Diss. ETH No. 14164

OBSTRUCTIVE AIRWAY DISEASES IN SWISS FARMERS

A dissertation submitted to the
SWISS FEDERAL INSTITUTE OF TECHNOLOGY
for the degree of
DOCTOR OF NATURAL SCIENCES

presented by

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Zürich 2001

Acknowledgements

This work was carried out during my appointment as an assistant at the Institute of Hygiene and Applied Physiology, ETH Zürich, a status which gave me the freedom to follow new ideas whenever they came up without the pressure of daily accountability as to the success of these new approaches.

I would like to express my thanks to all of those who contributed to this work,
in particular to:

1. Prof. Dr. Dr. Helmut Krueger, for his kind support and careful reviewing of this dissertation.
2. Dr. med. Brigitta Danuser of the Institute of Hygiene and Applied Physiology, ETH Zürich, who directed the entire work and provided many suggestions and the spiritual environment in which research is conducted in the sense mentioned above.
3. Dr. Rudolf Hauser for reviewing this thesis and for spontaneously accepting the coexamination.
4. Brigitte Wüthrich and Hanni Rebsamen, not only for the contributions they made with their Diploma-theses, but also for sharing my enthusiasm in the exciting field of obstructive lung diseases.
5. Prof. C. Bachert (formerly head of the ENT department, University Hospital Düsseldorf, Germany, now University of Gent, Belgium) for his advice in the study design in parts of the dissertation.
6. Special thanks to my wife Corinne who was always supporting my work.

The dissertation was supported by the Swiss National Science Foundation: NF-Number 3200-045997.95/1 and the BBW (Bundesamt für Bildung und Wissenschaft): Number 93.0283.

Christoph Weber

Zürich, January 2001

Table of Contents

Abstract

In the first part A of the study a questionnaire survey on general as well as work-related respiratory symptoms in relation to type of farming was carried out. This epidemiological study was performed with a representative sample of 1542 Swiss farmers using a self-administered questionnaire. The study was undertaken to assess the prevalence and risk factors of self reported asthma, symptoms of chronic bronchitis, hay fever, and work related respiratory symptoms as well as to compare the prevalence rates of respiratory symptoms of the Swiss farmers with the Swiss population (SAPALDIA-Study).

Because this first stage of the study has shown that poultry farmers were at highest risk for the development of respiratory symptoms it was decided to study them in more detail in this second part B of the survey. Therefore, it was the aim of this second part of the study to investigate the relationship between lung function and measures of exposure as well as farming characteristics and focuses on exposure parameters during work in animal confinement buildings. Therefore, 37 poultry farmers in Switzerland were chosen randomly and followed over one working day.

In the third part C the inflammatory response to a nasal Lipopolysaccharide (LPS) provocation in allergic and non-allergic subjects was evaluated. Low levels of Endotoxin have been measured indoors and a relationship between Endotoxin-levels and clinical severity of allergy has been shown. In poultry farmers which had a common nasal allergy (pollinosis) we found more work related symptoms when exposed to organic dust. Exposure to Endotoxin causes a release of proinflammatory mediators in the lower and upper airways. In healthy subjects it has been demonstrated, that interleukin (IL)-6, IL-1β and Tumor Necrosis Factor-α (TNF-α) increase in nasal lavage fluid a few hours after exposure to swine dust containing Endotoxin. According to a positive Phadiatop (>0.35 kU/l) and allergic history the subjects were grouped into seasonal allergic and non-allergics. 11 non-allergic and 12 allergic subject's noses were exposed during the pollen free season once to 20 μg LPS (*E. coli*) dissolved in a total of 10 ml 0.9% salt solution for 10 seconds. Just before and 20', 1 h, 6 h, and 23 h after the LPS-provocation nasal lavage samples were taken. Albumin, histamine, IL-1β, IL-4, IL-6, IL-8, TNF-

α, ECP (Eosinophil cationic Protein) and MPO (Myeloperoxidase) were determined. Symptoms were evaluated by a questionnaire.

In the epidemiological study (part A) a response rate of 86.3% was achieved. In farmers the prevalence rate was 16.0% for chronic bronchitis symptoms, 15.4% for asthma symptoms, and 42.0% for reporting at least one work related symptom. Using logistic regression analysis, adjusting for age and smoking habits, it was established that poultry farming and pig/cattle farming was a risk factor for reporting nasal irritation at work (OR 5.3, (1.6-18), OR 3.4 (1.0-10.9)). Poultry farmers showed in the most of the assessed symptoms the highest estimates. In plant farmers, the prevalence for chronic bronchitis was increased (OR 2.3 (1.0-5.2)). Over 4 hours spent per day in animal confinement buildings more than doubles the risk for reporting chronic bronchitis (OR 2.6 (1.0-6.8)) and phlegm (OR 2.3 (1.0-5.4)) independent of the type of farming. The comparison of the Swiss farmers with the Swiss population has shown a 2-fold elevated risk of reporting chronic bronchitis symptoms (OR 1.9 (1.3-3.0)) and a 4.5 fold elevated risk for bringing up phlegm regularly (OR 4.5 (3.3-6.7)) in farmers. The 10.0% prevalence of nasal allergies in farmers was lower than in the general population (OR 0.4 (0.3-0.6)).

The mean baseline spirometric results in poultry farmers for FEV_1 (% of predicted) was 100.2 ± 14.2. Baseline lung function results were shown to be significantly associated with ventilation of the animal houses. Furthermore, endotoxin content in total dust was shown to be a predictor for FVC and FEV_1 % pred. while $MMEF_{25/75}$ % pred. was more strongly related to the concentration of total bacteria. The total dust concentrations were found in Swiss poultry houses with median concentrations of 7.01 mg/m^3. The median airborne endotoxin concentrations in total dust was 258 ng/m^3 in Swiss poultry houses.

The allergic subjects in part C experienced more nose (41.6% vs. 18%) and more eye (33% vs. 18%) symptoms than the non-allergic subjects. Using analysis of variance the pattern of the measured mediators and the influence of the factor allergy was estimated. The concentrations of TNF-α and IL-4 were always below

detection limit. No effect of the LPS challenge on MPO and IL-8 was found. A significant effect was found for albumin, histamine, IL-1β, and IL-6. Maximum increase for albumin: 2, for histamine: 3.8, for IL-1β: 1.6, and for IL-6: 2,4 fold. No effect of allergy was found. Detailed analysis of IL-8, MPO, and ECP indicates that allergic subjects showed a slight response here.

Part A shows that agricultural work in Switzerland bears an elevated risk for reporting respiratory symptoms, especially pronounced in plant, and poultry farmers.

The results of part B indicate that there is a special need for threshold levels in respect to endotoxin and bacteria concentrations at the workplace. Beside that, prospective intervention studies using special ventilation control should be carried out. The median exposure levels for endotoxin and microorganisms found in the randomly chosen animal buildings exceeded recommended exposure standards.

In part C it was concluded that a nasal instillation of 20 μg LPS does produce an increase in IL-6, IL-1β, histamine, and albumin. The response pattern of allergic and non-allergic subjects in the determined proinflammatory and inflammatory mediators is similar although the allergics experienced more symptoms and show indications of an IL-8, MPO, and ECP response. The induced release of histamine could be a clue to explain the increased symptoms of allergics in endotoxin containing environments.

Zusammenfassung

Im ersten Teil (A) der Studie wurde eine Fragebogenumfrage über generelle und arbeitsassoziierte Atemwegssymptome durchgeführt. Diese repräsentative epidemiologische Studie wurde an 1542 Schweizer Bauern verschickt. Das Ziel der Studie war die Bestimmung der Prävalenz und Risikofaktoren von selbst genannten Asthmasymptomen, Symptomen von chronischer Bronchitis, Heufieber und arbeitsassoziierten Atemwegssymptomen. Zusätzlich wurde die Prävalenz von Atemwegssymptomen mit der Schweizer Bevölkerung (SPALDIA-Studie) verglichen.

Da der erste Teil der Studie gezeigt hat, dass Geflügelbauern das grösste Risiko aufweisen, Atemwegssymptome zu entwickeln, wurde entschieden, diese Gruppe im zweiten Teil (B) genauer zu untersuchen. Das Ziel dises zweiten Teiles der Studie war die Beziehung zwischen Lungenfunktion und Exposition bei der Arbeit, als auch die Charakteristik der Arbeitsweise mit Fokus auf die Belastung während der Arbeit im Stall aufzuzeigen. Dazu wurden 37 Schweizer Geflügelbauern zufällig ausgewählt und während eines Tages begleitet.

Im dritten Teil (C) wurde die Entzündungsreaktion nach einer Lipopolysaccharid-Provokation (LPS) an allergischen und nicht-allergischen Probanden untersucht. In Gebäuden wurden niedrige Konzentrationen von Endotoxin gemessen, und daraus konnte eine Beziehung zwischen Endotoxinkonzentrationen und klinischen stärke von Allergien aufgezeigt werden. Bei Geflügelbauern mit Heuschnupfen wurden häufiger arbeitsassoziierte Atemwegssymptome nach Exposition mit organischem Staub nachgewiesen. Die Exposition zu Endotoxin bewirkte die Ausschüttung von Entzündungsmediatoren in den unteren und oberen Atemwegen. Bei Gesunden Probanden wurde gezeigt, dass nach der Exposition mit Schweinestall-Staub ein Anstieg von Interleukin (IL)-6, IL-1β und Tumor Necrosis Faktor-α (TNF-α) in der nasalen Lavage-Flüssigkeit innert Stunden folgt. Die Probanden wurden anhand eines positiven Phadiatops (>0.35 kU/l) und deren allergischen Vorgeschichte in eine allergische und nich-allergische Gruppe aufgeteilt. 11 nicht-allergische und 12 allergische Nasen von Probanden wurden während der pollenfreien Jahreszeit für 10 Sekunden mit 10ml 0.9% NaCl-Lösung mit darin 20μg gelöstem LPS (E.

coli) exponiert. Gerade vor der LPS-Provokation und 20', 1 h, 6 h und 23 h danach, wurden nasale Lavage-Proben genommen. Darin wurden Albumin, Histamin, IL-1β, IL-4, IL-6, IL-8, TNF-α, ECP (Eosinophil cationic Protein) und MPO (Myeloperoxidase) bestimmt. Symptome wurden mittels eines Fragebogens erfasst.

In der epidemiologischen Studie (Teil A) wurde eine Rücklaufquote von 86.3% erreicht. Bei den Bauern war die Prävalenzrate der Symptome von chronischer Bronchitis 16%, die von Asthmasymptomen 15.4% und die von mindestens einem arbeitsassoziierten Symptom 42%. Mittels logistischer Regression - korrigiert für Alter und Rauchgewohnheiten - wurde gezeigt, dass Geflügel- und Schweine/Vieh-Bauern ein signifikant erhöhtes Risiko von selbstgenannten Nasensymptomen während der Arbeit haben (OR 5.3 (1.6-18), OR 3.4 (1.0-10.9)). Geflügelbauern weisen bei den meisten Symptomen die höchsten Werte auf. Bei Gemüsebauern war die Prävalenz von chronischer Bronchitis ebenfalls erhöht (OR 2.3 (1.0-5.2)). Wird mehr als 4 Stunden/Tag in einem Tierstall gearbeitet, erhöht sich das Risiko Symptome von chronischer Bronchitis (OR 2.6 (1.0-5.4)) und Auswurf (OR 2.3 (1.0-5.4)) anzugeben, unabhängig vom Typ des Bauernhofes. Der Vergleich von Schweizer Bauern mit der Schweizer Bevölkerung hat gezeigt, dass die Bauern ein doppelt so hohes Risiko besitzen, Symptome von chronischer Bronchitis (OR 1.9 (1.3-3.0)) zu nennen. Zusätzlich haben sie ein 4.5-fach so hohes Risiko, unter regelmässigem Auswurf zu leiden (OR 4.5 (3.3-6.7)). Bei den Bauern ist die Heuschnupfen-Prävalenz von 10% deutlich niedriger als die der Normalbevölkerung (OR 0.4 (0.3-0.6)).

Die Durchschnittlichen Basiswerte der spirometrischen Resultate bei den Geflügelbauern für FEV_1 (% von predicted) war 100.2 ± 14.2. Die durchschnittlichen Basis-Lungenfunktionswerte sind signifikant mit der Belüftung der Ställe assoziiert. Im Weiteren sind die Endotoxinwerte im Gesamtstaub mit FVC und FEV_1 % pred. assoziiert und $MMEF_{25/75}$ % pred. war verstärkt mit der Konzentration von Bakterien assoziiert. Der Median der Gesamtstaubkonzentrationen in Schweizer Geflügelställen betrug 7 mg/m^3. Der Median der Endotoxinkonzentrationen im Gesamtstaub betrug 258 ng/m^3.

Die Allergiker in Teil C gaben mehr Nasen- (41.6% vs. 18%) und mehr Augensymptome als Nicht-Allergiker an. Mittels Varianzanalyse wurde das Reaktionsmuster der gemessenen Mediatoren und der Einfluss vom Faktor „Allergie“ bestimmt. Die Konzentration von TNF-α und IL-4 waren immer unter der Nachweisgrenze. Keine Wirkung der LPS-Provokation auf MPO und IL-8 konnte festgestellt werden. Ein signifikanter Effekt wurde für Albumin, Histamin, IL-1β und IL-6 nachgewiesen. Die maximale Erhöhung von Albumin betrug 2, für Histamin 3.8, für IL-1β 1.6 und für IL-6 2.4-fach. Kein Effekt der Allergie wurde gefunden. Allergiker zeigten nach detaillierte Analysen eine schwache Reaktion auf IL-8, MPO und ECP.

Die landwirtschaftliche Arbeit in der Schweiz erhöht das Risiko Atemwegssymptome anzugeben (Teil A). Davon sind besonders die Geflügel- und Gemüsebauern betroffen.

Die Resultate von Teil B zeigen das Bedürfnis für die Festlegung von Grenzwerten für Endotoxin- und Bakterienkonzentrationen am Arbeitsplatz. Daneben sollten prospektive Interventionsstudien mit speziellen Belüftungskontrollen durchgeführt werden. Die Mediane der Expositionswerte für Endotoxin und Mikroorganismen in den gewählten Ställen übersteigen empfohlene Expositionsstandards.

In Teil C wurde gezeigt, dass die nasale Einbringung von 20μg gelöstem LPS ein Anstieg von Albumin, Histamin, IL-1β und IL-6 bewirkt. Das Reaktionsmuster der gemessenen Mediatoren bei Allergikern und Nicht-Allergikern war nicht unterschiedlich, obwohl die Allergiker vermehrt Symptome angegeben haben. Sie zeigen auch eine tendenziell erhöhte Reaktion bei IL-8, MPO und ECP. Die Ausschüttung von Histamin könnte ein Hinweis auf die häufigen Symptome von Allergikern in Endotoxinumgebungen sein.

General Introduction

Back in 1555 Olaus Magnus had already recognized that farmers health hazards are correlated to grain dusts (Schenker 1998). Today's epidemiological studies indicate an elevated risk of respiratory disorders in farmers compared to the non-farming population (Kogevinas et al. 1999). It is known that farmers are exposed to organic dust, including aeroallergens, bacteria, endotoxin, mites and fungi, insect antigens as well as hazardous gases such as ammonia, hydrogen sulfides and nitrogen oxides. These substances may affect parts in the respiratory system of the farmer and may induce obstructive lung diseases like allergic and non-allergic rhinitis (Terho et al. 1987; Melbostad et al. 1998), organic dust toxic syndrome (ODTS) (Rask-Andersen 1989; Melbostad et al. 1992) and may induce chronic airway inflammation such as chronic bronchitis (Rask-Andersen 1989), asthma (Terho et al. 1987), and asthma-like syndromes (Melbostad et al. 1997; Melbostad et al. 1998).

A Swiss mortality registered based study (Gassner et al. 1995) showed an elevated risk for farmers to die from obstructive lung diseases compared with the general population. Epidemiological studies in other countries reported that respiratory disorders are more frequent in farmers than in other occupations (Dosman et al. 1987; Husman et al. 1987; Vohlonen et al. 1987; Dalphin et al. 1989). Pig farmers especially have a particularly high risk of developing obstructive lung diseases (Donham et al. 1984; Dosman et al. 1988; Donham et al. 1990).

A European multicenter study on "Prevalence and risk factors of airway obstruction in farmers" (Nowak 1994) was carried out in seven centers in five countries (Denmark, Great Britain, Germany, Switzerland, Spain). In the first part of the study a questionnaire survey on general as well as work-related respiratory symptoms in relation to type of farming was carried out. In Switzerland 1542 randomly selected farmers were involved. In 1994 a total of about 230,000 persons on some 80,000 farms were working in Swiss agriculture. Swiss farms are small to medium sized (on average 13.5 hectares) and mainly of a mixed type, i.e. animals are kept and plants cultivated. Assessing risk factors for respiratory diseases in farmers is indicated both for preventive and economic reasons. In total, the cost of respiratory

disorders in Switzerland in 1990 was estimated to exceed 600 million Swiss Francs (Abt 1991).

So the aim in the first part of this dissertation is to assess the prevalence rates of respiratory symptoms in a cross-sectional sample of about 1500 Swiss farmers by the means of a standardized questionnaire. Then to evaluate risk factors, such as type of farming, or hours spent in animal confinement buildings related to reporting symptoms in Swiss farmers. Additionally to compare the respiratory symptoms of the farmers with the non-farming population of Switzerland.

Animal farmers (i.e. pigs, cattle, poultry, sheep) working in confinement houses were shown to be at highest risk for the development of respiratory symptoms in this multicenter study. Poultry farmers showed a significantly higher prevalence of wheezing compared to farmers not working with those animals (Weber et al. 1998). The results of this first part of the study are presented in detail in part A. Due to the high risk for poultry farmers to develop respiratory symptoms, it was decided to study them in more detail in the second part of the survey. It will focus on one subpopulation of farmers (about 40) in respect to low and high respiratory symptom levels. Therefore, it was the aim of this second part of the study to investigate the relationship between lung function and measures of exposure as well as farming characteristics and focuses on exposure parameters.

Low levels of Endotoxin have been measured indoors and a relationship between Endotoxin-levels and clinical severity of allergy has been shown. In poultry farmers which had a common nasal allergy (pollinosis) we found more work related symptoms when exposed to organic dust. Exposure to Endotoxin causes a release of proinflammatory mediators in the lower and upper airways. In healthy subjects it has been demonstrated, that interleukin (IL)-6, IL-1β and Tumor Necrosis Factor-α (TNF-α) increase in nasal lavage fluid a few hours after exposure to swine dust containing Endotoxin. The enhanced reaction to Endotoxin of allergic persons can be due to an altered baseline inflammatory mucosa response in persons once sensitized, to a hyperreactivity status of the mucosa and due to an altered response when both allergens and Endotoxin are present. In the third part the

important role of endotoxin for developing respiratory diseases is under investigation. The aim of this study was to evaluate the inflammatory response assessed by IL-1β, IL-6, IL-8, TNF-α, MPO, ECP, albumin, and histamine to a nasal Lipopolysaccharide (LPS) provocation in normal and allergic subjects. So we wanted to know if the baseline inflammatory mucosa response (assessed by IL-1β, IL-6, IL-8, TNF-α, MPO, ECP, albumin, and histamine) to Endotoxin is modified in allergic persons.

Part A: Respiratory symptoms in Swiss farmers: an epidemiological study of risk factors[1]

Introduction

A previous Swiss mortality registered based study (Gassner et al. 1995) showed an elevated risk for farmers to die from obstructive lung diseases compared with the general population. Epidemiological studies in other countries reported that respiratory disorders are more frequent in farmers than in other occupations (Dosman et al. 1987; Husman et al. 1987; Vohlonen et al. 1987; Dalphin et al. 1989). Pig farmers especially have a particularly high risk of developing obstructive lung diseases (Donham et al. 1984; Dosman et al. 1988; Donham et al. 1990).

Exposure on farms may affect parts of the respiratory tract provoking symptoms and/or diseases. Exposure consists of organic dust including aeroallergens, bacteria, endotoxins, mites and fungi, as well as hazardous gases such as ammonia, hydrogen sulfides and nitrogen oxides. These substances are known to cause allergic and non-allergic asthma and rhinitis (Terho et al. 1987; Melbostad et al. 1998) or organic toxic syndrome (ODTS) (Rask-Andersen 1989), and may induce chronic airway inflammation such as chronic bronchitis or mucus membrane irritation syndrome (Melbostad et al. 1997).

