Air contaminants in different European farming environments

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Abstract: Farmers are known to be at high risk from the development of occupational airway disease. The first stage of the European farmers’ study has shown that pig farmers in Denmark and Germany, poultry farmers in Switzerland and greenhouse workers in Spain were at highest risk for work-related respiratory symptoms. Therefore, the aim of this study was to determine exposure levels at relevant farm workplaces. Dust and endotoxin levels as well as microbiological concentrations were determined in 213 crop and animal farming environments by personal sampling. The highest total dust concentrations were found in poultry houses in Switzerland with median concentrations of 7.01 mg/m³. The median airborne endotoxin concentrations in total dust ranged between 0.36 ng/m³ in Spanish greenhouses and 257.58 ng/m³ in poultry houses in Switzerland. Likewise, the highest median concentrations of total (2.0 × 10⁷ cells/m³) and active fungi (4.4 × 10⁵ cfu/m³) have been found in Swiss poultry houses. The predominant fungus taxa discovered in poultry houses were Eurotium spp. and thermophilic fungi. Cladosporium and Botrytis were mainly detected in greenhouses. The exposure level found in this study might put the farmers at risk from respiratory diseases.

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Key words: swine confinement houses, poultry confinement houses, greenhouses, endotoxin, bacteria, fungi.

INTRODUCTION

Work in modern agriculture environments exposes the respiratory system to many different agents such as inorganic and organic dust containing endotoxin, bacteria, allergens, and fungi, as well as gases (e.g. NH₃) and chemicals (e.g., disinfectants, pesticides). Exposure to those substances may cause or exacerbate asthma, asthma-like syndrome, mucous membrane irritation, and chronic bronchitis. Additionally, extrinsic allergic alveolitis (EAA) is known to be caused by bacteria and fungi as well as animal allergens. However, EAA has not been excessively reported in modern livestock studies. Endotoxins may also cause organic dust toxic syndrome (ODTS) [1].

In most industries in the western world, measurements of airborne pollutants have to be conducted at a regular basis. In contrast, standards for most organic dust components exist in only few countries and routine measurements on farms for hygienic reasons are seldom carried out [1, 9, 33].
Because of the high prevalence of respiratory symptoms among pig farmers, several studies have been conducted to assess exposure levels in swine confinement buildings (e.g., [2, 5, 6, 13, 26, 29]). Limited environmental data are available for poultry confinement houses [6, 30, 31, 34]. There was a large scale European study on the concentrations of airborne dust, endotoxin and microorganisms in different livestock buildings [32, 33] but in that study, no personal measurements were made. Few studies were found regarding exposure levels to bioaerosols in greenhouses [3, 8]. Horticulture work is considered a hazardous occupation from a dermatological point of view [24] but less frequently as an occupational respiratory disease hazard [25].

In the first part of the European farmers’ study, the prevalence of occupational airway disease in farmers in Denmark, Germany, Switzerland, and Spain was assessed. It was shown that pig farmers have the highest prevalence of occupational airway disease regarding work-related respiratory symptoms (wheezing, cough and / or shortness of breath) and asthma-like syndrome [28]. In poultry farmers, we found a significantly increased risk for the development of occupational asthma symptoms [36]. In the group of crop farmers we found the highest prevalence in the subgroup cultivating flowers with regard to wheezing, asthma, chronic phlegm and symptoms of ODTS [21]. Therefore, it was the aim of the second part of the European study on “Prevalence and Risk Factors of Airway Obstruction in Farmers” [22] to investigate measures of exposure in the farming environments with a high risk for the development of occupational airway diseases. This paper focuses on the description of levels of exposure in swine and poultry confinement buildings as well as greenhouses in 4 European countries.

