarkable high proportion of PUP in pig stable dust extract raised questions whether our pig stable dust extracts were representative. However, we have found essentially identical results in several other dust extracts from other swine confinement buildings (not shown).

Animal feed components could also be a source of allergenic proteins in pig stable dust. However, due to the varying composition of the animal feed this will not be a consistent source of proteins compared to pig urine with a relatively constant composition. However, the additional 46 kDa band in pig stable dust could well be an animal feed derived allergen. Storage mites have been reported to be another important source of allergens in farming[7]. However, modern swine confinement buildings are not a suitable biotope for the growth of storage mites as a result of the environment consisting of concrete and iron and the regular cleaning and even disinfecting (Dr. Spieksma, personal communication).

Further inhibition experiments showed that these urinary allergens were serum-derived and should be present in substantial amounts, since pig serum showed 50% inhibition already at a concentration of approximately 0.15 μg/ml. Albumin, however, appeared to be no important IgE-binding allergen, as shown both by the immunoblotting and the inhibition EIA results. The exact nature of the 58 kDa and 33 kDa remains to be identified.

A positive IgE-titre against PUP allergens was associated with several factors possibly related to exposure. Farmers spending more time in pig farming, and farmers exposed to higher levels of NH_3 seemed to be more often sensitized to these pig related allergens. Associations with NH_3 exposure levels, and hours spent in pig farming per day may reflect exposure-response relationships, but the small number of IgE positive pig farmers and the cross-sectional study design precludes a more definite conclusion at this point.

Presence of IgE to pig-derived allergens was weakly associated with respiratory symptoms (OR 2.7, CI 0.8-9.5). In other studies no association between IgE antibodies against pig antigens and various respiratory health effects were observed[14,17,18]. In general, frequent contact with animals is an important risk factor for developing allergic diseases. However, large differences are observed between pig farmers in our study and for instance laboratory animal workers with respect to the occurrence of sensitization to urinary proteins and the relation with respiratory symptoms. In several surveys among laboratory animal workers prevalence rates of 15 to 30% of laboratory animal allergy has been described, and positive skin tests to animal urine was associated with respiratory symptoms[35]. The observed difference is not likely to be caused by a difference in exposure to urinary proteins but obviously there is a difference in potency between the several urinary proteins of different animals.

If both sensitization to pig-derived allergens and storage mite allergens are considered to be work related, then a strong association did exist with chronic respiratory symptoms. Since a statistically significant association of similar size remained after excluding pig farmers with sensitization to HDM, this association could not be attributed to potential cross-reactivity between house dust mite and storage mites[34]. This result suggest that sensitization to any work-related allergen may be a risk factor for chronic respiratory symptoms in pig farmers. It is, however, not clear whether sensitization to storage mites was exclusively related to working in pig farming. Analysis after incubation during 14 days of samples of settled dust, which were taken in 30 representative compartments in 10 farms, revealed that the number of storage mites in pig farms was very low (Spieksma, personal communication). Working conditions on specific farms, and other types of agricultural activities performed at pig farms may, however, be a source of exposure to storage mite allergens.

It can be concluded that serum IgE to PUP does occur more frequently than previously suggested, although it certainly does not account for the high prevalence of respiratory symptoms among pig farmers. However, type I allergy to PUP and storage mite allergens might significantly contribute to the occurrence and severity of respiratory symptoms.