In 1994 a total of 230,000 persons on 80,000 farms were working in Swiss agriculture. Swiss farms are small to medium sized (on average 13.5 hectares) and mainly of a mixed type, i.e. animals are kept and plants cultivated. Assessing risk factors for respiratory diseases in farmers is indicated both for preventive and economic reasons. In total, the cost of respiratory disorders in Switzerland in 1990 was estimated to exceed 600 million Swiss Francs (Abt 1991).

[1] Danuser B, Weber C, Künzli N, Schindler CH, Nowak D (2001) Respiratory symptoms in Swiss farmers: an epidemiological study of risk factors. Am J. Ind. Med. accepted.

As part of the EU-BIOMED project: "Prevalence and Risk Factors for Obstructive Airway Diseases in Farmers" the study had the aim of assessing the prevalence rates of respiratory symptoms and to evaluate risk factors, such as type of farming, or hours spent in animal confinement buildings related to reporting symptoms in Swiss farmers. Additionally it compared the respiratory symptoms of the farmers with the non-farming population of Switzerland. The data for the Swiss population was obtained from the "Swiss Study on Air Pollution and Lung Diseases in Adults" (SAPALDIA) (Zemp et al. 1999).

Methods

Study population

A random sample of 1542 German speaking farmers was taken, choosing every 150th farmer from an alphabetical list of German speaking farmers (230,000) provided by the national registry. 1330 (86%) of the questionnaires were returned. To achieve this response rate two reminders were distributed. The response rate of the different steps was 608, 271 and 451. From those, 276 (17.9%) were not farmers, 26 (1.7%) have died, 13 (0.8%) could not be identified, and 27 (1.8%) refused to participate. Among the participants only 48 (3.6%) were women. Due to this small sample size we did not include women in the analysis. Thus, the analysis is based on a total of 940 male farmers.

Questionnaire

The questionnaire contained questions about personal characteristics (e.g. smoking habits, age, sex), occupational characteristics (full or part time farming, type of farm, animal species, working hours in animal confinement buildings and plant species), and respiratory symptoms (Table 1). The respiratory questions were derived from the European Community Respiratory Health Survey, ECRHS (Burney et al. 1994). Additionally, five questions on work related respiratory symptoms indicative of the lower respiratory tract (F6.1, F6.2, F6.3) of allergy (F 6.4) and of the upper airways (F6.5) and one question on ODTS (F7) were included.

Question	Abbreviation used in text	Questionnaire
Have you had wheezing or whistling in your chest at any time in the past 12 months?	wheezing	F1/E1
Have you been woken by an attack of shortness of breath at any time in the last 12 months?	shortness of breath	F2/E5
Have you had an attack of asthma in the last 12 month?	asthma attack	F3/E13.5
Do you have any nasal allergies including hay fever?	nasal allergy	F4/E14
Do you usually bring up any phlegm from your chest during the day, or at night, in the winter?	phlegm	F5/E10
Do you bring up any phlegm from your chest on most days for as much as 3 months each year?	chronic bronchitis	F5.1/E10.1
Do you have during your work one or more of the following complaints: Breathlessness? cough without phlegm? cough with phlegm? wheezing? nasal irritation?	 breathless at work cough at work cough with phlegm at work wheezing at work nasal irritation at work	 F6.1 F6.2 F6.3 F6.4 F6.5
Two to six hours after a dust exposure, have you ever had a sudden onset of a flu-like illness with 2 or more of these symptoms: fever, chills, muscle ache, weakness, headache, cough, chest tightness, or shortness of breath?	ODTS (Organic Dust Toxic Syndrome)	F7

Table 1 Questions about respiratory symptoms used in Questionnaire. E= EC-Respiratory Health Survey Questionnaire and SAPALDIA, F=EU-BIOMED Questionnaire "Prevalence and Risk Factors of Obstructive Airway Diseases in Farmers", used in this study.

An affirmative answer to at least one of the questions ”wheezing”, ”shortness of breath”, ”asthma attack” (F1, F2, F3) (see table 1) was defined as ”asthma”. Giving the answer ‘yes’ in at least one of the questions ”breathless at work”, ”cough with phlegm at work”, ”cough at work”, or ”nasal irritation at work” (F6.1, F6.2, F6.3, F6.4, F6.5) was defined as ”work related symptom”.

Definition of farming categories

To investigate the effect of type of farming on reported symptoms the farmers were subdivided into seven groups. The grouping was made according to the time farmers spent in different animal confinement buildings as reported in the questionnaire. In Table 2 the grouping definitions which were used are shown. The validity of the definition of each farming type was confirmed by the ”Swiss Federal Research Station for Agricultur, Economics and Engineering” (FAT, CH-8356 Tänikon).

Category	Definition
Plant farming	working only with plants.
Pig farming	working ≥ 1 hour per day in enclosed buildings with pigs
Pigs/Cattle farming	working ≥ 1 hour per day with pigs and ≥ 2 hours with cattle in enclosed buildings
Cattle farming	working ≥ 2 hours per day in enclosed building with cattle
Poultry farming	working ≥ 1 hour per day in enclosed buildings with poultry
Mixed farming	having livestock and plant crops but not included in one of above categories
Small farms	working only part time on a farm and not long enough for an other category, they have no plants and have animals in a small number

Table 2: Definition of farming categories

Swiss farmers compared to the Swiss Population

The methods used in the SAPALDIA study were published (Martin et al. 1998). Shortly, the eight-center study population consisted of 9'651 participants of a random population sample of adult residents (18-60 years). Whereas the SAPALDIA data were collected by interview; the Swiss farmers study relies on a self-reporting questionnaire, issued by mail. Questions F1-F5.1 in the farmer questionnaire were also asked in the SAPALDIA study. The SAPALDIA interview used the same questionnaire as the ECRHS (Burney et al. 1994). Additional questions were asked, however, as the aim of SAPALDIA was to investigate the relationship between environmental factors and respiratory conditions. The phrasing of the work related symptoms were not comparable across the two studies. SAPALDIA exams where spread over twelve month, the Swiss Farmers were investigated during spring time only.

Statistical analysis

Analysis was performed with StatView 4.5 and SAS 6.12 by Abacus/SAS. The Fisher-Test was used for two-dimensional (2x2) and χ^2 test for multidimensional contingency tables. Multivariate analysis was performed by the methods of binary and multivariate logistic regression (Hosmer et al. 1989). Stratification was performed with the method of Mantel and Haenszel (Mantel et al. 1959).

Results

Swiss farmers

Socio-demographic data

Farming was a full time job for 738 (78.5%) and a part time job for 202 (21.5%). According to the grouping definition (table 2) 78 (8.3%) farmers did plant farming, 34 (3.6) mainly pig farming, 102 (10.9%) pig and cattle farming, 468 (49.8%) cattle farming, 37 (3.9%) mainly poultry farming, 115 (12.2%) mixed farming, and 106 (11.3) had small farms.

The average age for all farmers was 48.9 years with a standard deviation of 13 years. The prevalence of symptoms across four age categories is given in Table 3. I observed a significant increasing trend across the age groups for the symptoms ”phlegm” and ”chronic bronchitis” as well as for the work related questions ”cough with phlegm at work”, ”breathless at work”, ”wheezing at work”. For ODTS and ”nasal irritation at work” there was an inverse relationship with age.

544 (57.9%) of the farmers had never been smokers, 221 (23.5%) were current smokers and 175 (18.6) were former smokers. The prevalence of former smokers increased with age from 13.0 (21-40 years) to 25.1% (>60 years).

symptoms	age 21-40 n=277	age 41-50 n=254	age 51-60 n=217	age >60 n=191	all n=940	χ^2 p-value
wheezing	13.4 %	15.7 %	15.2 %	18.3 %	15.4 %	0.54
shortness of breath	3.97 %	7.48 %	8.29 %	7.85 %	6.70 %	0.18
asthma attack	2.53 %	1.97 %	2.76 %	3.66 %	2.66 %	0.74
phlegm	19.1 %	22.8 %	23.0 %	31.9 %	23.6 %	0.015*
chronic bronchitis	11.2 %	14.6 %	15.7 %	25.1 %	16.0 %	0.0007*
nasal allergy	12.6 %	9.84 %	6.91 %	7.85 %	9.57 %	0.54
nasal irritation at work	28.9 %	22.8 %	21.2 %	17.8 %	23.2 %	0.034*
cough at work	17.3 %	14.2 %	13.4 %	15.7 %	15.2 %	0.17
cough with phlegm at work	15.5 %	16.1 %	18.0 %	23.0 %	17.8 %	0.023*
breathless at work	3.97 %	4.72 %	9.68 %	11.5 %	7.02 %	0.003*
wheezing at work	4.69 %	7.09 %	10.6 %	11.5 %	8.09 %	0.02*
ODTS	32.6 %	25.2 %	25.3 %	20.4 %	24.7 %	0.46
asthma (one of questions F1-F3)	15.5 %	18.1 %	19.4 %	21.5 %	18.3 %	0.42
work related symptoms (one of questions F6.1-F6.5)	44.8 %	38.6 %	41.5 %	42.9 %	41.9 %	0.54

Table 3: Prevalence of self reported symptoms in Swiss farmers by age categories, p-value (χ^2) for a relation with age.

Responders

Using logistic regression analysis, the differences between the responders in the three steps were evaluated. Only minor differences were found. Step 2 responders consisted of more plant farmers compared with step 1 responders and indicated less work-related symptoms. Step 3 responders reported less ODTS symptoms and had a higher proportion of farmers from a mixed farming type than those in the two previous steps.

Effect of type of farming

Table 4 shows the prevalence rates of self reported symptoms in Swiss farmers by category of farming. In the univariate analysis only the prevalence of "nasal irritation at work" differed significantly across type of farming, being particularly high in poultry farmers. The prevalence rates of most symptoms were highest in the poultry-farming group. The highest prevalence of nasal allergy was reported in poultry farmers. The frequency of wheezing was about twice as high (17-19%) in

pig, cattle and poultry farmers as compared to those engaged in small and mixed farming (8-10%).

symptoms	plant farming n=78	pig farming n=34	pigs and cattle farming n=102	poultry farming n=37	cattle farming n=468	mixed farming n=115	small farming n=106	χ^2 p-value	all n=940
wheezing	15.4 %	17.6 %	13.7 %	18.9 %	18.4 %	9.57%	8.49 %	0.096	15.4 %
shortness of breath	6.41 %	8.82 %	2.94 %	13.5 %	7.48 %	6.09 %	4.72 %	0.37	6.70 %
asthma attack	2.56 %	2.94 %	3.92 %	2.70 %	3.42 %	0.87 %	0.00 %	0.42	2.66 %
phlegm	26.9 %	17.6 %	19.6 %	27.0 %	25.0 %	25.2 %	17.9 %	0.56	23.6 %
chronic bronchitis	24.4 %	11.8 %	11.8 %	18.9 %	16.0 %	16.5 %	13.2 %	0.34	16.0 %
nasal allergy	12.8 %	2.94 %	9.80 %	16.2 %	10.0 %	7.83 %	6.60 %	0.40	9.57 %
nasal irritation at work	20.5 %	14.7 %	25.5 %	40.5%	24.8 %	13.0 %	20.8 %	0.009*	23.2 %
cough at work	11.5 %	14.7 %	18.6 %	18.9 %	15.0 %	14.8 %	15.1 %	0.90	15.2 %
cough with phlegm at work	21.8 %	14.7 %	17.6 %	16.2 %	18.4 %	17.4 %	14.2 %	0.90	17.8 %
breathless at work	10.3 %	14.7 %	2.94 %	8.11 %	7.69 %	3.48 %	6.60 %	0.15	7.02 %
wheezing at work	11.5 %	5.88 %	8.82 %	10.8 %	8.97 %	3.48 %	5.66 %	0.37	8.09 %
ODTS	19.2 %	26.5 %	23.5 %	35.1 %	23.9 %	20.0 %	23.6 %	0.47	24.7 %
asthma (one of questions F1-F3)	19.2 %	17.6 %	14.7 %	27.0 %	20.4 %	13.9 %	11.3 %	0.13	18.3 %
work related symptoms (one of questions F6.1-F6.5)	50.0 %	38.2 %	44.1 %	54.1 %	42.5 %	34.8 %	36.8 %	0.26	41.9 %
mean time in confinement (h)	0.0	3.2	6.3	4.8	5.7	0.4	0.8		

Table 4: Prevalence of self reported symptoms in Swiss farmers by category of farm, as well as mean time spent in animal confinement buildings, p-value (χ^2) for a relation with categories

Multivariate Analysis

The multivariate logistic regression presented in Table 5 analyses the independent association of type of farming, and hours spent in animal confinement buildings with symptom prevalence, adjusted for age, and smoking status. Small farming was the reference for farming categories, age 21-40 was the reference for age, never having smoked was the reference for smoking status, 0-1 hour per day working in an animal confinement buildings was used as the reference for hours spent in confinement buildings. Estimates and confidence intervals are shown. With regard to type of farming, no clear pattern can be described. However, except for ”chronic bronchitis” and ”phlegm”, poultry farmers tend to show the highest estimate for the listed symptoms, being significant only for ”nasal irritation at work”. The highest estimate for ”chronic bronchitis” was seen in plant farmers (OR=2.32, p=0.04), which, in general, reported higher symptom prevalence rates compared to the reference group. The adjusted prevalence of wheezing was at least twice as high for cattle, poultry, pig, and plant farmers.

A clear dependence of symptom prevalence from the number of hours spent in animal confinement could be observed for ”chronic bronchitis” and ”phlegm” (Table 5), but also for ”breathlessness” ”asthma attacks”, and for the work related respiratory symptoms (data not shown). For ”nasal irritation at work” the trend was inverse.

Table 5 also shows that respiratory symptoms were most prevalent among the oldest age group, reaching statistical significance for ”chronic bronchitis” and ”phlegm”. Farmers over the age of 60 also had an elevated probability for reporting ”wheezing at work” (OR=2.67, p=0.009), and ”breathlessness at work” (age 51-60: OR=2.51, p=0.02; age >60: OR=3.21, p=0.003). For ”nasal irritation at work” the oldest age category had the lowest prevalence. Also ”nasal allergy” is less frequent in farmers older than 51 years of age.

The well-known cross-sectional associations of smoking status and symptoms could be confirmed.

	wheezing	chronic bronchitis	phlegm	asthma (F1,F2,F3)	nasal allergy	nasal irritation at work
plant farming n=78	1.96 (0.76-5.07)	2.32* (1.03-5.23)	1.85 (0.88-3.82)	1.94 (0.83-4.57)	2.03 (0.72-5.70)	0.97 (0.46-2.02)
pig farming n=34	2.35 (0.63-8.81)	0.61 (0.16-2.28)	0.68 (0.22-2.14)	1.50 (0.43-5.14)	0.74 (0.07-7.95)	1.10 (0.31-3.90)
pig and cattle farming n=102	1.80 (0.50-6.50)	0.47 (0.16-1.42)	0.66 (0.25-1.73)	1.30 (0.41-4.06)	4.19 (0.64-27.2)	3.37* (1.04-10.87)
poultry farming n=37	2.68 (0.68-10.6)	0.91 (0.27-3.06)	1.07 (0.36-3.17)	2.87 (0.86-9.56)	5.99 (0.95-37.7)	5.33* (1.57-18.05)
cattle farming n=468	2.42 (0.76-7.72)	0.64 (0.25-1.63)	0.86 (0.37-2.00)	1.88 (0.69-5.17)	4.35 (0.75-25.4)	2.87 (0.95-8.61)
mixed farming n=115	1.21 (0.48-3.10)	1.37 (0.64-2.97)	1.68 (0.86-3.29)	1.38 (0.61-3.12)	1.15 (0.41-3.25)	0.56 (0.27-1.15)
small farming n=106	1	1	1	1	1	1
age 21-40	1	1	1	1	1	1
age 41-50	1.19 (0.72-1.94)	1.36 (0.80-2.31)	1.22 (0.80-1.86)	1.17 (0.74-1.87)	0.72 (0.41-1.25)	0.72 (0.49-1.08)
age 51-60	1.09 (0.65-1.83)	1.28 (0.77-2.16)	1.18 (0.76-1.85)	1.25 (0.77-2.02)	0.46* (0.24-0.88)	0.66 (0.43-1.0)
age >60	1.53 (0.90-2.59)	2.40* (1.43-4.00)	1.87* (1.20-2.91)	1.54 (0.94-2.53)	0.63 (0.28-1.03)	0.57* (0.36-0.92)
never smoker	1	1	1	1	1	1
current smoker	1.88* (1.30-2.88)	1.15 (0.74-1.80)	1.46* (1.01-2.13)	2.14* (1.43-3.19)	1.09 (0.62-1.90)	1.12 (0.77-1.63)
former smoker	2.03* (1.30-3.20)	1.60* (1.03-2.48)	1.86* (1.27-2.75)	2.05* (1.34-3.14)	1.67 (0.96-2.90)	1.04 (0.68-1.57)
0-1 hour in confinement	1	1	1	1	1	1
>1-4 hours in confinement	0.99 (0.34-2.92)	2.08 (0.87-4.96)	1.85 (0.84-4.07)	1.25 (0.49-3.17)	0.36 (0.69-1.92)	0.41 (0.14-1.19)
>4 hours in confinement	1.12 (0.36-3.45)	2.61*(1.01-6.76)	2.30 (0.99-5.4)	1.34 (0.46-3.35)	0.30 (0.06-1.67)	0.38 (0.13-1.12)

*Table 5: OR with 95% confidence intervals by multivariate regression analysis model: respiratory symptom = farmer category + age + smoking habits + hours spent in confinement buildings. *=p<0.05*

Swiss Farmers compared to the Swiss Population

As seen in Table 6, the age distribution is different in the two populations. The SAPALDIA population includes more young and fewer older persons. Compared to the general population (SAPALDIA), current and former smoking was significantly less frequent in the farmer-population and never smoking was significant more frequent (Table 7).

Given the confounding by age and smoking status, Table 8 presents the crude and the age adjusted odds ratio of the symptom prevalence rates for never smokers. The asthma symptoms were not different in the two populations. Farmers had a 4.5 fold elevated risk for reporting "phlegm" and a 1.9 fold elevated risk for "chronic bronchitis". In contrast, their risk for reporting nasal allergies was less than half as high.

Age	Farmers (n=748)	SAPALDIA n=(4202)	Odds ratio (95% confidence limit)
age 21-40	37 %	46 %	0.70* (0.60-0.82)
age 41-50	34 %	30 %	1.16 (0.99-1.37)
age 51-60	29 %	24 %	1.32* (1.11-1.57)

Table 6: Distribution of age in farmers and general population (SAPALDIA), OR (= Fisher test is significant) and 95% confidence limits in parentheses.*

Smoking status	Farmers (n=748)	SAPALDIA n=(4202)	Crude odds ratio (95% confidence limit)	Mantel Haenszel adjusted odds ratio (95% confidence limit)
never smoker	58 %	34 %	2.58* (2.20-3.02)	2.72* (2.33-3.18)
current smoker	25 %	38 %	0.54* (0.45-0.64)	0.54* (0.46-0.65)
former smoker	17 %	28 %	0.56* (0.46-0.69)	0.52* (0.42-0.63)

Table 7: Distribution of smoking status in farmers and general population (SAPALDIA), Crude and Mantel Haenszel age adjusted OR (= Fisher test is significant) for age and 95% confidence limits in parentheses.*

Never smoker only	Farmers (n=442)	SAPALDIA n=(1460)	Crude odds ratio (95% confidence limit)	Mantel Haenszel adjusted odds ratio (95% confidence limit)
wheezing	11.5 %	9.5 %	1.29 (0.92-1.82)	1.23 (0.86-1.74)
shortness of breath	4.5 %	5.2 %	0.94 (0.57-1.57)	0.86 (0.52-1.44)
asthma attack	2.1 %	3.1 %	0.67 (0.32-1.38)	0.64 (0.31-1.34)
phlegm	18.3 %	4.8 %	4.98* (3.51-7.05)	4.50* (3.25-6.69)
chronic bronchitis	12.0 %	6.8 %	2.07* (1.44-2.95)	1.89* (1.32-2.95)
nasal allergy	10.0 %	21.5 %	0.38* (0.27-0.53)	0.40* (0.29-0.56)

Table 8: Never smokers only: Prevalence analysis and OR (= Fisher test is significant) from self reported symptoms of Swiss farmers (Farmer-Questionnaire) and Swiss population (SAPALDIA-Questionnaire), crude OR and OR adjusted for age, reference = Swiss population.*

Discussion

This study shows a two-fold elevated risk of reporting chronic bronchitis and a 4.5 fold (Mantel Haenszel) elevated risk for phlegm in farmers compared to the general Swiss population. These main results most likely indicate occupational disorders as the exposure-response relationship (hours spent in animal confinements) was particularly obvious for these symptoms.