**MATERIAL AND METHODS**

**Study population.** The groups of farmers were selected for further study based on the highest prevalence of respiratory symptoms at each of the study centers [21, 28]. Therefore, in Denmark (Aarhus) and Germany (Lower Saxony) farmers with primarily pig production were selected, and in Switzerland (Zurich) farmers with mainly poultry production were chosen. In Spain (Barcelona), farmers with chiefly greenhouse work (ornamental plant or flower production) were studied. In each participating country except Germany at least 35 farms were randomly selected from the target population. Study subjects in Germany were all pig farmers claiming compensation for another. Thus, in Spain the measurements were taken inside greenhouses and in the storage area. In Denmark and Switzerland the sampling time included work inside several animal houses, but all buildings housed the same kind of animals (pigs respectively poultry). In all centres the field work was carried out over all seasons.

**Dust.** Airborne dust (PM_{10}) was collected on pre-weighted (Technischer Überwachungsverein (TÜV) Hanover, Germany), 37 mm diameter glass fibre 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). Battery-operated pumps while moving from one building to another. Thus, in Spain the measurements were taken inside greenhouses and in the storage area. In Denmark and Switzerland the sampling time included work inside several animal houses, but all buildings housed the same kind of animals (pigs respectively poultry). In all centres the field work was carried out over all seasons.

**Endotoxin.** Endotoxin content of these dust samples was determined by a kinetic-turbidimetric Limulus assay as described by Hollander et al. [15] 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 microtitre-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 given as mg/m³.

**Ammonia, carbon dioxide, temperature, relative air humidity and air velocity.** Ammonia and carbon dioxide concentrations were measured with Draeger colorimetric detector tubes (Ammonia 5/a, CH 20501, 5–70 ppm;
Carbon Dioxide 100/a, 81 01811, 100–300 ppm; Draeger Sicherheitstechnik, Luebeck, Germany) with a manually operated pump (accuro, Draeger Sicherheitstechnik). In greenhouses in Spain only carbon dioxide was assessed. Temperature, relative air humidity and air velocity were taken by a multi-function instrument (Testo 400, Testo, Lenzkirch, Germany). The sampling points were located in the centre of the animal- or greenhouse at a point several meters from the overhead fan 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, Duesseldorf, 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) on the same day. In Germany, no airborne microorganism samples were collected.

The total concentration of airborne microorganisms was determined by the CAMNEA method utilizing an epifluorescence microscope [23] showing similar or slightly lower estimates of microorganisms than scanning electron microscopy or light microscopy [21]. Viable count estimation was carried out as described elsewhere [23].

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 (bioMerieux) for 30 secs and filtered through a dark 0.4 µm polycarbonate filter (Nuclepore, New York, USA). The number of microbial cells in 40 randomly chosen fields was counted by

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### Table 1. Farming characteristics: swine confinement houses, poultry confinement houses. For continuous variables median (range) are given. Dichotomous variables are given as frequencies.