References

1. Donham KJ, Zavala DC, Merchant JA. Respiratory symptoms and lung function among workers in Swine confinement buildings A cross-sectional epidemiological study. Study Arch Env Health 1984;39:96-101.
2. Dosman JA, Graham BI, Hall D, Pahwa P, McDuffie HH, Lucewitz M, To T. Respiratory symptoms and alterations in pulmonary function tests in swine producers in Saskatchewan. Results of a survey of farmers. J Occup Med 1988;30:715-20.
3. Heederik D, Brouwer R, Biersteker K, Boleij JSM. Relationship of airborne endotoxin and bacterial levels in pig farms with the lung function and respiratory symptoms of farmers. Int Arch Occup Environ Health 1991;62:595-601.
4. Iversen M, Dahl R, Korsgaard J, Hallas T, Jensen EJ. Respiratory symptoms in Danish farmers an epidemiological study of risk factors. Thorax 1988;43:872-7.
5. Donham K.J, Popendorf W, Palmgren U, Larsson L. Characterization of dusts collected from swine confinement buildings. Am J Ind Med 1986;10:294-7.
6. Rylander Y, Donham KJ, Hjort C, Brouwer R, Heederik D. Effects of exposure to dust in swine confinement buildings-a working group report. Scand J Work Environ Health 1989;15:309-12.
7. van Hage-Hamsten M, Johansson SGO, Zetterström O. Predominance of mite allergy to pollen and animal danders in a farming population. Clin Allergy 1987;17:417-23.

8. Terho EO, Vohlonen I, Husman K, Rautalahati M. Tukianen H, Vianden M. Sensitization to storage mites and other work-related and common allergens among Finish dairy farmers. Eur J Respir Dis 1987;71 (suppl 152):165-74.
9. Cuthbert OD, Jeffrey IG, McNeil HB, Wood J, Topping MD. Barn allergy among scottish farmers. Clin Allergy 1984;14:417-23.
10. Blainey AD, Topping MD, Olliers S, Davies RJ. Respiratory symptoms in farm workers role of storage mites. Thorax 1988;43:697-702.
11. Iversen M, Korsgaard J, Hallas T, Dahl R. Mite allergy and exposure to storage mites and house dust mites in farmers. Clin Exp Allergy 1990;20:211-9.
12. Brouwer R, Heederik D, Swieten P van. IgG_4 antibodies against pig-derived allergens. Am J Ind Med 1990;17:96-8.
13. Iversen M, Dahl R. Specific Antigens in dust from swine confinement buildings. Am J Ind Med 1994;25:49-51.
14. Katila MJ, Mäntyjärvi RA, Ojanen TH. Sensitization against environmental antigens and respiratory symptoms in swine workers. Br J Ind Med 1981;38:334-8.
15. Zhou C, Hurst TS, Cockcroft D, Dosman JA. Increased airways responsiveness in swine farmers. Chest 1991;99:941-4.
16. Larsson KA, Eklund G, Malmberg P, Belin L. Alterations in bronchoalveolar lavage fluid but not in lung function and bronchial responsiveness in swine confinement workers. Chest 1992;101:767-74.
17. Matson SC, Swanson MC, Reed CE, Yunginger JW. IgE and IgG-immune mechanisms do mediate occupational-related respiratory symptoms or systemic reactions in hog farmers. J Allergy Clin Immunol 1983;72:299-304.
18. Crook B, Robertson JF, Travers Glass SA, Botheroyd EM, Lacey J, Topping MD. Airborne dust, ammonium, microorganisms, and antigens in pig confinement houses and the respiratory health of exposed farm workers. Am Ind Hyg Assoc J 1991;52: 271-9.
19. Cormier Y, Boulet LP, Bedard G, Tremblay G. Respiratory health of workers exposed to swine confinement buildings only or to both swine confinement buildings and dairy barns. Scand J Work Environ Health 1991;17:269-75.
20. Zuskin E, Kanceljak B, Schachter EN et al. Immunological and respiratory findings in swine farmers. Environ Res 1991;56:120-30.
21. Harries, M.G. and O. Cromwell. Occupational asthma caused by allergy to pig's urine. Brit Med J 1982;284:867.
22. Brennan NJ. Pig butcher's asthma - case report and review of the literature. Irish Med J 1985;78:321-2.
23. Virtanen T, Kalliokoski P, Vilhunen P, Taivanen A, Mäntyjärvi R. Concentrations of specific dusts in swineries and the humoral response of swinery workers. Allergy 1990;45:354-62.
24. Berrens L, Dijk AG van, Bollebakker-Baars A, Huizinga M. Rast with animal dander, urine, saliva and serum. Am Allergy 1983;51:543-6.
25. Schou C. Defining allergens of mammalian origin. Clin Exp Allergy 1993;23:7-14.
26. Gordon S, Tee RD, Newman Taylor AJ. Analysis of rat urine proteins and allergens by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting. J Allergy Clin immunol 1993;92:298-305.
27. BMRC (British Medical Research Council). Standardized questionnaires on respiratory symptoms. Br Med J 1960;1665.
28. Biersteker K, Dijk WH van, Eissens JBMF, et. al. Ervaringen met geneeskundig onderzoek op CARA bij gemeentepersoneel te Rotterdam, 1970-1971. Tijdschr Soc Geneeskd 1974;52:158-62.