The high symptom prevalence rates among farmers are unlikely to be explained by participation bias. First, the three mailings indicated only minor overrepresentation among the fast responding farmers. Second, participation rate was high. Third, subjects with symptoms tended to be overrepresented as well in the comparison group of the SAPALDIA study (Luthi et al. 1997).

I was well aware of the inherent limitations of the cross-sectional design of this prevalence study. First, people with allergies and existing health problems might not start farming. Second, people with health problems, are more likely to quit farming earlier than those without, therefore underestimating the prevalence rates among current farmers (healthy worker). Thus the prevalence odds ratios for farmers versus the SAPALDIA population may even be underestimated.

The comparison of SAPALDIA with the Swiss farmers has inherent limitations due to the different methods of data collection. In SAPALDIA an interview was conducted, in Swiss farmers a self-reporting questionnaire was issued by mail. Data collection by interview tends to give more affirmative answers than self-reporting questionnaires (Rehm et al. 1993). Thus, it must be assumed that the farmer study even tends to underestimate the prevalence rates compared with SAPALDIA.

Compared with the general population farmers are at risk for reporting symptoms of chronic lower airway inflammation, chronic bronchitis and bringing up phlegm. This, as well as the magnitude of the effect, is in agreement with other published studies rhinitis occupations (Dosman et al. 1987; Terho et al. 1987; Dalphin et al. 1989).

Farmers are exposed to organic dust and gases, which are potential respiratory hazards (Rylander 1986). Bacteria, endotoxin, mites and fungi spores are the most important contaminants of dust in animal confinement buildings. It has been shown that organic dust of different agricultural origin evokes an inflammatory response in "in vitro" studies (Rylander 1993) and in experimental studies with endotoxin (Michel et al. 1995). The increasing risk found here for chronic bronchitis with prolonged time spent in animal confinement buildings confirms the hypotheses that the exposure conditions in these buildings are crucial for the respiratory health of the farmers.

In agreement with other recently published studies, no differences in asthma prevalence rates between animal farmers and non-farmers were found (Choudat et al. 1994; Susitaival 1994). Nasal allergies were significantly decreased in farmers. The "Swiss Study on Childhood Allergy and Respiratory Symptoms" (SCARPOL) (Braun-Fahrländer et al. 1999,) showed that farmers' children have less allergies than other children do. The reason that these rates, as well as those for adult farmers, are lower than among non-farmers is not yet understood. Kimbell-Dunn and co-workers (Kimbell-Dunn et al. 1999) concluded that the low prevalence of asthma and allergy among New Zealand farmers may be due to the

healthy worker effect. We doubt that this is the major reason in this study because most of the farmers under study were farm owners who may be less willing to change their occupation than employees (Thelin et al. 1994). Development of tolerance whereby farming lifestyle may play an important role seems more plausible as a likely influence on developing allergies.

Type of farming

The symptom pattern across type of farming may be subject to self-selection biases, which could not be controlled, in the cross-sectional design. Therefore, the farming type results, discussed in the following sections, may be interpreted with caution.

Poultry farmers reported the highest amount of work related symptoms but due to their small number only ”nasal irritation at work” was significantly elevated. A previous study of Swiss poultry farmers (Danuser et al. 1988) as well as a study from Croatia (Zuskin et al. 1995) showed a high prevalence of acute symptoms during work. Compared with other farming categories, the highest prevalence rate of nasal allergies was seen in poultry farmers. The prevalence OR doubled (from 2.5 to 5.3) with adjustment for the hours spent in animal confinement buildings, whereas this factor was inversely related to nasal irritation at work. This strong confounding may be due to strong self-selection of working habits, depending on the occurrence of nasal irritations (cross-sectional bias). Various potential aeroallergens in organic poultry dust especially from mites and the animal itself have been described (Lutsky et al. 1984; Müller et al. 1986; Perfetti et al. 1997). It is known that high concentrations of ammonia and organic dust are found in poultry houses (Danuser et al. 1988). In poultry confinement buildings, bacteria, endotoxin, and fungi contaminants, especially, are highest among different animal confinements buildings (Seedorf et al. 1998). Ammonia can additionally irritate nose and eye tissues (Verberk 1977). Ammonia attached to particles may not only affect the upper airways, it may reach the lower airways too. The finding of the high prevalence of ”phlegm” in poultry farmers indicates an involvement of the lower airways. In the second part of the European study on ”Prevalence and Risk Factors for Obstructive Airway Diseases in Farmers” the focus is on the investigation of the relationship between measures of exposure and health effects in poultry farmers.

In disagreement with other farming population based studies, Swiss pig farmers reported no elevated airway symptoms compared with other types of farming (Iversen et al. 1988). Donham and co-workers (Donham et al. 1984) have found prevalence rates of work related symptoms in pig farmers between 27 and 56%. The prevalence rate of chronic bronchitis in pig farmers found in other studies are much higher: 15.3% (Donham et al. 1989), 26% (Zejda et al. 1993) and 55% (Holness et al. 1987). Only Choudat et al. 1994 found prevalence rates in the same range as we did (6-15%). This discrepancy could be due to the small size of Swiss pig farms with, on average, only 18 pigs per farm. Keeping fewer pigs reduces the time per day necessary to spend in pig confinement buildings. As shown in our analyses, bronchitis symptoms increased with the number of hours spent with the animals.

In contrast to the Swiss mortality study (Gassner et al. 1995) where plant farmers showed a reduced risk of dying from chronic bronchitis, we found an elevated risk for them to report chronic bronchitis. First it is not clear to what extent our definition of plant farming corresponds to the one used in the mortality study. None of the farmers with symptoms worked in greenhouses. Most of them worked with grain, vegetables, root crops (potatoes) or fruits. The majority of them cultivated a combination of two or more of the above plants. Second mortality data are reflecting working conditions of the past, symptom prevalence data more the present situation. Changes in technology might have influenced the current plant-farming situation. Plant farmers may be exposed to pesticides and to organic dust due to unsafe handling and badly enclosed tractors. It is also possible that a self-selection took place. Animal farmers with health problems reduce the number of animals and may become plant farmers. In this study, fieldwork is recognized as a risk factor for reporting chronic bronchitis. Further studies should be carried out to investigate these results.

In conclusion, Swiss farmers are at increased risk to suffer from chronic respiratory symptoms. The prevalence of chronic cough and phlegm clearly increased with increasing number of hours spent in animal confinements, thus we consider the health impairment as occupational diseases. Preventive strategies should be developed to protect the health of farmers.[2]

[2] This study is part of the EU-BIOMED Project "Prevalence and Risk Factors for Obstructive Airway Diseases in Farmers". EU-Number: BMH1-CT94-1554. It was supported by the Swiss National Science Foundation: NF-Number 3200-045997.95/1 and the BBW (Bundesamt für Bildung und Wissenschaft): Number 93.0283.

SAPALDIA is a project of the Swiss National Science Foundation, and was part of the National Research Program NFP/PNR 26 (Grant 4026-028099). NK is a recipient of a National Science Foundation Advanced Scientist Fellowship (#3233-08922.96/1).

Part B: Air Contaminants and Respiratory Health Hazards in Poultry Farming Environments[3]

Introduction

It is known that animal farmers are exposed to organic dust, including aeroallergens, insect antigens, endotoxin, as well as hazardous gaseous exposures including ammonia, nitrogen oxides and hydrogen sulfide. These substances may affect one or more region in the respiratory system of the farmer and may induce diseases like allergic and non-allergic rhinitis (Terho et al. 1987), organic dust toxic syndrome (ODTS) (Melbostad et al. 1997; Schenker 1998), bronchitis (Rask-Andersen 1989), asthma (Terho et al. 1987), and asthma-like syndromes (Melbostad et al. 1998).

In the first part of the study a questionnaire survey on general as well as work-related respiratory symptoms in relation to type of farming was carried out. In Switzerland 1542 randomly selected farmers were involved. Animal farmers (pigs, cattle, poultry, sheep) working in confinement houses were shown to be at highest risk for the development of respiratory symptoms. Poultry farmers showed a significantly higher prevalence of wheezing compared to farmers not working with those animals (Weber et al. 1998). The results of this first part of the study are presented in detail in part A. Because this first stage of the study has shown that poultry farmers were at highest risk for the development of respiratory symptoms it was decided to study them in more detail in this second part of the survey.

Therefore, it was the aim of this second part of the study to investigate the relationship between lung function and measures of exposure as well as farming characteristics and focuses on exposure parameters.

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[3] adapted for Switzerland after Radon, K., D. Blainey, J. Blainey, B. Danuser, M. Iversen, E. Monso, U. Opravil, C. Weber and D. Nowak (1999D). "Respiratory symptoms in European farmers." Am J Respir Crit Care Med **159**: A297.
Radon, K., C. Weber, M. Iversen, B. Danuser, S. Pedersen and D. Nowak (2001). "Exposure assessment and lung function in pig and poultry farmers." Occup Environ Med **58**: 0-5.

Material and Methods

Study population

In Switzerland, the prevalence of work-related respiratory symptoms was highest in poultry farmers (Weber et al. 1998). Therefore 36 poultry farmers were randomly chosen out of the poultry farmers in part A and due to their little number (36) we also randomly selected part of them (15) out of the list of the regional professional farmers' organization. Combinations of the main production with other types of animal or plant farming were documented but were not a selection criteria.

Study design

Exposure to total dust, endotoxin and microorganisms was determined by means of personal sampling in the breathing zone. Samples were taken during the daily work inside the animal house. The management and work practices of the various farms required assessment in several rooms. Farmers carried out their usual task during measurements wearing the personal pumps while moving from one building to another. The sampling time included work inside several animal houses but all buildings housed the same kind of animals. The sampling time included also the time used moving from one building to another.

Questionnaire

Inventory of farm characteristics was done by visiting the farm and interviewing the participants about number and kind of animals, heating and ventilation system, type of floor, frequency of cleaning, and location of air exhaust. A special kind of ventilation is porous ventilation. Porous ventilation as porous walls or ceilings is characterized by big porous surfaces with plenty of small wholes, e.g. mineral wool or perforated plates. In animal houses with automatic ventilation control a temperature sensor mostly controls the ventilation flow. A particular type of automatic ventilation control is regulation via humidity sensors used in houses where supplementary heat is needed. In these buildings a humidity sensor is used additionally to the temperature sensor. This means that the temperature sensor influencing the air exchange in the animal house controls the indoor temperature but if the indoor relative air humidity increases above the set level, additional heat is supplied. Subsequently the temperature rises and the ventilation flow increases

due to temperature control. The results of the farm characteristics have been documented elsewhere (Radon et al. 1999D).

Structured interviews were additionally performed with questions on respiratory symptoms within the preceding year, smoking habits, and standardized questions on chronic bronchitis (Medical Research Council Criteria). Asthma was defined as having been woken by an attack of shortness of breath during the last year, reporting at least one asthma attack during the last year, or currently taking asthma medication. Subjects reporting cough and phlegm on most days for at least 3 months during the preceding year were defined as having chronic bronchitis. Special emphasis was given to respiratory symptoms during work that suggested airway narrowing or irritation (shortness of breath, wheezing and dry cough). The questionnaires were tested for comprehensibility and translated, with back translation into English.

Dust

Airborne dust was collected on pre-weighted (Technischer Überwachungsverein (TÜV) Hanover, Germany), 37 mm diameter glass fiber filters (SKC, Müllheim, Germany) fixed in threaded holders (GSP, Personal air sampler, "GSA Meßgerätebau Neuss", Germany). Battery-operated pumps (224 PCXR 7 KB, SKC, Müllheim, Germany) provided a constant airflow of 3.5 l/min. All exposed filters were subsequently re-weighed at the laboratory of TÜV Hanover (Germany). Before weighing and re-weighing all filters were desiccated for 24 hours under defined conditions (23 °C, 50 % air humidity). The lower detection limit was 0.09 mg/filter. The results were related to air volume and given as mg/m^3.

Endotoxin

Endotoxin content of dust samples was determined by a kinetic-turbidimetric Limulus assay as described by Hollander et al. 1993 in the laboratory of the Institute of Animal Hygiene and Animal Welfare (School of Veterinary Medicine Hanover, Germany). Briefly, each filter was extracted by rapid shaking with endotoxin-free water (Acila, Pyroquant Diagnostik GmbH, Walldorf, Germany) for one hour. From a diluted aliquot, 100 µl were added to a microtiter-plate well (96 wells, NUNC) and assayed with 100 µl LAL reagent (Kinetic-QCL, BioWhittaker, Verviers, Belgium) at 37 °C. A standard calibration curve (50, 5, 0.5, 0.05, 0.005 EU / ml) was performed on each plate. Each sample was spiked by 0.5 EU EC 6 standard (EC = *Escherichia Coli*). Optical density at 405 nm was measured by an automatic reader (Autos Reader hat III, Biowhittaker). Results were related to air volume and expressed as ng/m^3 (EC 6 standard, 8 EU = 1 ng). The lower detection limit was 0.005 EU.

Ammonia, carbon dioxide, temperature, relative air humidity and air velocity

Temperature, relative air humidity, Ammonia and carbon dioxide concentrations were measured with the Metrosonics aq-5000 indoor air quality monitor (Metrosonics, Rochester, NY, USA). Air velocity was taken by a multi-function instrument (Testo 400, Testo, Lenzkirch, Germany). The sampling points were located in the center of the animal house at a point several meters from the overhead than in the passageway, 1.5 m above the floor. All parameters were assessed once in the morning when the farmer was entering the building.

Airborne microorganisms

Polycarbonate filters with a pore size of 0.4 µm and a diameter of 25 mm were placed on cellulose support pads and sealed in pre-sterilized carbon-filled polypropylene air monitoring cassettes (Pegasus Labor, Düsseldorf, Germany). The filter holders were connected to portable battery-operated pumps (224 PCXR 7 KB, SKC, Muellheim, Germany) calibrated for an airflow of 1 l/min. All samples were sent to the laboratory (Pegasus Labor) within the same day of collection. In Germany no airborne microorganism samples were collected.

The total concentration of airborne microorganisms was determined by the CAMNEA method utilizing an epifluorescence microscope (Palmgren et al. 1986) showing similar or slightly lower estimates of microorganisms than scanning electron microscopy or light microscopy (Eduard et al. 1990). Viable count estimation was done as described elsewhere (Palmgren et al. 1986).

In short, before analyzing the microorganisms, the polycarbonate filters were extracted in the filter cassettes by adding 5.0 ml 0.05 % Tween 80 solution and shaking for 15 min at room temperature. Samples were immediately used for plating and analysis by epifluorescence microscopy. Counting by epifluorescence microscopy was carried out by staining 1 ml extraction fluid with 0.3 ml 0.01 % acridine orange in acetate buffer (bioMedieux) for 30 s and filtered through a dark 0.4 µm polycarbonate filter (Nuclepore, New York, USA). The number of microbial cells in forty randomly chosen fields was counted by epifluorescence microscopy at 1250 times magnification, grouping into bacterial rods, bacterial spores and fungal spores. Counts were related to air volume and expressed as the logarithm of colony forming units/m^3 of sampled air (log CFU/m^3). The lowest countable concentration of microorganisms was 3 * 10^3 counts per sample (= 3.5 log counts per sample). Using this method, viable and non-viable microorganisms were enumerated.

In order to get the number of viable microorganisms, cultivable bacteria and fungi were quantified by inoculation of suitable dilutions of the extraction fluid from the filters on plates with selective media. After incubation, CFU were counted and the concentration was calculated as CFU/m^3 air. The minimum detectable concentration was 50 CFU/filter. Different groups of microorganisms were isolated using the following media:

2. Maltextract agar with penicillin and streptomycin (20 g maltextract (Oxoid), 20 g agar (Fluka), 2 ml penicillin-streptomycin solution, 1 l aqua dest).
3. DG 18-agar with chloramphenicol (31.5 g DG18-Agar (Oxoid), 220 ml Glycerin (Merck), 1 ml chloramphenicol solution (10 g chloramphenicol (Fluka), 100 ml 95 % Ethanol), 10 g agar No 2, 1 l aqua dest).
4. Tryptone glucose extract agar (TGE-Agar) with delvocid (24 g tryptone glucose extract agar (Oxoid), 0.1 g delvocid (Gist Brocades), 1 l aqua dest).

5. Tryptone glucose extract agar (TGE-Agar) with delvocid and saccharose (24 g tryptone glucose extract agar (Oxoid), 400 g Saccharose (BDH), 0.1 g delvocid (Gist Brocades), 1 l aqua dest).

The incubation temperatures used for fungi were 21 °C (mesophilic) and 45 °C (thermophilic), bacteria cultures were incubated at 21 °C (mesophilic) and 55 °C (thermophilic). The incubation time was 7 days, for Actinomycetes 7 and 14 days.
For the determination of fungi maltextract and DG 18 was used, for bacteria TGE and TGE with saccharose was used.
All colonies were examined microscopically. Cultivation of selected isolates was performed by classical microbiological principles. The following genus were identified:

I. Fungi: *Absidia, Alternaria, Aspergillus, Botrytis, Cladosporium, Eurotium, Candida, Mucor, Penicillium, Trichoderma, Ulocladium, thermophilic fungi*
II. Bacteria: *Bacillus, Streptomyces, thermophilic bacteria.*

When microorganisms of a certain genus were detected in a sample, the sample was expressed as positive for this type of microorganisms. Therefore, results of the different genus of microorganisms are expressed as frequencies of positive samples. In the final analysis only bacteria or fungi detectable in at least 10 buildings were included.

Lung Function Tests

Lung function tests were performed immediately before and after feeding of the animals in the morning. A portable spirometer (MultiSPIRO-PC, Biotrine, Woburn, MA, USA) was used after daily calibration. *Forced vital capacity* (FVC*), forced expiratory volume in 1 second* (FEV_1) and *mid expiratory flow rate* ($MMEF_{25/75}$) were measured. All results were analyzed blindly by the same person according to the ATS standardization criteria, that is of three acceptable flow-volume curves the largest and the second largest values of FVC and FEV_1-values were not allowed to vary by more than 200 ml or 10 %. $MMEF_{25/75}$ values were recorded from the maneuver with the largest sum of FEV_1 and FVC (1995). Lung function results were compared to age and height adjusted reference values as proposed by the ECSC

(Quanjer et al. 1993) and given as % of predicted (% pred.). The decline in lung function parameters over the feeding period was calculated in % of the baseline value. As pre- and post-exposure measurements were taken on all subjects, each individual served as his or her own control.

Environmental Measurements

The measurements were taken during the cold season. Personal monitors were used to collect samples for each farmer during the daily work inside the animal buildings resulting in a median sampling time of 30 minutes (Radon et al. 1999D). Farmers carried out their usual task during measurements wearing the personal pumps while moving from one building to another. The collected samples were analyzed for total dust, endotoxin concentration in total dust, and microbial contamination (total and viable bacteria and fungi). Additionally, a point measurement of ammonia, carbon dioxide, temperature, air humidity and air velocity was performed in each of the animal houses under study. Details of the air sampling and laboratory analyses are described elsewhere (Radon et al. 1999D).

Analysis

The treatment of the data was performed with a statistical package for personal computers (Statistica©, Tulsa, USA).

Due to the non-normal distribution of the data the results are given as median with range. Results of the different groups of microorganisms are given as relative frequencies. Standard tests for non-normal distributed variables were used as median-test for continuous variables and Chi-square test for dichotomous variables.

The t-test for dependent variables was used to compare lung function prior to and after feeding. Spearman´s rank correlation analysis was used to study the relationship between baseline lung function results and concentration of occupational exposures. Due to the wide range of the measures of exposures, the latter variables were log-transformed. Lung function data from poultry farmers was collapsed into one analysis for comparison with environmental contaminants to

determine if an exposure response could be observed. Additionally, correlation models with statistically significant results in the univariate analysis were included in a multiple linear regression model using the stepwise forward method. In the multiple linear regression models autocorrelation of the independent parameters was excluded using Durbin-Watson-statistics.

Differences in farming methods and levels of exposure between symptomatic (wheezing, breathlessness, or cough without phlegm at work) and asymptomatic farmers were tested using Fisher's exact test and t-test for independent variables. All statistical tests were done at $\alpha = 0.05$ significance level.