<table>
<thead>
<tr>
<th></th>
<th>Swine confinement houses</th>
<th>Poultry confinement houses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Denmark</td>
<td>Germany</td>
</tr>
<tr>
<td>Number of farmers</td>
<td>40</td>
<td>100</td>
</tr>
<tr>
<td>Area (m²)</td>
<td>200 (97–404)</td>
<td>140 (40–840)</td>
</tr>
<tr>
<td>Volume (m³)</td>
<td>771 (233–2182)</td>
<td>389 (112–2940)</td>
</tr>
<tr>
<td>Non-pregnant and carrying sows per farm (number/farm)</td>
<td>135 (0–530)</td>
<td>14 (0–330)</td>
</tr>
<tr>
<td>Yielding sows and piglets (number/farm)</td>
<td>378 (0–10000)</td>
<td>6 (0–113)</td>
</tr>
<tr>
<td>Young sows (number/farm)</td>
<td>20 (0–540)</td>
<td>0 (0–300)</td>
</tr>
<tr>
<td>Weaners (number/farm)</td>
<td>520 (0–2600)</td>
<td>0 (0–652)</td>
</tr>
<tr>
<td>Porkers (number/farm)</td>
<td>120 (0–1500)</td>
<td>200 (0–1330)</td>
</tr>
<tr>
<td>Boars (number/farm)</td>
<td>4 (0–13)</td>
<td>1 (0–4)</td>
</tr>
<tr>
<td>Group stall (n)</td>
<td>39</td>
<td>81</td>
</tr>
<tr>
<td>Laying hens (number/farm)</td>
<td>2100 (0–16000)</td>
<td></td>
</tr>
<tr>
<td>Chicks (number/farm)</td>
<td>0 (0–20000)</td>
<td></td>
</tr>
<tr>
<td>Cocks (number/farm)</td>
<td>0 (0–3000)</td>
<td></td>
</tr>
<tr>
<td>Fattening poultry (number/farm)</td>
<td>0 (0–11500)</td>
<td></td>
</tr>
<tr>
<td>Free-range conditions</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Concrete floor (n)</td>
<td>6</td>
<td>17</td>
</tr>
<tr>
<td>Pellet feeding (n)</td>
<td>29</td>
<td>23</td>
</tr>
<tr>
<td>Manual feeding (n)</td>
<td>34</td>
<td>56</td>
</tr>
<tr>
<td>Natural ventilation (n)</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>Air inlet: porous channel (n)</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Ventilation control: humidity sensor (n)</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>Heating (n)</td>
<td>37</td>
<td>50</td>
</tr>
<tr>
<td>Storage time of liquid manure &gt; 1 month (n)</td>
<td>5</td>
<td>52</td>
</tr>
<tr>
<td>Interval of cleaning/use of disinfectants &gt; 1 month (n)</td>
<td>6</td>
<td>81</td>
</tr>
</tbody>
</table>

n – number of samples
epifluorescence microscopy at 1,250 × magnification. Counts were related to air volume and expressed as colony forming units/m\(^3\) of sampled air (cfu/m\(^3\)). The lowest countable concentration of microorganisms was 3 × 10\(^3\) counts per sample. Using this method, viable and non-viable microorganisms were enumerated.

In order to obtain the number of viable microorganisms, culturable 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:

- Malt extract agar with penicillin and streptomycin (20 g maltextract (Oxoid), 20 g agar (Fluka), 2 ml penicillin-streptomycin solution, 1 l aqua dest).
- 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).
- 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).
- Maltextract agar and DG 18-agar were used to identify fungi, bacteria were identified on Tryptone glucose extract agar. 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). All colonies were examined microscopically. Cultivation of selected isolates was performed by classical microbiological principles. The following genus were identified:
  - Fungi: Absidia, Alternaria, Aspergillus, Botrytis, Cladosporium, Eurotium, Candida, Mucor, Penicillium, Trichoderma, Ulocladium, thermophilic fungi.
  - Bacteria: Bacillus, Streptomyces, thermophilic bacteria.

When microorganisms of a certain genera 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.

**Analysis.** Computations were completed with the aid of a statistical package for personal computers (Statistica©). Due to the non-normal distribution of the data the results for each study center are given as median with range. Results of the different groups of microorganisms are given as relative frequencies.

**RESULTS**

**Farming characteristics.** The number of farmers and the farming characteristics of each study centre are given in Table 1 and Table 2.

Pig houses in Denmark were larger than in Germany with higher median numbers of animals (Tab. 1). The main difference between the Danish and German type of pig farming was a longer storage time of liquid manure and a longer interval of cleaning in Germany. Additionally, German animal houses more often had natural ventilation (windows, ventilation flaps), and ventilation control via air humidity sensor was not used in Germany (none of the German farmers vs. 25 out of 40 in Denmark).

The Swiss poultry houses under study had a median volume of 749 m\(^3\) with up to 20,000 animals per farm (Tab. 1). Most of the farms had several poultry houses. The interval of cleaning in poultry houses was longer than in pig houses (35 of the 36 farmers with a interval of cleaning longer than 1 month) whereas the storage time of manure was comparable to German pig houses.