29. Willems JJH, Hofschreuder P. A passive monitor for measuring ammonia. In: Allegrini I, Febo A, Perrino C, editors. Proceedings of the workshop on Field intercomparison exercise on ammonia an ammonium measurement, April 29 to May 4, 1990, Rome. Commission of the European Communities, Directorate-General for Science, Research and Development. Air Polution Research Report 37;113-18.
30. Smid T, Heederik D, Mensink G, Houba R, Boleij JSM. Exposure to dust, endotoxins, and fungi in the animal feed industry. Am Ind Hyg Ass J 1992;53:362-68.
31. Hollander A, Heederik D, Versloot P, Douwes J. Inhibition and enhancement in the analysis of airborne endotoxin levels in various occupational environments. Am Ind Hyg Assoc J 1993;54:647-53.
32. Doekes G, Ieperen-van Dijk AG van. Allergens of Pityrosporum ovale and Candida albicans I. Cross-reactivity of IgE-binding components. Allergy 1993;48:394-400.
33. Laemli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;227:680-5.
34. Johansson E, Borga A, Johansson SGO, Hage-Hamsten M van. Immunoblot multi-allergen inhibition studies of allergenic cross-reactivity of the dust mites *Lepidoglyphus destructor* and *Dermatophagoides pteronyssinus*. J Immunol 1991;147:972-9.
35. Venables KM, Tee RD, Hawkins ER et al. Laboratory animal allergy in a pharmaceutical company. Br J Ind Med 1988;45:660-6.

Dankwoord

Het onderzoek dat in dit proefschrift is beschreven had zijn oorsprong bij de Gezondheidsdienst voor Dieren in Boxtel - als opdracht voor de Vereniging van Varkenshouders - en zijn afronding bij de vakgroep Humane Epidemiologie en Gezondheidsleer en de vakgroep Luchtkwaliteit van de LU Wageningen. Zonder de bijdrage van veel mensen op de verschillende werkplekken had dit boekje er niet gelegen.

Als eerste gaat mijn dank uit naar Dick Heederik. Dick, jij hebt een onmisbare inbreng gehad. Door je vele ideeën en je overoptimistische tijdsplanningen heb je mij altijd weten blijven motiveren om dit proefschrift te schrijven. Daarnaast heb jij, daar waar het onderzoeksbudget niet toereikend was, kunnen regelen dat een aantal essentiële gegevens alsnog verzameld kon worden. Aan mijn promotoren Jan Boleij en Martin Tielen heb ik in de eerste plaats te danken dat ik dit onderzoek kon gaan doen, terwijl zij ook in het verdere verloop de voorwaarden wisten te creëren om tot deze afronding te komen. Jan en Martin, bedankt voor jullie steun en vertrouwen.

De bijdragen van Hans Kromhout en Gert Doekes zijn in verschillende stadia essentieel geweest. Hans, door jouw rotsvaste geloof in modellering van de blootstelling heb ik ook in epidemiologisch opzicht veel meer met dit onderzoek kunnen doen dan zich in eerste instantie liet aanzien. Gert, gelukkig had jij aan kleine aanwijzingen voldoende om het onderzoek een heel interessante wending te geven, waarbij het accent veel meer op de immunologie kwam te liggen.