Results

Farming characteristics

The number of farmers and the farming characteristics are given in Table 9. The Swiss poultry houses under study had a median volume of 749 m^3 with a median number of 2,100 animals per farm (Table 9). Most of the farms had several poultry houses. The interval of cleaning in poultry houses was in 35 of the 36 farmers longer than 1 month.

Measurements

The median total dust concentrations, ammonia, carbon dioxide, temperature, air velocity and concentrations of airborne microorganisms are given in Table 10. *Bacillus* spp. were found in nearly one third of all specimen.

Subjects

Descriptive data and lung function values of poultry farmers as age, duration of work on a farm, prevalence of respiratory symptoms and smoking habits are shown in Table 11. Lung function results after work inside the animal buildings did not differ significantly compared to the pre-exposure values ($p_{\text{paired-sample t-Test}} > 0.05$).

Number of farmers	36
	Median (range)
Area (m^2)	300 (36 – 700)
Volume (m^3)	749 (90 - 2100)
Laying hens (number / farm)	2100 (0 – 16000)
Chicks (number / farm)	0 (0 – 20000)
Cocks (number / farm)	0 (0 – 3000)
Fattening poultry (number / farm)	0 (0 – 11500)
	N
Free-range conditions	26
Concrete floor	31
Pellet like feeding	4
Manual feeding	1
Natural ventilation	4
Air inlet: porous channel	15
Ventilation control: humidity sensor	2
Heating*	13
Storage time of liquid manure > 1 month	20
Interval of cleaning > 1 month	35

*Table 9: Farming characteristics in investigated Swiss poultry confinement houses. For continuous variables median (range) are given. Dichotomous variables are given as frequencies. *Due to breeding.*

n	36
Total dust sampling time (minutes)	30 (11 – 133)
Microorganism sampling time (minutes)	30 (11 – 85)
Total dust **(mg / m^3)**	7.01 0.42 – 21.8
Endotoxin (in total dust samples) **(ng / m^3)**	258 19.0 – 1635
Ammonia **(ppm)**	12 <5 – 40
Carbon dioxide **(ppm)**	2100 600 - >3000
Temperature (°C)	16.2 4.2 – 25.4
Air humidity (%)	71.1 54.0 - 96.0
Air velocity (m/s)	0.01 0.00 – 0.29
Total fungi **(log cells / m^3)**	7.46 <DL – 9.04
Active fungi **(log CFU/m^3)**	5.64 4.15 – 8.02
Total bacteria **(log cells / m^3)**	9.67 7.43 – 10.62
Active bacteria **(log CFU/m^3)**	7.90 5.75 – 9.20

Table 10: Concentrations and physical parameters of the environmental measurements (median and range) (DL = detection limit)

	Poultry farmers
Number	36
Male gender (%)	24 (67%)
Current smokers (%)	11 (31%)
Ex-smokers (%)	5 (14%)
Asthma symptoms (%)[#]	3 (9%)
Symptoms of chronic bronchitis (%)[&]	4 (14%)
Work-related respiratory symptoms (%)	21 (58%)
	Mean ± SD
Age (years)	41 ± 13
Duration of work as a farmer (years)	20 ± 14
FVC pre-exposure (% pred.)	101.4 ± 14.9
FVC post-exposure (% of pred.)	102.6 ± 14.0
FEV_1 pre-exposure (% of pred.)	100.2 ± 14.2
FEV_1 post-exposure (% of pred.)	101.0 ± 13.6
$MMEF_{25/75}$ pre-exposure (% of pred.)	88.8 ± 20.4
$MMEF_{25/75}$ post-exposure (% of pred.)	89.1 ± 22.2

Table 11: Descriptive data and lung function values of poultry farmers
number in parenthesis = % of total population
 # woken by an attack of shortness of breath during the last year, asthma attack during the last year, or taking asthma medication presently
& cough and phlegm on most days for at least 3 months during the preceding year

Associations between farm characteristics and baseline lung function results

Table 12 shows that farming characteristics are significantly associated with lung function results prior to the feeding period using Mann-Whitney-U-test for independent variables for poultry farmers. In poultry houses the presence of an air inlet through porous inlets was significantly negatively associated with results of FVC % pred.

		n	FVC % pred.	FEV_1 % pred.	$MMEF_{25/75}$ % pred.
Automatic feeding	**No**	1	number of farmers without automatic feeding too low		
	Yes	35			
Storage time liquid manure > 1 months	**No**	16	96.9 ± 13.4	96.9 ± 13.7	88.9 ± 20.7
	Yes	20	105.0 ± 15.45	102.8 ± 14.3	88.7 ± 20.7
Air inlet: Porous inlet	**No**	15	108.5 ± 15.8*	105.3 ±13.1	87.5 ± 15.2
	Yes	21	96.4 ± 12.2	96.6 ± 14.1	89.7 ± 23.9
Control: humidity sensor	**No**	34	number of farmers with humidity sensor too low		
	Yes	2			
Heating	**No**	22	100.3 ± 11.7	99.3 ± 12.9	87.8 ± 18.5
	Yes	13	103.9 ± 19.8	102.6 ± 16.6	91.1 ± 24.3

Table 12: Univariate associations between farm characteristics and lung function results for poultry farmers. $^{*}p_{U\text{-}test} < 0.05$

Associations between environmental measurements and spirometric results

No significant relationship between environmental measurements and spirometric results could be found. But endotoxin content in total dust had the tendency to be negatively associated with FVC % pred. (data not shown).

Discussion

This study illustrates:

1. high concentrations of dust and endotoxin in randomly selected poultry confinement buildings compared to normal indoor air.
2. elevated levels of bacteria and molds in poultry houses compared to normal indoor air.
3. factors related to operation in the confinement areas for poultry (parameters of ventilation and feeding management) were significantly associated with decrements in lung function
4. airborne concentrations of total bacteria and endotoxin had the tendency to bee negatively associated with lung function in poultry farmers.

Air Contaminants

The given farming characteristics reflect the wide spectrum of animal confinement buildings resulting in different exposure conditions inside these buildings. Due to the random sampling procedure it could be assumed that these farms represent a typical range of farming characteristics and exposure conditions in Switzerland.

Dust

A former study (Danuser et al. 1988) found in Swiss poultry farmers levels of total dust averaged 5.0 mg/m^3. They were not using personal sampling. Measurements were taken 1.6 meters above the ground inside the animal houses, during 5 minues 6 times a day. Ammonia levels averaged in this study about 13 ppm with peak values of about 50 ppm. This findings do not differ much with our results. Hauser 1990 found in 16 Swiss poultry houses lower levels of total dust (1.20 – 12.1 mg/m^3) using the photometric method. The range of total dust in some studies are shown in Table 13.

Author	total dust (mg/m^3)
Gebhart (1973)	1.94 - 2.18
Grüter (1975)	1.0 - 4.15
Nauke et al. (1981)	2.18 - 5.27
Clark et al. (1983)	1.13 - 3.68
Grüter (1975)	1.7 - 44.3
Nauke et al. (1981)	2.18 - 5.27
Clark et al. (1983)	1.13 - 3.68
Danuser (1988)	average: 5.0
Hagmar (1990)	average: 6.3
Schunk (1990)	6 - 35
Thelin (1984)	5 - 23
Danuser (1995)	average: 2.4
Takai (1998)	4 – 10
Takai (1998)	1 – 4.8
Jones (1984)	7.6 - 11
Oelenchock (1982)	11 - 24
Hartung (1983)	0.14 - 0.41
Jellen (1984)	0.86 - 1.86
Hauser (1985)	0.81 - 1.41
Hauser (1990)	1.20 - 12.1
This study	**0.42 – 21.8**

Table 13: Total dust concentrations in different studies adapted after Jellen 1984, Hauser et al. 1985 and Danuser 2000.

Comparing the total dust concentrations inside animal confinement houses to other published data did not differ much (Clark et al. 1983; Attwood et al. 1987; Donham et al. 1989; Preller et al. 1995; Takai et al. 1998; Donham et al. 1999). Specific limits of 2.4 mg/m^3 for total dust in livestock buildings were suggested by Donham et al. 1999. These limits were exceeded in 80 % of the animal houses under study.

Endotoxin

Our results on the endotoxin concentrations in animal confinement units show good agreement with some recent studies (Clark et al. 1983; Attwood et al. 1987; Donham et al. 1989; Seedorf et al. 1998; Vogelzang et al. 1998). Not all of these studies were done on a personal base but (Donham et al. 1995) found that personal sampling was more strongly related to pulmonary function than area sampling. The major contributors to endotoxin-contaminated organic dusts are animal feces and bacteria-contaminated plant materials like grain or cotton. There are various suggestions for an exposure standard ranging from 5 to 200 ng/m^3. The National Health Council of the Netherlands has recently proposed an exposure limit of 4.5 ng/m^3. If this limit represents the range of possible respiratory health hazards all of the randomly selected farming environments in this study exceeded this threshold.

Author	**endotoxin (ng/m^3)**
Clark (1983)	310
Thelin (1984)	310 - 1090
Seedorf (1998)	860
Seedorf (1998)	780
This study	**19 – 1635**

Table 14: Endotoxin concentrations in different studies adapted after Danuser 2000.

Bacteria and molds

Whereas the viability of moulds and bacteria is probably of less importance in the work environment it cannot be ruled out that viable microorganisms may induce a stronger response if, after deposition in the lung, they produce antigens that are not present in dead microorganisms (Eduard et al. 1990). Methods detecting viable microorganisms have the largest potential for the identification of bacterial species. Therefore it seems successful to determine viable and total amount of microorganisms at workplaces on a personal base.

It is well known that bacteria and fungi play a major role for the development of extrinsic allergic alveolitis (Malmberg 1990; do Pico 1992) and some fungi for occupational asthma in farmers. As in our study, Seedorf et al. 1998 found the high levels of fungi and bacteria in poultry houses. The levels of microorganisms found in the study of Seedorf et al. 1998 were lower than in our survey but these were collected on an area basis. Based on respiratory symptoms Donham et al. 1989 recommended exposure thresholds in swine buildings of log 5.8 CFU per m^3 for bacteria and log 4.1 CFU per m^3 for moulds. These recommended levels for microorganisms were clearly exceeded at all sampling sites of the study presented here.

The species of fungi found inside the farming buildings characterize the climatic conditions in these buildings. *Aspergillus* spp. and *Eurotium* spp. (part of the *Aspergillus glaucus* group) grow best under climatic environments with high air humidity and high temperature and were thus mostly detected in animal confinement houses. Therefore, our results indicate a high prevalence of microorganisms, which may provoke type I and type III allergies inside randomly selected farming environments (Danuser 2000).

Ammonia and carbon dioxide

Inside the poultry confinement houses the air velocity was low resulting in a lower air exchange rate in these buildings. Therefore, the high ammonia and carbon dioxide concentrations in poultry houses may be related to this finding. Using Spearman's rank correlation It was found a weak but significant negative relationship between air velocity and ammonia concentration inside poultry houses ($r = -0.35$; $p = 0.04$; data not shown).

In conclusion, the personal measurements of dust, endotoxin and microorganisms under standardized conditions in a wide range of farming environments have shown that in randomly chosen farms farmers were exposed to possible hazardous levels of air contaminants like dust, endotoxins, and microorganisms.

Lung function

The two main findings of the investigation in lung function of poultry farmers were:

1. factors related to operation in the confinement buildings for poultry (parameters of ventilation and feeding management) were significantly associated with decrements in lung function, and
2. airborne concentrations of total bacteria and endotoxin had the tendency to be negatively associated with lung function in poultry farmers.

Using questionnaires on farm characteristics seems useful because they remain rather stable for a long duration and therefore reflect exposure during recent years.

The limitation of our study was the low number of poultry farmers. In order to detect farming characteristics resulting in small lung function changes higher numbers of farmers would be needed to get sufficient statistical power. Nevertheless, we have shown parameters of ventilation and feeding related to changes in lung function. The finding of this study that ventilation is an important factor for the development of occupational airway disease in farmers is compatible with data of the previous studies of Bongers et al. 1987, Vogelzang et al. 1996; Vogelzang et al. 1997) as well as a study on cattle farmers (Radon et al. 1999B). In the latter study It was also detected a tendency of a negative influence of heating on respiratory symptoms. The associations between ventilation as well as heating may indicate that higher temperatures all over the year and lower air exchange rates may result in higher concentrations of mites, endotoxin and glucans in animal houses and thus in higher respiratory morbidity among farmers. In the univariate regression model air velocity was shown to have a significant influence on FEV_1 and $MMEF_{25/75}$ % pred.. Because air velocity and measures of exposure were highly negatively correlated, air velocity did not improve the multivariate regression model.

While Vogelzang et al. (Vogelzang et al. 1996; Vogelzang et al. 1997) and Bongers et al. (Bongers et al. 1987) have found lower lung function results associated with automated feeding, in our study automated feeding had the tendency to be associated with better $MMEF_{25/75}$ % pred. Therefore, it may be speculated that farmers feeding manually work closer to the animals than farmers without. Analyzing the association between farm characteristics and prevalence of mild

bronchial hyperresponsiveness, Vogelzang et al. 1997 found that automated dry feeding was associated with the highest prevalence of bronchial hyperresponsiveness. They concluded that the combination of automation with dry feed is associated with adverse health effects.

Contrary to many industrial workers, farmers may have some influence on their own work environment. Farmers with respiratory symptoms might try to invest in technical improvement as dust reducing techniques. They could also split the time of exposure between different people. Or they simply reduce air temperature which decreases the air pollution (lower ammonia and bacteria concentrations). Therefore, farming characteristics on some farms with symptomatic farmers might be as modern or even more modern than on farms with asymptomatic farmers. Thus, it is not surprising that no significant differences in farming characteristics and environmental measurements were seen between asymptomatic and symptomatic farmers. In order to detect such differences, higher numbers of farmers would be needed to get sufficient statistical power.

The possible relationship between endotoxin concentration in total dust and lung function impairment shown in this study has previously been observed by Heederik et al. 1991 in pig farmers. Due to the low number of poultry farmers no significance could be shown. Using an interaction term of bacteria and endotoxin, originating of gram-negative bacteria, this term was shown to have the tendency to influence on FEV_1 and $MMEF_{25/75}$ % pred.. Endotoxin is capable of causing toxic alveolitis which may result in slightly restrictive lung function changes. This may explain why the endotoxin concentration in total dust was negatively related to FVC % pred. and FEV_1 % pred.. No relationship was seen in our study between total dust concentrations and lung function results. Thus, exposure to organic dust cannot be defined only by its level in terms of a gravimetric exposure measurement. The exposure pattern and the constituents of the dust should also be taken into account. There is a special need for a threshold level in respect to endotoxin concentrations and content of bacteria at the workplace.
No significant change in lung function values was seen over the feeding period. This lack of change is probably due to the circadian rhythm of lung function values

with lowest values in the early morning and highest in the afternoon (Wegner et al. 1997). Other studies have shown a significant decline over the feeding period. In poultry farmers, Thelin et al. 1984 found an average decrease in FEV_1 of 0.11 liter studying an exposure period of one working day but one has to bear in mind that the median exposure time in our study was only 30 minutes in poultry houses. In our study it was impossible to obtain longer exposure periods due to the fact that the farmers normally work only for a short time in the morning and in the evening inside the animal houses. Thus, the circadian rhythm of lung function probably covered the expected effect of exposure. Nevertheless, in the subgroup of symptomatic farmers a significant lung function decline over the feeding period would have been expected as it was shown in other surveys (Zuskin et al. 1992; Radon et al. 1999B). Nevertheless, we saw a tendency towards a lower increase in FEV_1 over the feeding period in the symptomatic farmers.

Conclusion

In this study, a possible dose-response relationship between the level of endotoxin as well as bacteria and lung function results was observed indicating the importance of threshold levels for endotoxin and bacteria. Beside this, a high standard of ventilation control inside the animal houses was observed as the best possibility to avoid long-term lung function impairment. Prospective intervention studies are essential to estimate the effects of such measures.[4]

[4] This study was supported by the European Union (BMH1-CT94-1554), Swiss National Science Foundation (NF 3200-045997.95/1), BBW Switzerland (93.0283), Lieselotte und Dr. Karl Otto Winkler-Stiftung für Arbeitsmedizin.

Part C: Nasal inflammatory mediator Response to instilled Lipopolysaccharides in Allergic and Non-Allergic Subjects[5]

Introduction

Low levels of endotoxin have been measured indoors and a relationship between Endotoxin-levels and clinical severity of allergic asthmatics has been shown previously (Michel et al. 1991), due to the low level or absence of exposure this could not be demonstrated for allergic rhinitis (Michel et al. 1996). In poultry farmers who had a common nasal allergy (pollinosis) more work related symptoms were reported when exposed to organic dust (Danuser et al. 1995) compared with non-allergic farmers. The enhanced reaction to endotoxin of allergic persons can be due to an altered baseline inflammatory mucosa response in persons once sensitized, to a hyperreactivity status of the mucosa or due to an altered response when both allergens and endotoxin are present (Hunt et al. 1994). In a dose-response investigation to inhaled Lipopolysaccharides (LPS) in normal subjects an increase in ECP but not in eosinophil count in the induced sputum was found (Michel et al. 1997). In vitro a small amount (<0.1 ng/ml) of LPS is able to enhance human eosinophil survival as well as release of several cytokines (Takanaski et al. 1994). The involvement of ECP in an LPS response could be of great interest because of the suggestion that LPS modifies eosiniphilic inflammation (Michel et al. 1997) and could thereby extend allergic subjects symptoms.

Exposure to endotoxin causes a release of proinflammatory mediators in the lower and upper airways (Clapp et al. 1994; Wang et al. 1997). LPS are considered as the inflammatory compound of endotoxin (Ulmer 1997). In healthy subjects it has been demonstrated, that Interleukin (IL)-6, IL-1β, Tumor Necrosis Factor-α (TNF-α)

[5] C. Weber, B. Danuser. (1999) Inflammatory Response in Nasal Lavage to LPS-Provocation in Normal and Allergic Subjects. Proceedings Indoor Air 99, 4: 1114-1119

and albumin increase in nasal lavage fluid a few hours after exposure to swine dust containing endotoxin (Wang et al. 1997). No data exist of release of nasal inflammatory mediators due to pure LPS.

IL-4 is a factor for grow and differentiation process of B-lymphocytes and can be released by basophiles especially after IgE stimulation (Arock et al. 1993) and is considered as "Atopy associated". Myeloperoxidase (MPO) is a hemoproteine stored in the granulas of neutrophiles. In inflammatory defense situations MPO is released and can therefore be used as a marker of neutrophiles activation (Repine et al. 1994) and it was shown to increase in the induced sputum after an LPS inhalation challenge. Histamine is one of the hallmarks of the allergic reaction, but it is known that histamine can be released also upon a second stimulation of basophiles by chemokines for example IL-8 (Reddigari et al. 1992).

We wanted to know if the baseline inflammatory nasal mucosa response, assessed by IL-1β, IL-6, IL-8, TNF-α, MPO, ECP, albumin, and histamine, to LPS is modified in persons with seasonal rhinitis in the absence of allergens.

Methods

The study population consisted of 26 volunteers, 13 females and 13 males from age 19 to 42. Three of them were excluded; one due to smoking, current rhinitis (one) and taking medication (one). The study was undertaken during the pollen free season. All subjects gave written informed consent, and the local Ethic Committee approved the study.

Study design

The subjects arrived at 8.00 am, in the morning, filled in the basic questionnaire thus allowing to get acclimatized. At 8.30 the nose was inspected and cleaned two times with isotonic NaCl, then a baseline nasal lavage sample was taken followed by spirometry. A blood sample for IgE determination was taken. The procedure for nasal lavage previously described by Bascom (Bascom et al. 1988) was used. At 9.00 the nasal instillation of 20 μg LPS dissolved in 10 ml NaCl, 5 ml in each nostril was performed for 10 seconds. The instillation and the normal lavage sampling time were identical. After 20 minutes, 1 hour, 6 hours and 23 hours

lavage samples were taken followed by spirometry and assessment of symptoms by the symptom questionnaire. The sessions were performed in a climatized room at 22° C and 40% relative humidity. Between the nasal lavages the subjects stayed in the same or in a neighbor building and they have been informed to keep away from known sources of nasal irritants. During the night they stayed at their own home.

Questionnaires

A questionnaire was used to evaluate medication, any exposure to endotoxin rich environments in the past 24 hours, nasal allergy symptoms, and smoking habits. After each nasal lavage a symptom questionnaire was used to determine acute symptoms of nose irritation, eye irritation, airway irritation and general symptoms on a 10 grade scale with 1 meaning for example clearly unblocked nose or no runny nose to 10 = totally blocked or runny nose.