The Spanish greenhouse workers (Tab. 2) were working inside greenhouses and/or the storage area of the farm. In this area, flowers and ornamental plants were prepared prior to transport. The main kinds of flowers cultivated in the greenhouses were Rosa sp., Gerbera jamesonii and Dianthus caryophyllus. The most important among a wide range of tasks for the farmers included work in the storage area, cutting flowers, watering plants, and spraying pesticides. Pesticides were used regularly (≥ once a week) in 28 out of 37 greenhouses.

**Dust and endotoxin concentrations.** The median total dust concentrations in each study centre are given in Table 3. Comparable dust concentrations were seen in swine confinement buildings and poultry houses. The total dust concentrations were lowest in greenhouses. The
endotoxin concentrations in total dust were highest in poultry houses and lowest in greenhouses.

**Ammonia, carbon dioxide, temperature, relative air humidity, and air velocity.** Ammonia and carbon dioxide was found to be highest in the poultry confinement buildings (Tab. 3). Inside these buildings, temperature and air velocity were lowest.

**Airborne microorganisms.** Concentrations of airborne microorganisms were measured in pig houses (Denmark), poultry houses (Switzerland) and greenhouses (Spain). The findings give a current overview of the microbiological status of the air in farming environments (Tab. 3, Fig. 1) with respect to potential hazardous microorganisms.

The highest total and active fungus concentrations were detected in poultry houses compared to pig houses and greenhouses. Comparing the different taxa of fungi, in poultry houses *Eurotium* spp. (52.8%, 30.8%, and 2.7%, respectively) and thermophilic fungi (19.4%, 5.1%, and 2.9%, respectively) were more frequently detected than in pig or greenhouses. The predominant fungus taxa recovered in greenhouses were species of *Cladosporium* (83.8%) and *Botrytis* (32.4%).

Bacteria concentrations were high in all animal houses. *Bacillus* spp. were found in nearly one third of all specimen whereas *Streptomyces* spp. were more often detectable in pig houses than in poultry confinement houses or greenhouses (59.0% vs. 27.8% and 16.2%, respectively).
DISCUSSION

This study illustrates the range of the air quality in working environments in swine and poultry barns as well as in greenhouses in 4 European countries, high concentrations of dust and endotoxins in randomly selected swine and poultry confinement buildings, and elevated levels of bacteria and moulds, not only in animal houses, but also in flower and plant production.

The given farming characteristics reflect the wide spectrum of animal confinement buildings and greenhouses resulting in different exposure conditions inside these buildings. Due to the random sampling procedure in Denmark, Switzerland and Spain it could be assumed that these farms represent a typical range of farming characteristics and exposure conditions in the respective area. The comparison to German swine confinement houses may be biased due to differences in recruiting subjects. The selected German farmers complained of work-related respiratory symptoms. Thus, one might speculate that the less “hygienic” confinement houses causing a higher risk for respiratory diseases are over-represented in the German sample. On the other hand, these farmers may be, e.g., more sensitive to the exposures in the work environment. Overall, we saw a longer cleaning intervals inside the poultry houses, and differences in ventilation in poultry confinement houses compared to swine confinement buildings. Additionally, inside the poultry confinement houses the air velocity was lower resulting in a lower air exchange rate in these buildings. Therefore, the higher ammonia and carbon dioxide concentrations in poultry houses may be related to

this finding. Using Spearman’s rank correlation we 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). Inside greenhouses, the frequent use of pesticides seems to be important. In a cross-sectional survey Wilkins et al. [37] reported recently that involvement with pesticides may induce symptoms of cough.

A single measurement of gas exposure was performed inside all working areas. One might argue that such a single measurement is not representative for the working day. However, in the Swiss poultry houses and German swine confinement areas a second measurement of NH$_3$ and CO$_2$ was performed in the early afternoon. These measurements were slightly lower than the morning measurements (median (range) of the German and Swiss measurements combined: NH$_3$ 10 (<5–50) ppm vs. 10 (<5–60) ppm, respectively, CO$_2$ 1,500 (400–>3,000) vs. 1,600 (300–>3,000) ppm, respectively).