Stijn Gielen en Peter Vogelzang wil ik bedanken voor de samenwerking en voor het gebruik kunnen maken van de longfunctiedata en de gegevens over bronchiale reactiviteit, die in het kader van het medisch deel van het onderzoek verzameld zijn. Verder hebben Marcel Willemsen, Hans van Gisteren, Jossie Garthoff, Sander Monte en Roel Vermeulen tijdens hun studenten-projecten er voor gezorgd dat er een schat aan data werd verzameld op basis waarvan ik heb kunnen werken.

Veel mensen van de Gezondheidsdienst voor Dieren, onder andere van de afdeling VGZ en het microbiologisch lab, hebben zich ingezet voor het onderzoek. Vooral de manier waarop zij zijn bijgesprongen bij de feitelijke blootstellingsmetingen, zonder een wanklank naar mij ondanks de lange werkdagen, vond ik geweldig. Speciale dank gaat uit naar Rudolf Gerrits. Rudolf, bedankt voor je inzet op een groot aantal terreinen.

Jan Burema, Jeroen Douwes, Jacques Spithoven, Pieter Versloot, Joop Willems, Siegfried de Wind en alle andere collega's die op één of andere manier hebben bijgedragen aan het resultaat of die er voor hebben gezorgd dat ik mijn werk naar behoren heb kunnen doen wil ik daarvoor bedanken.

Een woord van dank is ook op zijn plaats aan de varkenshouders die aan het onderzoek hebben meegedaan. Dit geldt met name de varkenshouders en hun familieleden die wij vaak hebben lastig gevallen voor onze metingen en waar ik altijd gastvrij ben ontvangen.

De leuke sfeer waarin ik heb kunnen werken is voor mij één van de redenen geweest waarom ik de laatste vijf jaar met plezier naar mijn werk ben gegaan. Daar hebben veel collega's op John Snow en de Dreijenborch, maar ook de kamergenoten in Boxtel voor gezorgd. Voor allemaal geldt: ik heb jullie gezelschap en vriendschap enorm op prijs gesteld.

Als laatste wil ik Jacco bedanken. Jacco, jouw mentale ondersteuning en relativeringsvermogen waren goud waard. Gelukkig heb ik dat tot de laatste letter van dit proefschrift kunnen ondervinden, want hoe anders had het kunnen zijn!

Curriculum Vitae

Liesbeth (Elisabeth Antoinette) Preller werd geboren op 26 mei 1963 in Borne (O). In 1981 behaalde zij het VWO diploma aan de scholengemeenschap College Noetsele te Nijverdal. Zij studeerde van 1981 tot 1988 Milieuhygiëne aan de Landbouwuniversiteit in Wageningen. In de doctoraalfase deed zij vakken Gezondheidsleer en Luchtverontreiniging en -hygiëne, gericht op arbeidsomstandigheden en het algemene milieu. Na haar afstuderen werkte zij eerst anderhalf jaar als toegevoegd onderzoeker bij de vakgroepen Humane Epidemiologie en Gezondheidsleer en Luchtkwaliteit van de Landbouwuniversiteit aan een arbeidsepidemiologisch onderzoek onder kantoorwerknemers. In maart 1990 startte ze bij de Gezondheidsdienst voor Dieren in Zuid-Nederland te Boxtel met het onderzoek naar de gezondheid van varkenshouders in relatie tot hun werkomstandigheden. Tijdens het onderzoek was zij voor bepaalde periode ook aangesteld bij de Landbouwuniversiteit, vakgroep Luchtkwaliteit. Sinds mei 1995 werkt zij voor de onderzoeksschool 'Netherlands Institute for Health Sciences' bij het Centrum voor Chronische Ziekten en Milieu-Epidemiologie van het Rijksinstituut voor Volksgezondheid en Milieuhygiëne te Bilthoven.