Pulmonary function testing

After each lavage the pulmonary function was measured (MultiSPIRO-PC, Biotrine Corporation, Woburn, MA, USA). These maneuvers were performed using standard protocols and the American Thoracic Society guidelines.

Nasal lavage

The procedure for nasal lavage previously described by Bascom (Bascom et al. 1988) was used. During the lavage, the subject was seated with the neck extended to an angle of approximately 45° and with the soft palate closed. Five ml of 0.9% NaCl was instilled into each nostril, using a syringe without needle. After 10 s (no breathing possible), the subject flexed the neck forward and expelled the liquid into a plastic basin, which was placed on ice during processing. The volume of the combined lavage portions was measured and centrifuged at 300 xg for 10 min at +4°C. The supernatant was aliquoted and kept frozen at -80°C until the analysis.

LPS solution

LPS form *E.coli* serotype 026:B6 (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) was used. For preparation of the LPS solution 2 mg LPS was added to 1000 ml 0.9% NaCl (Braun AG, Emmenbrücken CH) resulting in LPS concentration

of 2 μg per ml NaCl and stored at +4°C. The LPS solution was allowed to warm up to room temperature for 30 min.

Analysis of nasal lavage fluid

The concentrations of IL-1β, IL-4, Il-6, IL-8 and TNF-α in lavage fluids were measured in duplicate by enzyme-linked immunosorbent assay (ELISA). A Quantikine TM high sensitivity, two-site (sandwich) ELISA kit (R&D Systems, Minneapolis, USA) was used. The Quantikine HS immunoassay uses an enzyme amplification system with alkaline phosphatase. The lower limit of the assay was for IL-1β = 0,125 pg/ml, IL-4 = 0,25 pg/ml, IL-6 = 0.156 pg/ml, IL-8 = 31.2 pg/ml, TNF-α = 0.5 pg/ml. Histamine was measured by a ELISA kit (Immunotech, Marseille, France) with a lower detection limit of 0,5 nM/ml. Absorbance was read at 450 nm using a Microplate Reader (BioRad, California, USA).

ECP and MPO were measured by RIA (Radio Immuno Assay) (R&D Systems, Minneapolis, USA), detection limits MPO = 1,6 ng/ml, ECP = 2 μg/ml.

Albumin was measured with a BCA Protein Assay Reagent (Pierce, Rockford, Illinois, USA) detection limit = 31,3 μg/ml. All analysis were made by the laboratory of the ENT unit University Hospital, Düsseldorf, Germany.

IgE Determination

The IgE determination was made in a approved medical laboratory (Dr. Violier AG, Zurich, CH) using the Phadiatop FEIA CAP system (Pharmacia & Upjohn, Sweden) according to manufacturers instruction.

Statistics

Results are presented as box plots in figures and as means and standard deviation (SD) in tables. The effect of LPS, allergy and sex was analysed with analysis of variance with repeated design by StatView 4.5, Abacus. For further analysis differences between measurement points were estimated with Wilcoxon sign-rank test. A p-value less than 0.05 was considered significant.

Results

Subjects

Average age of all subjects was 26.6 years (median 26), with a standard deviation (SD) of 6.8 years. Average age of female subjects was 25.4 years (SD 8.2) and for male subjects 24.2 years (SD 2.8). According to a positive Phadiatop SX1 (>class 1 or >0.35 kU/l) and allergic history the subjects were grouped in allergics and non-allergics. 11 subjects were non-allergic (7 female, 4 male) and 12 allergic (5 female, 7 male). Average age for allergic subjects was 23.3 years (SD=3.0 years, median = 24.5 years) and for non-allergic subjects 28.7 (SD=8.1 years, median = 28.0 years).

Symptoms

The non-allergic subjects indicated in the mean higher symptom scores prior to the LPS provocation. An increase in the 10 graded score >2 was considered as significant increase. Table 15 lists the number and percentage of individuals grouped by allergy, which experienced nose, eye, throat or airway symptoms. No general symptoms were ever indicated. Most of the symptoms were indicated 6 hours after the LPS provocation. 41.6% of the allergic individuals experienced nose symptoms compared to 18% of the non-allergics, and 33% of the allergics experienced eye symptoms compared with 18% of the non-allergic.

Nose		Eye		Airways		Throat	
Allergic	Non-allergic	Allergic	Non-allergic	Allergic	Non-allergic	Allergic	Non-allergic
5	2	4	2	2	1	1	2
41.6%	18%	33%	18%	16.6%	9.8%	8.3%	18%

Table 15: Reported increase in Symptoms: number and percentage of individuals which experienced an increase in symptoms

Lung function

The FVC and FEV_1 values of allergic subjects (FVC 101.1 (8.1) FEV_1 103.3 (4.7)) are not different from the non-allergic subjects (FVC 103.2 (6.4) FEV_1 104.5 (8.0)) expressed in percentage of predicted values (Knudson). No effect has been found of the nasal LPS instillation on the lung function values measured at the different time points (Table 16).

Time	Prechallenge		20 minutes		1 hour		6 hour		23 hours	
	FVC	FEV1	FVC	FEV1	FVC	FEV1	FVC	FEV1	FVC	FEV1
	% pred.		% pred		% pred		% pred		% pred	
Allergic	101.1	103.3	100.9	103.8	100.4	102.9	100.0	102.5	99.6	103.2
	(8.14)	(4.75)	(8.18)	(6.21)	(7.85)	(7.75)	(8.03)	(7.96)	(7.04)	(7.27)
Non-allergic	103.2	104.5	102.6	105.4	104.5	105.0	104.3	105.8	104.5	105.4
	(6.39)	(8.01)	(5.67)	(9.04)	(7.47)	(8.82)	(8.27)	(9.1)	(7.04)	(8.64)

Table 16: Lung function measurements: Mean and SD of spirometric lung function values in percent of predicted (Knudson) of allergic and non-allergic subjects at the different measurement points.

Nasal lavage analysis

Of the 10 ml instilled liquid the mean expelled liquid for all subjects and all lavage samples was 7.46 ml (SD 0.58). No differences in expelled nasal liquid at the different measurement points was found. The concentrations of TNF-α and IL-4 were in all subjects anytime below detection limit.

Figure 1 presents box plots of the determined values of IL-6, IL-1β, histamine, albumin, MPO, and IL-8 for all subjects and grouped by allergy at the different measurement points. The upper or lower detection limits are indicated. 3 subjects (3 males, 2 allergic, 1 non-allergic) showed histamine levels anytime over the upper detection limit and those values were excluded for further analysis. In most of the samples ECP values were below the lower detection limits of 2 μg/ml. Before LPS only in 8 (5 allergic) samples ECP could be determined. 20 minutes after LPS in 5 (3 allergic), 1 hour after in 9 (5 allergic), 6 hours after LPS in 12 (8 allergic) and 23 hours after LPS in 12 (7 allergic) samples. Individual values for the allergic group is shown in Figure 2.

Table 17 presents means and SD for IL-6, histamine, albumin, IL-8, and MPO. No effect of the LPS provocation in the analysis of variance with repeated design could be found for MPO, and IL-8. A significant effect was found for albumin ($p<0.001$), histamine ($p<0.001$), IL-1β ($p<0.01$), IL-6 ($p<0.001$). The mean maximum increase for all subjects was for albumin 2 fold, for histamine 3.8 fold, for IL-1β 1.6 fold and for IL-6 2.4 fold. In IL-6, histamine and albumin the maximum increase was found 6 hours after provocation. The maximum increase in IL-1β was found 23 hours after the LPS provocation. No effect of allergy could be shown in the analysis of variance. The time-pattern of IL-6 concentrations shows a gender effect. Female subjects demonstrated an earlier response to the LPS provocation than males (Figure 3).

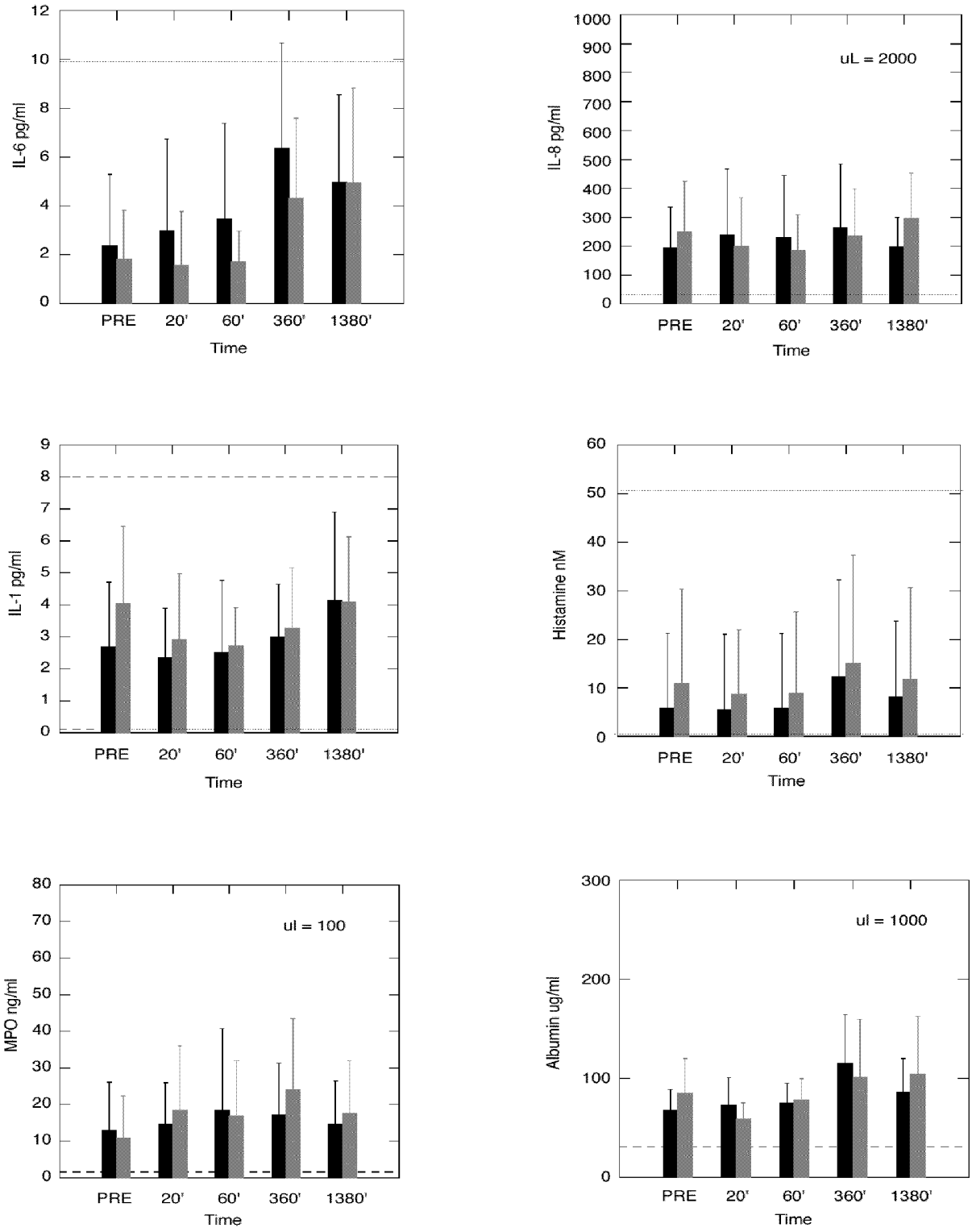

Figure 1: Means and SD, grouped by Allergy for IL-6, IL-8, IL1-b, Histamine, MPO and Albumin. Black bars represent values on non-allergic, gray bars of allergic subjects. Dashed lines = upper or lower detection limit. μl = upper detection limit.

Time	Pre			20'			1 h			6 h			23 h			RANOVA
Sub-jects	all	NA	A	all	NA	A	all	NA	A	all	NA	A	all	NA	A	p-value
								IL-6								
mean	2.09	2.38	1.82	2.25	2.98	1.57	2.56	3.47	1.73	5.30	6.37	4.4	4.95	4.95	4.94	<0.001
SD	2.32	2.76	1.91	2.91	3.57	2.1	2.79	3.72	1.18	3.69	4.1	3.1	3.49	3.43	3.69	<0.05*
								IL-1β								
mean	3.40	2.69	4.06	2.65	2.36	2.92	2.63	2.52	2.73	3.15	3.00	3.28	4.11	4.14	4.10	<0.01
SD	2.19	1.91	2.30	1.72	1.45	1.96	1.65	2.13	1.14	1.65	3.00	1.79	2.24	2.62	1.93	
								Histamine								
mean	2.27	1.33	3.12	2.37	1.09	3.53	1.93	1.24	2.55	8.75	8.91	8.59	4.34	4.30	4.35	<0.001
SD	2.58	1.28	3.18	3.08	0.76	3.92	1.45	0.80	1.64	13.6	14.2	13.6	3.89	5.04	2.69	
								Albumin								
mean	76.4	67.4	84.6	65.3	73.1	58.2	76.7	74.8	78.4	123	115	130	95.4	86.2	104	<0.001
SD	28.8	20.4	33.7	22.4	26.1	16.3	19.5	19.4	20.2	85.9	46.8	113	45.8	31.9	55.7	
								IL-8								
mean	257	195	314	219	240	200	209	232	187	249	264	235	327	361	297	ns
SD	220	134	270	186	217	160	162	204	117	180	209	156	384	546	149	
								MPO								
mean	11.8	12.8	10.8	16.6	14.6	18.4	17.4	18.3	17.0	20.8	17.1	24.0	16.2	14.6	17.6	ns
SD	11.5	12.5	10.9	14.0	10.8	16.8	17.5	21.2	14.3	16.3	13.4	18.5	12.3	11.2	13.6	

*Table 17: Means and SD and RANOVA for IL-6, IL-1b, Histamine, Albumin, IL-8, and MPO: Means and SD of the measured mediators at the different measurement points. (*indicates a time-gender interaction), NA= no-allergic, A= allergic.*

For further analysis the differences between the measurements points in MPO, IL-8 and ECP was tested with Wilcoxon sign-rank test. Compared with the pre-challenge values the MPO values 1 and 6 hours after LPS challenge for the allergic group was increased (one sided also at 20' and 23 hours). For IL-8 the values at 20' and 1 hour after challenge decreased compared with pre-challenge values and at 23 hours the values increased compared with 20' after challenge in the allergic group only (indicated in

Figure 1). The ECP values of the allergic group 20' after challenge were lower (one-sided) than pre-challenge values, and increased afterwards compared with the 20' values (indicated in Figure 2). There are insufficent data in the non-allergic group for testing.

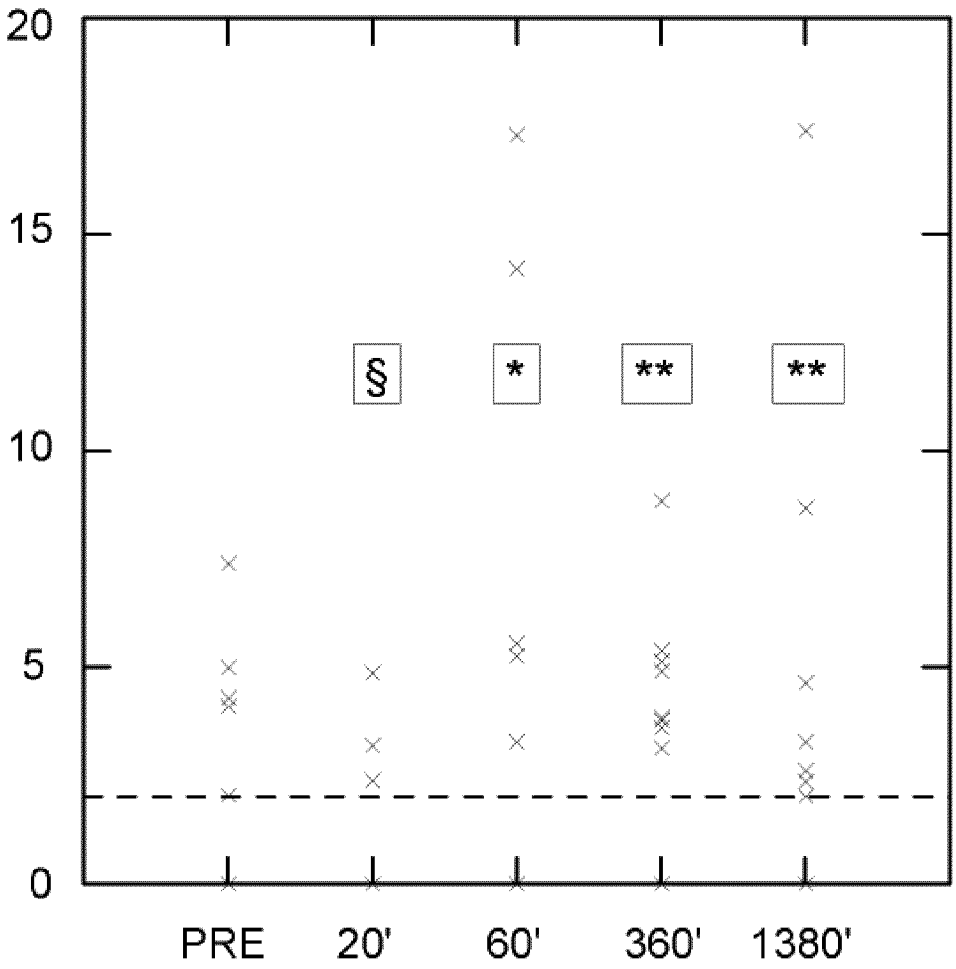

*Figure 2: ECP values measured at the different time points of allergic subjects. § = one-sided different form pre-challenge values, * = one-sided different from 20 min values, ** = two-sided different from 20 min values.*

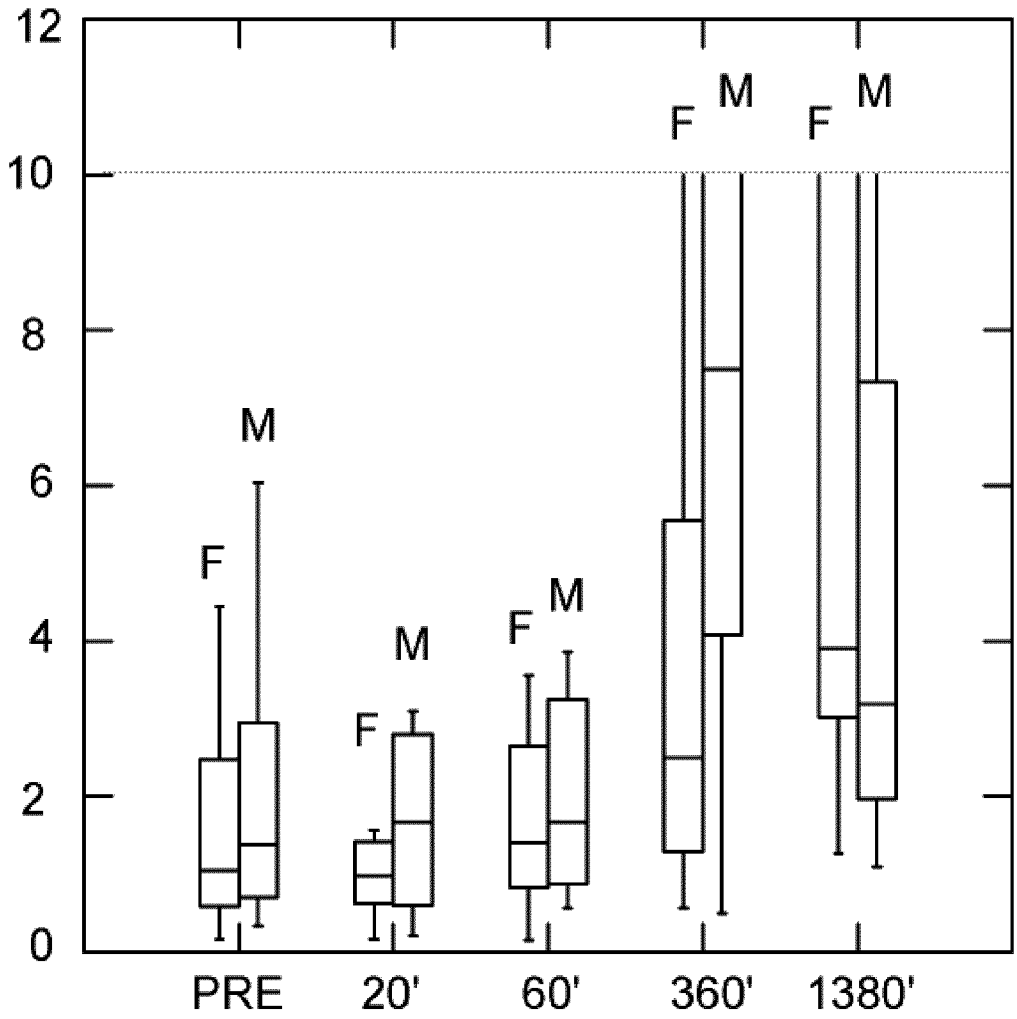

Figure 3: IL-6 values grouped by sex. "F" represent values of females, "M" represent values of males .