Comparing the total dust concentrations inside animal confinement houses to published data no differences could be observed [2, 5, 6, 9, 12, 13, 26, 33]. In general, there are no exposure limits specific for organic dust in the working environment. Specific limits of 2.4 mg/m$^3$ for total dust in livestock buildings were suggested by Donham and Cumro [9]. These limits were exceeded in 80% of the animal houses under study. As expected, dust concentrations in greenhouses were low.

Our results on the endotoxin concentrations in animal confinement units show good agreement with some recent studies [2, 6, 12, 32, 35]. Not all of these studies were conducted on a personal basis, but Donham et al. [11] found that personal sampling was more strongly related to pulmonary function than area sampling. In our study we were only able to perform the endotoxin measurements once. Additionally, sampling was undertaken over multiple seasons. Preller et al. (1995) [27] found a large day-to-day variability in endotoxin measurements taken in swine confinement buildings. However, the large variability of endotoxin measurements seen between swine confinement buildings, poultry houses and greenhouses is estimated to be higher than the intrapersonal variability. The major contributors to endotoxin-contaminated organic dusts are animal feces and bacteria-contaminated plant materials such as grain or cotton. Therefore, the low amounts of endotoxins in greenhouses and storage areas in Spain are not surprising but no data on endotoxin contents in greenhouses have been published before. Recently, no exposure limits are available for endotoxins. There are various suggestions for an exposure standard ranging from 5–200 ng/m$^3$. The National Health Council of the Netherlands has recently proposed an exposure limit of 4.5 ng/m$^3$ while the International Commission on Occupational Health proposed an occupational exposure limit of 12.5 ng/m$^3$.

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 [14]. Methods detecting viable microorganisms have the largest potential for the identification of bacterial species. Therefore, it seems important 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, and some fungi for occupational asthma in farmers [18]. As in our study, Seedorf and co-workers [32] found the highest levels of fungi and bacteria in poultry houses. The levels of microorganisms found in the study of Seedorf et al. [32] were lower than in our survey but these were collected on an area basis. In contrast, Blomquist [3] described airborne fungal spores of more than $1 \times 10^8$ cells per m$^3$ in pig houses but only between $1 \times 10^7$–$1 \times 10^8$ cells per m$^3$ in greenhouses.

The species of fungi found inside the farm buildings characterize the climatic conditions in these buildings. Aspergillus spp. and Eurotium spp. (part of the Aspergillus glauces group) grow best under climatic environments with high air humidity and high temperature and were thus mostly detected in animal confinement houses. On the other hand, Botrytis spp. were mainly found inside greenhouses. These species grow best in warm regions with the growing of fruits and vegetables [18], conditions represented in the Spanish greenhouses. Beside these fungi, Cladosporium spp. and Alternaria spp., types of outdoor particles, predominated inside greenhouses, probably originating from the outside air [16]. The highest prevalence of Streptomyces spp. was seen inside swine confinement buildings. As described by Dalphin et al. [7], for dairy farmers the prevalence of Streptomyces spp., in the confinement house depends on the mode of storage and drying of hay at the farm. Therefore, our results indicate a high prevalence of microorganisms which may provoke type I and type III allergies inside randomly selected farming environments [19, 20]. It is important to bear in mind that these microorganisms were also found in greenhouses.

In conclusion, the personal measurements of dust, endotoxins and microorganisms under standardized conditions in a wide range of farming environments have shown that on randomly chosen farms farmers were exposed to potentially hazardous levels of air contaminants such as dust, endotoxins, and microorganisms. Further analyses are to be done to describe the association between farm characteristics, measures of exposure and respiratory health of the respective farmers. These results could provide the necessary information for the development of intervention strategies in order to reduce potential health hazards in the farming environment.

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