Discussion

The study demonstrates an increase in IL-6, IL-1β, histamine, and albumin in the nose after an LPS instillation of 20 μg for 10 seconds. The allergic subjects experienced more symptoms after the LPS provocation than the non-allergic but the inflammatory response of both groups shows the same magnitude and pattern except for minor findings concerning IL-8 and ECP. All of the allergic participants had a seasonal rhinitis and their symptoms are limited to the pollen season. The study took place in January and no pollen flight was recorded during the study period. The absence of measurable IL-4 confirms that during testing time no allergens to which the subjects are sensitized have been present.

This is the first study, which shows a histamine response in the nose to an LPS challenge. No histamine could be measured in BAL following an exposure to corn dust extract containing endotoxin resulting in an inhaled dose between 30-60 μg endotoxin, although the same assay method was used (Hunt et al. 1994). The not measurable histamine levels in the lower airways upon a LPS challenge in a comparable dose range implies that the LPS caused inflammatory response of the nose is different from the lower airways.

The observed histamine increase 6 hours after LPS provocation is of the same magnitude as after a low level of allergen challenge in allergic subjects (Jacobi et al. 1998). The study was designed to detect differences of the response of allergic and non-allergic subjects and no control challenge was made. Therefore it has to be considered if repeated nasal lavage could be the cause of the histamine increase. Repeated nasal lavages with physiological saline do not cause an increase in histamine levels (Krayenbuhl et al. 1989; Reddigari et al. 1992), whereas challenges with hyperosmolar saline does induce a histamine response in allergic subjects (Krayenbuhl et al. 1989). The increase in histamine here is observed in both study groups and physiological saline was used, and we are not aware of another systematic histamine stimulus so we conclude that the histamine increase is caused by the LPS challenge. After allergen or methacholine challenge the histamine increases immediately (Davies et al. 1987; Jacobi et al. 1998). We

can not exactly determine the time point when histamine started to increase, because no samples in-between 1-6 hours after LPS challenge were taken, but after 20' and 1 hour the histamine concentrations clearly stayed on prechallenge levels. Histamine is used as a mast cell/basophil activation marker. A pool of histamine in the unaffected human nose which can be transferred to lavage fluid during glandular hypersecretion induced by some exogen stimuli like methacholine is suggested (Jacobi et al. 1998). We can not define the origin of the measured histamine in this study. To carry along tryptase determinations would give reference to mast cell activation and should be taken in consideration in further studies.

Prechallenge levels of histamine are high and varying (Naclerio et al. 1983), our protocol included 2 washout lavages in order to reduce the level of mediators before prechallenge sampling. Still three subjects showed histamine values, which were over the upper detection limit of 50 nM. The values of those subjects remained as high over the whole sampling period, indicating that more washouts as proposed by Naclerio and coworkers (Naclerio et al. 1983) would not have lowered them.

The increase in histamine may in part explain why nasal allergics do report more symptoms after exposure to dust containing endotoxin. Application of histamine to the nasal mucosa can induce symptoms such as allergic rhinits in humans and animals (Okuda et al. 1983). Especially when the mucosa is activated additional histamine can evoke symptoms (Raphael et al. 1989).

The increase in IL-6, Il-1β and albumin is in agreement with the results of Wang and co-workers (Wang et al. 1997). Additionally they found a TNF-α response 7 hours after endotoxin (1.2 $\mu g/m^3$) containing swine dust exposure in the nasal lavage. Neither before nor after LPS challenges could we measure TNF-α. IL-6 was long time considered exclusively as proinflammatory mediator but newly also anti-inflammatory properties have been described (Tilg et al. 1994). Il-6 can prevent the synthesis of IL-1β and TNF-α in macrophages and induces their antagonists.

The playing together of pro- and anti-inflammatory mediators especially in relation to different LPS doses remains to be investigated.

MPO is released from PMN's granules and is involved in the production of oxygen radical's (Repine et al. 1994). MPO was analysed as a marker for activated PMN's. Michel (Michel et al. 1997) found an increase in MPO in sputum after inhalation of 5 μg LPS (*e. coli*), and more pronounced after 50 μg LPS. It is well known that IL-8 activates PMN's (Baggiolini et al. 1992). We were unable to demonstrate neither an IL-8 nor a MPO response in the main analysis. In the allergic group the IL-8 values decreased first significantly and increased later indicating that in this group a IL-8 response took place. Additionally the allergic showed an increase in MPO. But the IL-8 and MPO response of the allergic group was not strong enough to induce a significant interaction in the main analysis. Therefore we consider this results as observations which have to be investigated in more details.

Although the number of lavage samples in which ECP could be detected increased 6 hours after 20 μg LPS challenge (Figure 2), no clear effect over all subjects could be shown in this study. In the allergic group a decrease followed by an increase of ECP values is seen. In a previous study the increase in ECP got only significant after 50 μg inhaled LPS in the induced sputum in normal subjects (Wang et al. 1997) suggesting that higher LPS doses are necessary to induce an ECP response in non-allergic subjects. Our results indicate that allergic subjects have an ECP response to lower LPS doses than non-allergic subjects. Because of the decrease shortly after LPS challenge it is not likely that the seen increase is due to an enhanced eosinophil survival as observed in a *in vitro* study (Takanaski et al. 1994). We suggest that LPS activates inflammatory pathways which are tracked by allergy which in turn would predispose modification of an allergic reaction by LPS (Clapp et al. 1994; Wang et al. 1997).

We conclude that a nasal instillation of 20 μg LPS does produce an increase in IL-6, IL-1β, histamine, and albumin. The response pattern of allergic and non-allergic subjects in the determined proinflammatory mediators is similar although the allergics experienced more symptoms and show indications of an additional IL-8, MPO and ECP response. The release of histamine can partly explain the increase in symptoms of nasal allergics in endotoxin containing environments especially when hyperreactivity is already present.[6]

[6] This study was founded by the Swiss National Science Foundation NF 3200-45997. We would like to thank Prof. C. Bachert (formerly head of the ENT department, University Hospital Düsseldorf, Germany, now University of Gent, Belgium) for his advice in the study design.

General discussion

Farming is associated with exposure to a variety of biohazards, the most relevant being dust, bacteria, endotoxin, mites, fungi, methane, hydrogen sulfide and ammonia. Inhalation injury may result in airways inflammatory reactions (bronchitis, asthma and bronchiolitis) or in parenchyma reactions (alveolitis or pulmonary edema). Epidemiological studies indicate a greater risk of respiratory disorders in farmers than in non-farming occupations. In Switzerland and California, the standardized mortality ratio (SMR) for chronic bronchitis and asthma of farmers is significantly elevated as compared with the general population (Carlson et al. 1978; Minder 1993; Gassner et al. 1995). Therefore, we have to consider chronic bronchitis and asthma as occupational diseases in farmers.

The farmer's hazardous exposures to biohazards are reviewed in detail in (do Pico 1992). Agricultural dusts are a complex mixture of materials derived from cereal grain and vegetation, containing natural contaminants such as insect parts, mites, animal hair, fungi, bacteria and fungal toxins (Farant 1989; Donham et al. 1993; Rylander 1993).

Organic dust levels varies with type of farming, source of product, state of decomposition, temperature, humidity and individual handling functions (do Pico 1992). It is known that the air quality in animal houses is also very depending on keeping systems, how the animals are kept and the season when measurements were taken even between the same types of keeping systems we often find significant differences in air quality (Danuser et al. 1988; Hauser et al. 1988; Takai et al. 1998). Additionally (Takai et al. 1998) found significant difference between European countries. Also the endotoxin levels vary widely from 0.01 to 100 µg/m3 (Lundholm et al. 1986). In addition to the different exposure conditions also the type of exposure measurements varies. Therefore exposure measurements are hardly comparable.

If you are taking the MAK-values (MAK=maximum workplace value) of ammonium (Switzerland: 25 ppm, Germany: 20 ppm) for comparison then they are often overridden. In opposite, breathable dust is in MAK-limits (Switzerland: 6 mg/m^3;

Germany: 4 mg/m^3 total dust, 1.5 mg/m^3 breathable dust). In Switzerland there is no MAK-value for organic dust and endotoxin. In Germany and Denmark there is a surveillance value for endotoxin of 0.1-0.2 µg/m^3. This value is exceeded in all studies. Bacteria and fungi concentrations in animal houses are also high. Bacteria values of 5'000-10'000 CFU/m^3 are considered as acceptable. All results are fare above this range. At this time there is no MAK-value for fungi. So the air quality in respect of bacteria, fungi and endotoxin in animal houses must be considered as elevated.

Some predisposing individual conditions are known for certain diseases and exposure settings. Smoking has an interactive effect with organic dust on the prevalence of symptoms and pulmonary function changes (Dopico et al. 1984; Iversen et al. 1990). Smokers report more symptoms than non-smokers. Farmers tend to smoke less than the general population (part A, (Dalphin et al. 1989). Smoking was a major determinant of work-related symptoms in farmers (part A). Retired farmers over the age of 65 suffered 5.5 times more often from asthmatic diseases as a control group of farmers who were not yet retired (Yesalis et al. 1985). Former pig farmers report asthma 4 times more often than people still active in pig farming (Wilhelmsson et al. 1989). This findings suggest a strong selection process or a healthy worker effect and a delayed onset of the disease.

It has been shown that agricultural work in Switzerland bears an elevated risk for reporting respiratory symptoms, especially pronounced in poultry farmers. The comparison of Swiss farmers with the Swiss population has shown a 2-fold elevated risk of reporting chronic bronchitis and a 4.5 fold elevated risk for bringing up phlegm regularly. Particularly poultry farming increased the risk for reporting nasal irritation at work more than 5 fold. Poultry farmers showed in the most of the assessed symptoms the highest estimates. Over 4 hours spent per day in animal confinement buildings more than doubles the risk for reporting chronic bronchitis independent of the type of farming. The high leves of dust, endotoxin, bacteria and molds in poultry confinement buildings are the most important parameters for developing respiratory symptoms. So it was shown that airborne concentrations of total bacteria and endotoxin were negativeley associated with lung function in

poultry farmers. Endotoxin content in total dust was shown to be a predictor for FVC and FEV_1 % pred. while $MMEF_{25/75}$ % pred. was more strongly related to the concentration of total bacteria. The total dust concentrations were found in investigated poultry houses with median concentrations of 7.01 mg/m^3 and the median airborne endotoxin concentrations in total dust was 258 ng/m^3.

Exposure to endotoxin causes a release of proinflammatory mediators in the lower and upper airways. LPS are considered as the inflammatory compound of endotoxin. In healthy subjects it has been demonstrated, that Interleukin (IL)-6, IL-1β, Tumor Necrosis Factor-α (TNF-α) and albumin increase in nasal lavage fluid a few hours after exposure to swine dust containing endotoxin. The nasal instillation of 20 μg LPS does produce an increase in IL-6, IL-1β, histamine, and albumin. A significant effect was found for albumin, histamine, IL-1β, and IL-6. Maximum increase for albumin: 2, for histamine: 3.8, for IL-1β: 1.6, and for IL-6: 2,4 fold. No effect of allergy was found. The response pattern of allergic and non-allergic subjects in the determined proinflammatory and inflammatory mediators is similar although the allergics experienced more symptoms and show indications of an IL-8, MPO, and ECP response. The induced release of histamine could be a clue to explain the increased symptoms of allergics in endotoxin containing environments.

The cytokine levels of IL-8 found in the nasal lavage fluid in 8 (Wüthrich 1998) and 16 poultry farmers (Weber 1999, not published) are in the same range as the values of patients with viral rhinitis (Roseler et al. 1995) or allergic patients (Bachert et al. 1995) (Figure 4). It must be assumed that high levels of IL-8 indicate that a nasal inflammation is taking place in farmer's noses. This could be due to high levels of endotoxin in the farming environment.

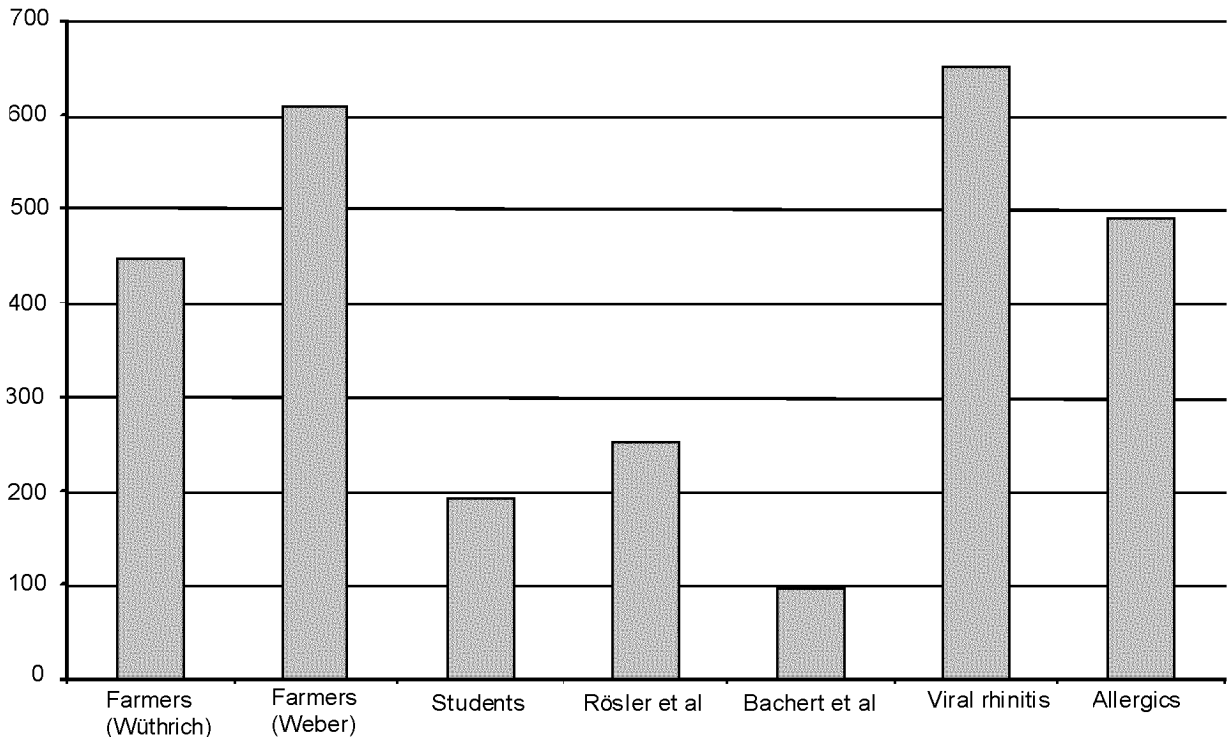

Figure 4: Measured IL-8 values (ng/mg) in nasal lavage fluid of poultry farmers (Wüthrich 1998), Weber (not published), compared with students (Wüthrich 1998), (Roseler et al. 1995), (Bachert et al. 1995), an patient with viral rhinitis (Roseler et al. 1995) and allergics (Bachert et al. 1995).

The results indicate that there is a special need for reducing the air pollution in animal houses. Inventig threshold levels in respect to endotoxin and bacteria concentrations at the workplace could be one brick in the puzzle. Beside that, prospective intervention studies using special ventilation control should be carried out.

Future studies should focus on endotoxin and bacteria levels in animal houses. The relationship between endotoxin and respiratory symptoms must be further investigated.

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Appendix

Appendix A	Questionnaire Swiss farmers
Appendix B 1	Self reported Symptoms in Swiss farmers and SAPALDIA
Appendix B 2	Self reported Symptoms in Swiss farmers and SAPALDIA divided into age categories (never smoker only)
Appendix C	Questionnaire poultry farmers
Appendix D	Steps farmer measurements
Appendix E	Summary equations for lung volumes and ventilatory flows for adults aged 18 - 70 years
Appendix F	Protocoll endotoxin provocation
Appendix G	Questionnaire syptoms endotoxin provocation
Appendix H	Abbreviations

Appendix A: Questionnaire Swiss farmers

Fragebogen

		Nein	Ja
1	Haben Sie in den letzten 12 Monaten irgendwann ein pfeifendes Atemgeräusch in der Brust gehabt? („Pfeifendes Atemgeräusch“ bedeutet ein mattes Keuchen. Es kann hoch oder tief klingen).	☐	☐
2	Sind Sie in den letzten 12 Monaten jemals aufgewacht, weil Sie plötzlich Atemnot gehabt haben?	☐	☐
3	Haben Sie in den letzten 12 Monaten einen Asthma-Anfall gehabt?	☐	☐
4	Haben Sie allergischen Schnupfen, zum Beispiel „Heuschnupfen“?	☐	☐
5	Haben Sie normalerweise im Winter tagsüber oder nachts Auswurf?	☐	☐
5.1	Haben sie solchen Auswurf fast täglich für mindestens 3 Monate pro Jahr?	☐	☐

6 Haben Sie während der Arbeit eine oder mehrere der folgenden Beschwerden:

			Nein	Ja
6.1	Atemlosigkeit	6.1	☐	☐
6.2	Husten ohne Auswurf?	6.2	☐	☐
6.3	Husten mit Auswurf?	6.3	☐	☐
6.4	pfeifendes Geräusch?	6.4	☐	☐
6.5	gereizte Nase?	6.5	☐	☐

		Nein	Ja
7	Hatten Sie irgendeinmal zwei bis sechs Stunden nachdem Sie Staub ausgesetzt waren, eine plötzlich auftretende grippeähnliche Krankheit mit 2 oder mehreren der folgenden Symptome: Fieber, Schüttelfrost, Muskelschmerzen, Schwächeanfälle, Kopfweh, Husten, Engegefühl in der Brust, Atemnot?	☐	☐
8	Ist die Landwirtschaft Ihr Hauptberuf?	☐	☐
9	Arbeiten Sie		
		Nein	Ja
	9.1 in der Viehwirtschaft?	☐	☐
	9.2 im Pflanzenbau?	☐	☐
10	Arbeiten Sie regelmässig mit folgenden Tieren: („Ja“ heisst mit mehr als 10 Tieren)	Nein ☐	Ja ☐
	10.1 Schweinen? 10.1	☐	☐
	10.2 Milchvieh? 10.2	☐	☐
	10.3 Mastvieh? 10.3	☐	☐
	10.4 Kälbern? 10.4	☐	☐
	10.5 Schafen? 10.5	☐	☐
	10.6 Geflügel? 10.6	☐	☐
	10.7 Kaninchen? 10.7	☐	☐
	10.8 anderen Tieren? 10.8	☐	☐
11	Arbeiten Sie mit Tieren in geschlossenen Ställen? Falls „ja“: Wieviele <u>Stunden</u> pro Tag mit:	Nein ☐	Ja ☐
	11.1 Schweinen? Stunden/Tag	☐	
	11.2 Vieh? Stunden/Tag	☐	
	11.3 Geflügel? Stunden/Tag	☐	
	11.4 Anderen Tieren? Stunden/Tag	☐	

12 Welche Pflanzen bauen Sie regelmässig an:

			Nein	Ja
12.1	Getreide?	12.1	☐	☐
12.2	Gemüse?	12.2	☐	☐
12.3	Wurzelgewächse (inkl. Kartoffeln)?	12.3	☐	☐
12.4	Oelpflanzen?	12.4	☐	☐
12.5	Tabak?	12.5	☐	☐
12.6	Hopfen?	12.6	☐	☐
12.7	Früchte?	12.7	☐	☐
12.8	Tomaten?	12.8	☐	☐
12.9	Pilze?	12.9	☐	☐
12.10	Blumen?	12.10	☐	☐
12.11	Nüsse (inkl. Mandeln)?	12.11	☐	☐
12.12	andere Pflanzen?	12.12	☐	☐
12.13	lokale Spezialitäten?	12.13	☐	☐

13 Arbeiten Sie, Pflanzenbau betreibend in Treibhäusern? Falls „ja“: Wieviele Stunden pro Tag mit

Nein ☐ Ja ☐

13.1	Gemüse?	Stunden/Tag	☐
13.2	Früchten?	Stunden/Tag	☐
13.3	Tomaten?	Stunden/Tag	☐
13.4	Pilzen?	Stunden/Tag	☐
13.5	Blumen?	Stunden/Tag	☐

14 Haben Sie schon einmal mindestens ein Jahr lang geraucht? („Ja“ heisst: mindestens 20 Zigarettenpackungen oder 360g Tabak im ganzen Leben. ODER: mindestens eine Zigarette pro Tag, oder eine Zigarre pro Woche für ein Jahr).

Nein ☐ Ja ☐

Falls „ja“:

		Nein	Ja
14.1	Rauchen Sie zur Zeit (im letzten Monat)?	☐	☐

15 Wann ist Ihr Geburtsdatum?

Tag ☐ Monat ☐ Jahr ☐

16 Sind sie ein Mann oder eine Frau?

Mann ☐ Frau ☐

Appendix B 1: Self reported Symptoms in Swiss farmers and SAPALDIA

Symptoms	Age 21-60			Age 21-40			Age 41-50			Age 51-60		
	Prevalence	*n*	*OR*	*Prevalence*		*OR*	*Prevalence*	*n*	*OR*	*Prevalence*	*n*	*OR*
wheeze 12 mo	S: 15.8% F: 14.7%	S: 4202 F: 748	0.92 (0.74-1.15)	S: 15.1% F: 13.4%	S: 1922 F: 277	0.86 (0.60-1.25)	S: 15.3% F: 15.7%	S: 1287 F: 254	1.03 (0.71-1.50)	S: 17.6% F: 15.2%	S: 993 F: 217	0.84 (0.56-1.26)
woken by attack shorten breath 12 mo	S: 4.76% F: 6.42%	S: 4202 F: 748	1.37 (0.99-1.90)	S: 3.33% F: 3.97%	S: 1922 F: 277	1.20 (0.63-2.31)	S: 5.13% F: 7.48%	S: 1287 F: 254	1.50 (0.88-2.54)	S: 7.05% F: 8.29%	S: 993 F: 217	1.19 (0.69-2.05)
attack asthma 12 mo	S: 2.59% F: 2.41%	S: 4199 F: 748	0.95 (0.57-1.58)	S: 2.66% F: 2.53%	S: 1919 F: 277	0.95 (0.43-2.11)	S: 2.33% F: 1.97%	S: 1287 F: 254	0.84 (0.32-2.19)	S: 2.52% F: 2.76%	S: 980 F: 217	1.10 (0.45-2.72)
phlegm day-night	S: 6.10% F: 21.5%	S: 4200 F: 748	4.23* (3.41-5.24)	S: 5.41% F: 19.1%	S: 1921 F: 277	4.13* (2.89-5.92)	S: 5.84% F: 22.8%	S: 1268 F: 254	4.77* (3.28-6.95)	S: 7.85% F: 23.0%	S: 993 F: 217	3.51* (2.37-5.20)
phlegm 3 mo per year	S: 10.3% F: 13.6%	S: 4102 F: 748	1.38* (1.09-1.74)	S: 8.32% F: 11.2%	S: 1886 F: 277	1.39 (0.92-2.09)	S: 10.1% F: 14.6%	S: 1250 F: 254	1.52* (1.03-2.26)	S: 14.4% F: 15.7%	S: 966 F: 217	1.11 (0.74-1.66)
hay fever	S: 18.2% F: 10. 0%	S: 4201 F: 748	0.45* (0.39-0.64)	S: 22.6% F: 12.6%	S: 1921 F: 277	0.49* (0.34-0.72)	S: 13.8% F: 9.84%	S: 1287 F: 254	0.68 (0.44-1.07)	S: 15.5% F: 6.91%	S: 980 F: 217	0.40* (0.23-0.70)
runny nose symptom at work or nasal irritation at work	S: 1.29% F: 24.6%	S: 4192 F: 748	25.0* (18.2-34.3)	S: 1.72% F: 28.9%	S: 1917 F: 277	23.2* (15.1-35.7)	S: 1.01% F: 22.8%	S: 1285 F: 254	29.0* (15.6-53.8)	S: 0.81% F: 21.2%	S: 990 F: 217	33.0* (15.3-71.2)
cough symptom at work	S: 1.38% F: 26.2%	S: 4191 F: 748	25.3* (18.6-34.4)	S: 1.51% F: 26.7%	S: 1916 F: 277	23.7* (15.1-37.3)	S: 1.32% F: 25.6%	S: 1285 F: 254	25.7* (14.7-44.7)	S: 1.21% F: 26.3%	S: 990 F: 217	29.0* (15.2-55.3)
cough w/o phlegm at work	S: 1.39% F: 15.1%	S: 4191 F: 748	12.7* (9.14-17.6)	S: 1.51% F: 17.3%	S: 1916 F: 277	13.6* (8.43-22.1)	S: 1.32% F: 14.2%	S: 1285 F: 254	12.3* (6.80-22.3)	S: 1.21% F: 13.4%	S: 990 F: 217	12.6* (6.30-25.1)
cough w phlegm at work	S: 1.38% F: 16.4%	S: 4191 F: 748	14.0* (10.1-19.4)	S: 1.51% F: 15.5%	S: 1916 F: 277	12.0* (7.32-19.5)	S: 1.32% F: 16.1%	S: 1285 F: 254	14.4* (8.00-25.7)	S: 1.21% F: 18.0%	S: 990 F: 217	17.9* (9.17-34.8)
shortness breath at work	S: 0.86% F: 5.75%	S: 4191 F: 748	7.04* (4.49-11.0)	S: 0.78% F: 3.97%	S: 1916 F: 277	5.24* (2.38-11.5)	S: 0.93% F: 4.72%	S: 1285 F: 254	5.26* (2.34-11.8)	S: 0.91% F: 9.68%	S: 990 F: 217	11.7* (5.27-25.9)
wheezing symptom at work	S: 0.60% F: 7.22%	S: 4191 F: 748	13.0* (8.02-21.0)	S: 0.52% F: 4.69%	S: 1916 F: 277	9.39* (4.07-21.6)	S: 0.62% F: 7.09%	S: 1285 F: 254	12.2* (5.23-28.3)	S: 0.71% F: 10.6%	S: 990 F: 217	16.6* (7.05-39.3)
never smoker	S: 34.8% F: 57.9%	S: 4197 F: 748	2.58* (2.20-3.02)	S: 42.1% F: 61.7%	S: 1919 F: 277	2.22* (1.72-2.88)	S: 29.1% F: 57.9%	S: 1287 F: 254	3.35* (2.54-4.42)	S: 28.2% F: 53.0%	S: 991 F: 217	2.88* (2.13-3.89)
current smoker	S: 38.5% F: 25.1%	S: 4197 F: 748	0.54* (0.45-0.64)	S: 40.2% F: 25.3%	S: 1919 F: 277	0.50* (0.38-0.67)	S: 38.5% F: 26.8%	S: 1287 F: 254	0.58* (0.43-0.79)	S: 34.9% F: 23.0%	S: 991 F: 217	0.56* (0.40-0.79)
former smoker	S: 26.8% F: 17.0%	S: 4197 F: 748	0.56* (0.46-0.69)	S: 17.7% F: 13.0%	S: 1919 F: 277	0.69 (0.48-1.00)	S: 32.4% F: 15.4%	S: 1287 F: 254	0.38* (0.26-0.54)	S: 36.9% F: 24.0%	S: 991 F: 217	0.54* (0.38-0.75)

OR (if Fisher Test is significant) and Prevalence analysis from self reported symptoms of Swiss Farmers (F) (Farmer-Questionnaire) and Swiss Population (S) (SAPALDIA-Questionnaire, Swiss Study on Air Pollution an Lung Diseases in Adults, NFP 26). OR comparing Swiss Farmers with Swiss Population (reference) and 95% confidence limits in parentheses. E= EC-Respiratory Health Survey Questionnaire.*

Appendix B 2: Self reported Symptoms in Swiss farmers and SAPALDIA divided into age categories (never smoker only)

Symptoms	Age 21-60			Age 21-40			Age 41-50			Age 51-60		
Symptoms of never smoker	*Prevalence*	*n*	*OR*	*Prevalence*	*n*	*OR*	*Prevalence*	*n*	*OR*	*Prevalence*	*n*	*OR*
wheeze 12 mo	S: 9.18 % F: 11.5 %	S: 1460 F: 442	1.29 (0.92-1.82)	S: 8.30 % F: 7.39 %	S: 807 F: 176	0.88 (0.47-1.63)	S: 9.09% F: 15.4%	S: 374 F: 149	1.83* (1.04-3.22)	S: 11.8 % F: 12.8 %	S: 279 F: 117	1.10 (0.57-2.11)
woken by attack shorten breath 12 mo	S: 4.79% F: 4.52%	S: 1460 F: 442	0.94 (0.57-1.57)	S: 3.72% F: 2.84%	S: 807 F: 176	0.76 (0.29-1.98)	S: 4.81% F: 6.71%	S: 374 F: 149	1.42 (0.64-3.16)	S: 7.89% F: 4.27%	S: 279 F: 117	0.52 (0.19-1.41)
phlegm day-night	S: 4.32% F: 18.3%	S: 1460 F: 442	4.98* (3.51-7.05)	S: 2.85 % F: 14.8 %	S: 807 F: 176	5.91* (3.28-10.6)	S: 4.81% F: 22.1%	S: 374 F: 149	5.63* (3.05-10.4)	S: 7.89% F: 18.8%	S: 279 F: 117	2.71* (1.43-5.11)
phlegm 3 mo per year	S: 6.19% F: 12.0%	S: 1438 F: 442	2.07* (1.44-2.95)	S: 4.51% F: 8.52%	S: 798 F: 176	1.97* (1.05-3.69)	S: 6.54% F: 15.4%	S: 367 F: 149	2.61* (1.42-4.79)	S: 10.6% F: 12.8%	S: 273 F: 117	1.24 (0.64-2.41)
hay fever	S: 22.7% F: 10.0 %	S: 1460 F: 442	0.38* (0.27-0.53)	S: 26.1% F: 10.8 %	S: 807 F: 442	0.34* (0.21-0.56)	S: 19.3% F: 12.1%	S: 374 F: 149	0.58 (0.33-1.00)	S: 17.2% F: 6.001%	S: 279 F: 117	0.31* (0.13-0.70)
runny nose symptom at work or nasal irritation at work	S: 1.31% F: 25.1%	S: 1453 F: 442	25.3* (15.3-41.8)	S: 1.87% F: 29.0%	S: 803 F: 176	21.4* (11.7-39.3)	S: 1.07% F: 22.1%	S: 373 F: 149	26.2* (9.11-75.6)	S: 0.36% F: 23.1%	S: 277 F: 117	83.1* (11.1-620)
cough symptom at work	S: 1.24% F: 23.8%	S: 1452 F: 442	24.8* (14.8-41.5)	S: 1.50% F: 22.2%	S: 802 F: 176	18.7* (9.57-36.7)	S: 1.34% F: 23.5%	S: 373 F: 149	22.6* (8.65-59.0)	S: 0.36% F: 26.5%	S: 277 F: 117	99.5* (13.4-740)
cough w/o phlegm at work	S: 1.24% F: 13.8%	S: 1452 F: 442	12.8* (7.45-21.8)	S: 1.50% F: 14.8%	S: 802 F: 176	11.4* (5.63-23.1)	S: 1.34% F: 13.4%	S: 373 F: 149	11.4* (4.20-31.0)	S: 1.21% F: 13.4%	S: 277 F: 117	40.6* (5.29-311)
cough w phlegm at work	S: 1.24% F: 14.54%	S: 1452 F: 442	13.5* (7.90-23.0)	S: 1.50% F: 12.5%	S: 802 F: 176	9.40* (4.56-19.4)	S: 1.34% F: 15.4%	S: 373 F: 149	13.4* (5.00-36.1)	S: 0.36% F: 16.2%	S: 277 F: 117	53.5* (7.07-405)
shortness breath at work	S: 0.62% F: 4.52%	S: 1452 F: 442	7.60* (3.43-16.8)	S: 0.50% F: 1.70%	S: 802 F: 176	3.46 (0.77-15.6)	S: 1.07% F: 4.70%	S: 373 F: 149	4.55* (1.31-15.8)	S: 0.36% F: 8.55%	S: 277 F: 117	25.8* (3.26-204)
wheezing symptom at work	S: 0.48% F: 6.56%	S: 1452 F: 442	14.5* (6.30-33.3)	S: 0.37% F: 2.84%	S: 802 F: 176	7.79* (1.84-32.9)	S: 1.07% F: 8.05%	S: 373 F: 149	8.08* (2.56-25.5)	S: 0.00% F: 10.3%	S: 277 F: 117	31.5* (4.05-246)

*Never smoker: ORs (*if Fisher Test is significant) and Prevalence analysis from self reported symptoms of Swiss Farmers (F) (Farmer-Questionnaire) and Swiss Population (S) (SAPALDIA-Questionnaire, Swiss Study on Air Pollution an Lung Diseases in Adults, NFP 26). OR comparing Swiss Farmers with Swiss Population (reference) and 95% confidence limits in parentheses. E= EC-Respiratory Health Survey Questionnaire.*

Appendix C: Questionnaire poultry farmers

Datum:
ausgefüllt von:

Untersuchungsprotokoll zur arbeitsmedizinischen Beurteilung der Belastungs- und Beanspruchungssituation des Atemtrakts bei Landwirten - Geflügel -

Allgemeine Betriebsdaten

Aktenzeichen der HLBG:

Name:

Vorname:

Geb.datum:

Geschlecht:

Anschrift:

Telefon:

Lungenfunktion: Test

Körperlänge in cm
Spirometer ans Stromnetz anschließen und 5 Minuten warten
Bestimmung folgender Parameter:
Temperatur
Luftfeuchte
Luftdruck

Fragen unmittelbar vor der Lufu:

1.	Haben Sie zur Zeit eine Erkältung?	ja/nein
2.	Haben Sie heute Medikamente für die Atmung eingenommen?	ja/nein
2.1	Wenn ja: Welche? (Name, Dosis)	
2.2	Wann zuletzt? (Uhrzeit)	
3.	Haben Sie innerhalb der letzten Stunde geraucht?	ja/nein
4.	Kalibrierung	
5.	Eingabe der Patientendaten	

Lungenfunktion vor der Stallarbeit morgens
Fluss-Volumen-Kurve.

Stalluft-Messungen morgens und abends

Gemessen wurden in welchem Stall:

Filter-Nummern, Laufzeit:

	Filter-Nummern	Laufzeit (min)
Personenbezogen Feinstaub; 2 l/min		+
Personenbezogen Gesamtstaub; 3,5 l/min		+
Personenbezogen Mikroorganismen; 1 l/min		+

Ammoniak, Kohlendioxid (Kurzzeit-Röhrchen):

	Morgens		Abends	
	Sofort bei Arbeitsbeginn	Nach 20 min. Stallarbeit	Sofort bei Arbeitsbeginn	Nach 20 Min. Stallarbeit
Ammoniak (ppm)				
Kohlendioxid (ppm)				

Temperatur, Luftfeuchtigkeit, Luftgeschwindigkeit:

	Morgens	Abends
Temperatur (°C)		
Luftfeuchtigkeit (%)		
Luftgeschwindigkeit (m/s)		

Lungenfunktion nach der Stallarbeit morgens

Stallerhebungsbogen

Tierbestand

Tierart: Geflügel	Art	Anzahl im gemessenen Stall	Anzahl insgesamt
1.1	Legehennen	.....	.....
1.2	Küken	.....	.....
1.3	Hähne	.....	.....
1.4	Mastgeflügel	.....	.....

Übriger Tierbestand (geschätzt)

		Anzahl
2.1	Milchkühe	.....
2.2	Mastbullen	.....
2.3	Kälber (bis 150 kg)	.....
2.4	Schweine	.....

3. Aufstallungsart

3.1 Bodenhaltung O 3.2 Käfighaltung O
3.3 Volierenhaltung O 3.4 Auslaufhaltung O

4. Der für die Messungen ausgewählte Stall:

4.1 Wärmegedämmt O 4.2 Nicht wärmegedämmt O

4.3 Stallänge: ..... m 4.4 Breite: ..... m
4.5 Fläche: ..... m2 4.6 Höhe: ..... m

5. Stallfußboden, Einstreu

5.1 Fußboden planbefestigt O 5.2 Teilspalten O
5.3 Vollspalten O

5.4 Einstreu mit Stroh O 5.5 Andere Einstreu O
5.6 Keine Einstreu O

6. Fütterungssystem

6.1 Trockenfütterung O 6.2 Flüssigfütterung O
6.3 Automatisch O 6.4 Manuell O
6.5 Ad lib O 6.6 Restriktiv O

7. Welche Futtermittel werden eingesetzt?

7.1 Allein-Futtermittel:
7.1.1 Getreideprodukte O 7.1.2 O
7.1.3 O

7.2 Misch-Futtermittel:
7.2.1 geschrotet O 7.2.2 pelletiert O
7.2.3 andere O

8. Häufigkeit und Zeitpunkt der Fütterung am Tag

8.1 einmal täglich O 8.2 zweimal täglich O
8.3 öfter O

9. Fütterung immer durch dieselbe Person?

9.1 ja O 9.2 nein O

10. Mistsystem:

10.1. Lagerdauer des Mistes im Stall:

10.1.1	eine Woche	O	10.1.2	zwei Wochen	O
10.1.3	drei bis vier Wochen	O	10.1.4	> 4 Wochen	O

11. Reinigungsintervalle

11.1	täglich	O	11.2	wöchentlich	O
11.3	monatlich	O	11.4	seltener	O

Desinfektionsintervalle

11.5	täglich	O	11.6	wöchentlich	O
11.7	monatlich	O	11.8	seltener	O

12. Stalluft

12.1 Lüftungssystem:

12.1.1	Freie Lüftung	O	12.1.2	Zwangslüftung	O
12.1.3	Überdruck	O	12.1.4	Unterdruck	O
12.1.5	Gleichdruck	O			

12.2 Zuluftöffnungen in:

12.2.1	Wand	O	12.2.2	Decke	O
12.2.3	Poren Wand	O	12.2.4	Poren Decke	O
12.2.5	Poren Kanal	O	12.2.6	Andere	O

12.3 Abluftöffnungen in:

12.3.1	Wand	O	12.3.2	Decke	O
12.3.3	Spaltenboden	O	12.3.4	Andere	O

12.4 Möglichkeiten der Regelung:

12.4.1	Drehzahlregelung	O	12.4.2	Stufenschaltung	O

12.5 Steuerung

12.5.1	Thermostat	O	12.5.2	Hygrostat	O
12.5.3	andere	O	12.5.4	manuell	O

12.6 Heizsystem

12.6.1	vorhanden	O	12.6.2	nicht vorhanden	O

13. Management

13.1	Rein-Raus-Verfahren	O	13.2	Kontinuierliche Belegung	O

14. Anzahl Herkunftsbetriebe

14.1	Geschlossenes System = Eigener Betrieb				O
14.2	Zwei Betriebe	O	14.3	Drei Betriebe	O
14.4	Mehr als drei Betriebe	O			

15. Welche Erkrankungen treten öfter / regelmäßig im Tierbestand auf?

15.1	Atemwegserkrankungen	O	15.2	Magen-Darmerkrankungen	O

Medizinischer Fragebogen

Zur Beantwortung der Fragen kreuzen sie bitte das zutreffende Kästchen an. Wenn Sie unsicher sind, wählen Sie bitte "NEIN".

Teil 1 - Atembeschwerden

1. Haben Sie jemals in den letzten 12 Monaten ein pfeifendes oder brummendes Geräusch in Ihrem Brustkorb gehört? NEIN O JA O

   WENN "NEIN", GEHEN SIE BITTE ZU FRAGE 2, WENN "JA":

   1.1. Fühlten Sie sich jemals außer Atem, als dieses Geräusch auftrat? NEIN O JA O

   1.2. Hatten Sie dieses Pfeifen oder Brummen, wenn Sie nicht erkältet waren? NEIN O JA O

2. Sind Sie irgendwann in den letzten 12 Monaten mit einem Engegefühl im Brustkorb aufgewacht? NEIN O JA O

3. Sind Sie irgendwann in den letzten 12 Monaten durch einen Anfall von Luftnot aufgewacht? NEIN O JA O

4. Sind Sie irgendwann in den letzten 12 Monaten wegen eines Hustenanfalls aufgewacht? NEIN O JA O

5. Haben Sie in den letzten 12 Monaten einen Asthmaanfall gehabt? NEIN O JA O

6. Nehmen Sie derzeit irgendeine Medizin (zum Beispiel Inhalationen, Dosieraerosole (Sprays) oder Tabletten) gegen Asthma? NEIN O JA O

7. Haben Sie allergischen Schnupfen, zum Beispiel "Heuschnupfen"? NEIN O JA O

8. Husten Sie gewöhnlich im Winter als erstes nach dem Aufstehen? NEIN O JA O

   (WENN "JA" ODER UNSICHER, BEANTWORTEN SIE BITTE FRAGE 9.1 ZUR ERGÄNZUNG)

9. Husten Sie gewöhnlich im Winter während des Tages, oder in der Nacht? NEIN O JA O

   WENN "NEIN", GEHEN SIE BITTE ZU FRAGE 10, WENN "JA":

   9.1. Husten Sie derart meistens für mindestens 3 Monate jährlich? NEIN O JA O

10. Haben Sie im Winter gewöhnlich als erstes am Morgen Auswurf? NEIN O JA O

    (WENN "JA" ODER UNSICHER, BEANTWORTEN SIE BITTE FRAGE 11.1 ZUR ERGÄNZUNG)

11. Haben Sie im Winter gewöhnlich Auswurf tagsüber oder nachts? NEIN JA
O O

WENN "NEIN", GEHEN SIE BITTE ZU FRAGE 12, WENN "JA":

11.1. Haben Sie solchen Auswurf an den meisten Tagen für mindestens 3 Monate jährlich?

NEIN JA
O O

Teil 2 - Landwirtschaftliche Produktion

12. Wie viele Jahre arbeiten Sie schon in der Landwirtschaft?....._ _
13. In welchen Jahren haben Sie folgende Produktion /Tierhaltung betrieben bzw. betreiben sie noch:

13.1 Schweinehaltung.....	von	(Jahr) _ _ _	bis	_ _ _	(Jahr)
13.2 Rinderhaltung	von	(Jahr) _ _ _	bis	_ _ _	(Jahr)
13.3 Legehennenhaltung.....	von	(Jahr) _ _ _	bis	_ _ _	(Jahr)
13.4 Geflügelmast.....	von	(Jahr) _ _ _	bis	_ _ _	(Jahr)
13.5 Schafhaltung.....	von	(Jahr) _ _ _	bis	_ _ _	(Jahr)
13.6 Pferdehaltung.....	von	(Jahr) _ _ _	bis	_ _ _	(Jahr)
13.7 Andere.....	von	(Jahr) _ _ _	bis	_ _ _	(Jahr)

14. Tierzahlen: siehe Stall-Erhebungsbogen. Hier keine Angaben erforderlich.

15. Führen Sie folgende Arbeiten selbst aus?	NEIN	JA
15.1 Schroten.....	O	O
15.2 Schrot von Hand vorgeben.....	O	O
15.3 Ausmisten von Hand.....	O	O
15.4 Gülle ausbringen	O	O
15.5 Pflanzenschutzmittel spritzen.....	O	O
15.6 Mineraldünger streuen.....	O	O

Teil 3 - Atembeschwerden bei der Arbeit

16. Haben Sie bei der Arbeit im Stall eine oder mehrere der folgenden Beschwerden?

	NEIN	JA
16.1 Kurzluftig, außer Atem	O	O
16.2 Reizhusten (Husten ohne Auswurf)	O	O
16.3 Pfeifende oder brummende Atemgeräusche	O	O

17. Wie war der Verlauf dieser Beschwerden während der letzten 10 Jahre?
BITTE SETZEN SIE FOLGENDE HÄUFIGKEITSANGABEN IN JEDES KÄSTCHEN EIN:

HÄUFIGKEIT DER ATEMBESCHWERDEN BEI DER ARBEIT:

1 = OHNE BESCHWERDEN
2 = SELTENER ALS EINMAL IM MONAT
3 = EINMAL IM MONAT
4 = EINMAL IM MONAT BIS EINMAL IN DER WOCHE
5 = EINMAL IN DER WOCHE
6 = TÄGLICH

Bis vor 10 J.	Bis vor 8 J.	Bis vor 6 J.	Bis vor 4 J.	Bis vor 2 J.	Jetzt
O	O	O	O	O	O

18. Haben Sie während der Arbeit mit Getreide, Heu oder Stroh folgende Beschwerden **(BITTE ANKREUZEN):**

	Getreide	Heu	Stroh
18.1 Kurzluftig, außer Atem			
18.2 Reizhusten			
18.3 Pfeifende oder brummende Atemgeräusche			

19. Haben Sie bei den nachfolgend genannten Tätigkeiten folgende Beschwerden **(BITTE ANKREUZEN):**

	Schroten	Schrot von Hand vorgeben	Ausmisten von Hand
19.1 Kurzluftig, außer Atem			
19.2 Reizhusten			
19.3 Pfeifende oder brummende Atemgeräusche			

20. Haben Sie bei den nachfolgend genannten Tätigkeiten folgende Beschwerden **(BITTE ANKREUZEN):**

	Gülle ausbringen	Pflanzen-schutz spritzen	Mineral-dünger streuen
20.1 Kurzluftig, außer Atem			
20.2 Reizhusten			
20.3 Pfeifende oder brummende Atemgeräusche			

21. **WENN SIE FRAGE 16 ODER 18 MIT "JA" BEANTWORTET HABEN, KREUZEN SIE BITTE AN, WANN DIE ATEMBESCHWERDEN BEI DER ARBEIT BEGINNEN UND VERSCHWINDEN:**

BEGINN von Atembeschwerden bei der Arbeit	im Stall	mit Getreide	mit Heu	mit Stroh
21.1 In den ersten Minuten				
21.2 In der ersten Stunde				
21.3 Nach mehr als einer Stunde				
21.4 Nach Arbeitsende				

VERSCHWINDEN von Atembeschwerden nach der Arbeit	im Stall	mit Getreide	mit Heu	mit Stroh
21.5 In den ersten Minuten				
21.6 In der ersten Stunde				
21.7 Nach mehr als einer Stunde				
21.8 Nach Arbeitsende				

22. Verwenden Sie ein Atemschutzgerät bei der Arbeit im Stall? NEIN O JA O

WENN "JA": 22.1 Etwa wieviele Stunden pro Woche?....._ _

Teil 4 - Rauchen

23. Haben Sie schon einmal ein Jahr lang geraucht? NEIN O JA O

("JA" BEDEUTET MINDESTENS 20 PÄCKCHEN ZIGARETTEN IM LEBEN ODER 2 PÄCKCHEN TABAK IN IHREM LEBEN, ODER EIN JAHR LANG MINDESTENS EINE ZIGARETTE PRO TAG ODER EINE ZIGARRE PRO WOCHE)

WENN "NEIN", IST DER FRAGEBOGEN BEENDET, WENN "JA":

23.1 Wie alt waren Sie, als Sie anfingen zu rauchen?..... _ _

23.2 Rauchen Sie jetzt (bzw. bis vor einem Monat) ? NEIN O JA O

WENN "NEIN", GEHEN SIE ZU FRAGE 23.3.1, WENN "JA" :

23.2.1-4 Wieviel rauchen Sie jetzt durchschnittlich?

23.2.1 Zigaretten pro Tag..... _ _ _

23.2.2 Zigarillos pro Tag..... _ _ _

23.2.3 Zigarren am Tag..... _ _ _

23.2.4 Pfeifentabak in Gramm pro Woche..... _ _ _

23.3 Haben Sie das Rauchen aufgegeben oder reduziert? NEIN O JA O

WENN "NEIN", IST DER FRAGEBOGEN BEENDET, WENN "JA" :

23.3.1 Wie alt waren Sie, als Sie das Rauchen reduziert bzw. aufgegeben haben? _ _

23.3.2.1-4 Wieviel rauchten Sie früher durchschnittlich, bezogen auf die ganze Zeit, die Sie rauchten, bevor Sie reduziert bzw. aufgaben?

23.3.2.1 Zigaretten pro Tag..... _ _ _

23.3.2.2 Zigarillos pro Tag..... _ _ _

23.3.2.3 Zigarren pro Tag..... _ _ _

23.3.2.4 Pfeifentabak in Gramm pro Woche..... _ _ _

Lungenfunktion vor der Stallarbeit abends

1. Haben Sie heute Medikamente für die Atmung eingenommen? ja/nein

1.1 Wenn ja: Welche? (Name, Dosis)

1.2 Wann zuletzt? (Uhrzeit)

Lungenfunktion nach der Stallarbeit abends

Appendix D: Steps farmer measurement

Durchführung der Untersuchungen bei den Landwirten

1. Vorbereitung

1.1 Pumpen nach dem Aufladen mit Rotameter kalibrieren, dazu:

- Rotameter ins Lot bringen
- Schlauch oben mit Pumpe verbinden
- Ablesen an der Oberkante des schwimmenden Kegels
- Volumen Gesamtstaubpumpe: 3,5 l/min
  Feinstaubpumpe: 2 l/min
  Mikroorganismenpumpe: 1 l/min

1.2 Filter in Sammler einlegen

- Gesamtstaub: Filterkante oben, Filter unten
  Schlauch nach unten mit Pumpe verbinden
- Feinstaub: Filterkante oben, Filter unten
  Sieb ganz oben
  Probenahme von oben
  Schlauch nach unten mit Pumpe verbinden
- Mikroorganismen: blauer Stecker nach oben, roter Stecker nach unten, Schlauch von unten mit Pumpe verbinden Filterschatulle beschriften

Filternummern notieren!

2. Auf dem Hof

2.1 Lungenfunktion vor der Arbeit morgens:

1. Spirometer anschließen, kalibrieren, Patientendaten eingeben
2. Probanden Grösse messen
3. Lungenfunktion nach dem „Lung Function Protocol“ ohne Stativ

2.2 vor der Fütterung:

1. geeigneten Stall aussuchen
2. Pumpen umlegen und einschalten
   Laufzeit: Feinstaub und Gesamtstaub jeweils Dauer der Fütterung,
   Mikrorganismen max. 1 Stunde
3. CO_2-Messung
4. NH_3-Messung
5. Temperatur, Luftfeuchtigkeit und Luftgeschwindigkeit messen

2.3 20 min nach Betreten des Stalls:

1. CO_2-Messung
2. NH_3-Messung

2.4 nach der Fütterung

1. Gerät abschalten und Zeit notieren
2. Mikroorganismenpumpe nach 1 h abschalten und roten gegen blauen Stopfen austauschen
3. Lungenfunktion
4. Fragebogen

Mikroorganismenproben *sofort* an: Dr. Palmgren
Pegasus Labor GmbH
Adersstr. 24
40215 Düsseldorf

Abends:

2.5 vor der Fütterung:

1. Lungenfunktion vor dem Füttern
2. Pumpen für Feinstaub und Gesamtstaub umlegen und einschalten mit dem gleichen Filter wie morgens! (**Abends keine Mikroorganismen**)
3. CO_2-Messung vor dem Füttern
4. NH_3-Messung
5. Temperatur, Luftfeuchtigkeit und Luftgeschwindigkeit messen

2.6 20 min nach Betreten des Stalls:

1. CO_2-Messung
2. NH_3-Messung

2.7 nach der Fütterung

1. Gerät abschalten und Zeit notieren

3. zu Hause

1. Filter über Kopf entnehmen und in Filterschachtel mit entsprechender Nummer legen
2. Filterschachtel mit Tesafilm verschließen
3. Filter gesammelt an Zentralinstitut für Arbeitsmedizin in Hamburg schicken

Flow sheet: Step 2	Morning Feeding	Work with plant crops	Evening Feeding
Spirometry	| | 20 min		| | 20 min
Ammonia	| |		| |
Carbon monoxide	20 min | |		20 min | |
Total dust, endotoxin	|_____|		|_____|
Respirable dust, endotoxin	|_____|		|_____|
Microorganism	|_____|		|_____|

Appendix E: Summary equations for lung volumes and ventilatory flows for adults aged 18 - 70 years[1]

Variable	Unit	Regression equation	RSD	1,64 RSD
		Men		
IVC	l	6,10H-0,028A-4,65	0,56	0,92
FVC	l	5,76H-0,026A-4,34	0,61	1,00
TLC	l	7,99H-7,08	0,70	1,15
RV	l	1,31H+0,022A-1,23	0,41	0,67
FRC	l	2,34H+0,009A-1,09	0,6	0,99
RV/TLC	%	0,39A+13,96	5,46	9,0
FRC/TLC	%	0,21A+43,8	6,74	11,1
FEV_1	l	4,30H-0,029A-2,49	0,51	0,84
FEV_1/VC	%	-0,18A+87,21	7,17	11,8
$FEF_{25-75\%}$	l/s	1,94H-0,043A+2,70	1,04	1,71
PEF	l/s	6,14H-0,043A+0,15	1,21	1,99
MEF_{75}	l/s	5,46H-0,029A-0,47	1,71	2,81
MEF_{50}	l/s	3,79H-0,031A-0,35	1,32	2,17
MEF_{25}	l/s	2,61H-0,026A-1,34	0,78	1,28
		Women		
IVC	l	4,66H-0,024A-3,28	0,42	0,69
FVC	l	4,43H-0,026A-2,89	0,43	0,71
TLC	l	6,60H-5,79	0,60	0,99
RV	l	1,81H+0,016A-2,00	0,35	0,58
FRC	l	2,24H+0,001A-1,00	0,50	0,82
RV/TLC	%	0,34A+18,96	5,83	9,6
FRC/TLC	%	0,16A+45,1	5,93	9,8
FEV_1	l	3,95H-0,025A-2,60	0,38	0,62
FEV_1/VC	%	-0,19A+89,10	6,51	10,7
$FEF_{25-75\%}$	l/s	1,25H-0,034A+2,92	0,85	1,40
PEF	l/s	5,50H-0,030A-1,11	0,90	1,48
MEF_{75}	l/s	3,22H-0,025A+1,60	1,35	2,22
MEF_{50}	l/s	2,45H-0,025A+1,16	1,10	1,81
MEF_{25}	l/s	1,05H-0,025A+1,11	0,69	1,13

H: Height (m); A: Age (Years); RSD: residual standard deviation

[1] between 18 and 25 yr substitute 25 yr in the equations

Appendix F: Protocoll endotoxin provocation

Versuchsprotokoll

Name: .....

Datum: .....

	Zeit Vorgabe	Zeit real	Menge (ml)	Proben-Nr.
Nasenreinigung	08:45			
Baseline - Lavage	08:50			
Lungenfunktion	08:55			
Provokation	09:00			
Lavage 1	09:20			
Lungenfunktion	09:25			
Lavage 2	10:00			
Lungenfunktion	10:05			
Lavage 3	15:00			
Lungenfunktion	15:05			
Lavage 4	08:00			
Lungenfunktion	08:05			

Appendix G: Questionnaire syptoms endotoxin provocation

Symptome Endotoxin-Provokation

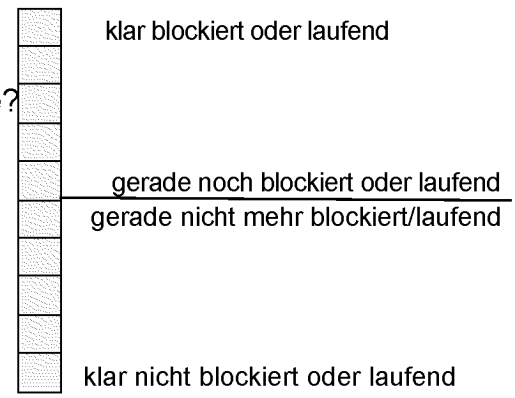
Datum **und** Uhrzeit: .....

Name: .....

Nasenreizung

2. Haben Sie eine laufende oder blockierte Nase?

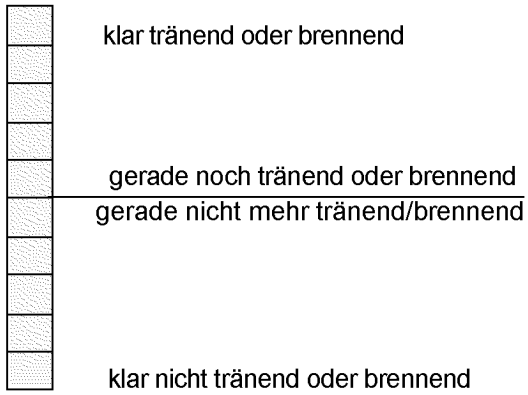
Bitte kreuzen Sie ein Feld an



Augenreizung

2. Haben Sie gereizte, das heisst tränende oder brennende Augen?

   Bitte kreuzen Sie ein Feld an

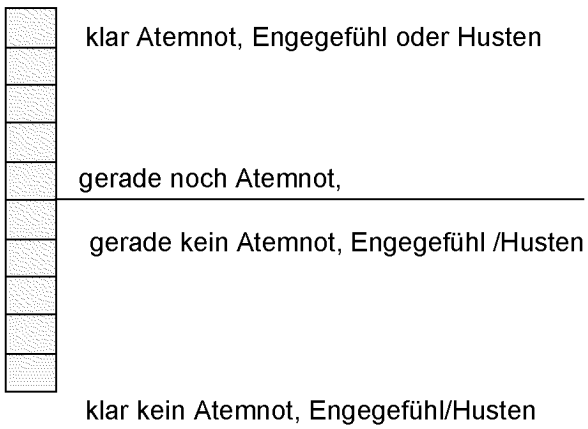


Lungenreizung

2. Haben Sie Atemnot, ein Engegefühl in der Brust oder Husten?

   Engegefühl/Husten

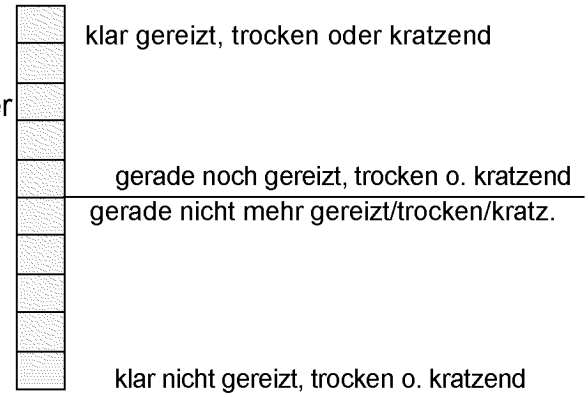
   Bitte kreuzen Sie ein Feld an



Halsreizung

2. Haben Sie einen gereizten, trockenen oder kratzenden Rachen?

   Bitte kreuzen Sie ein Feld an



Appendix H: Abbreviations

CFU	Colony forming Units
EC	Escherichia Coli
ECP	Eosinophil cationic Protein
ECRHS	European Community Respiratory Health Survey
ECSC	European community for coal and steel
ENT	Ear, Nose, Throat (Otolaryngology)
FAT	Swiss Federal Research Station for Agricultur, Economics and Engineering
FEV_1	Forced Expiratory Volume in 1 second
FVC	Forced Vital Capacity
IL	Interleukin
LPS	Lipopolysaccharide
MAK	Maximum Workplace Value
$MMEF_{25/75}$	Mid Expiratory Flow Rate
MPO	Myeloperoxidase
ODTS	Organic Dust Toxic Syndrome
OR	Odds Ratio
SAPALDIA	Swiss Study on Air Pollution and Lung Diseases in Adults
SCARPOL	Swiss Study on Childhood Allergy and Respiratory Symptoms
SD	Standard Deviation
TNF-α	Tumor Necrosis Factor-α

Curriculum vitae

Christoph Weber, born on the 10th January 1968 in Zürich, Switzerland

1975 – 1981	Primary school in Oberwil/Nürensdorf, ZH, Switzerland.
1981 – 1984	Swiss Federal Baccalaureate, Type B, Rychenberg, Winterthur, Switzerland.
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1989 – 1995	Studies at the Department of Environmental Science, Swiss Federal Institute of Technology Zürich. Graduation with the degree dipl. sc. nat. ETH
1995 - 2000	Phd. Student, at the Institute of Hygiene and Applied Physiology ETH Zürich, Professor Dr. Dr. Helmut Krueger and Dr. med. Brigitta Danuser
1996-1997	Postgraduate Study in occupational health at the Swiss Federal Institute of Technology Zürich and the University of Lausanne. Graduation with the degree Dipl. NDS ETHZ, EPG UNIL in Arbeit und Gesundheit