Chemical and Biological Hazards Prevention

Studies and Research Projects

REPORT **R-845**

Archaea in Bioaerosols in Dairy Farms, Poultry Houses and Wastewater Treatment Plants and Their Role in Lung Inflammation

Pascale Blais Lecours
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PEER REVIEW

In compliance with IRSST policy, the research results published in this document have been peer-reviewed.

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ABSTRACT

Bioaerosols are aerosols composed of solid particles or liquid droplets measuring 0.002 to 100 µm and carrying live microorganisms or molecules derived from living organisms. These aerosols are found in high concentrations in many work environments. Though many occupational respiratory diseases are associated with bioaerosols, their etiology is often unknown. The exact composition of the bioaerosols responsible for these respiratory diseases must be clearly determined so we can understand to what exactly workers are exposed.

We recently documented unsuspected airborne microorganisms (archaea) in swine confinement buildings. In this study, molecular biology methods were used to describe the biodiversity of airborne bacteria and archaea in a number of work environments so bioaerosol exposure of workers could be determined. We thus characterized the bacterial and archaeal content of bioaerosols in dairy barns, poultry houses and wastewater treatment plants and determined the exposure of dairy-farm workers to *Saccharopolyspora rectivirgula* (SR), the agent responsible for Farmer's Lung. In addition, we used a mouse model of chronic airway exposure to characterize the immunogenicity of two species of airborne archaea found in different work environments, *Methanobrevibacter smithii* (MBS) and *Methanosphaera stadtmanae* (MSS).

The quantitative polymerase chain reaction (qPCR) technique was used to quantify airborne bacteria and archaea in dairy barns, poultry houses and wastewater treatment plants. Denaturing gradient gel electrophoresis (DGGE) was used for biodiversity characterization of these microorganisms. The ELISA technique was used to measure MBS-, MSS- and SR-specific antibodies in workers' blood plasma. To study the proinflammatory effects of archaea, C57BI/6 mice were instilled intranasally with three different concentrations of two archaeal species (MBS and MSS) three times a week for three consecutive weeks.

Up to 10^8 total bacteria and 10^6 total archaea per m^3 of air were detected in the dairy barns. Similar quantities of airborne archaea were found in the poultry houses, whereas up to 10^8 total bacteria and 10^4 total archaea per m^3 of air were found in the wastewater treatment plants. MBS and MSS, two immunogenic archaeal species, were also detected in the farm air sampled. Despite recommendations to farmers regarding storage of hay, up to 10^7 copies of the SR 16S rRNA gene per m^3 of air were detected. In fact, exposure to this actinomycete was greater in dairy farm workers than in the control group. Histopathologic studies of the lungs of mice exposed to archaea demonstrated lung alterations, and these were more severe in mice instilled with MSS. The mouse model also made it possible to demonstrate that MSS induces greater production of activated myeloid dendritic cells in the lungs than MBS.

These results demonstrate the complexity of bioaerosols in agricultural and industrial environments, some components of which (such as archaea) may play a role in the development of occupational respiratory diseases. These microorganisms can cause lung inflammation, the intensity depending on the species of archaea. We are just beginning to explore the presence of these archaea in our environment and to understand our response to these little known agents. Their role as protective, immunostimulatory, proinflammatory or tolerated agents merits further exploration.

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1. INTRODUCTION

1.1 Bioaerosols

Bioaerosols are suspensions of airborne particles 0.002 to 100 µm in aerodynamic diameter that originate from or depend on living organisms (e.g., plants, fungi, bacteria, archaea, animals, humans, protists or viruses).[1] The particles may consist of entire microorganisms or fragments thereof, proteins, toxins, metabolic waste or microscopic plant structures (e.g., pollen, spores or plant fragments). Bioaerosols can affect the health of living organisms exposed to them because of their infectivity, allergenicity, toxicity or pharmacological properties.[2]

1.2 Bioaerosol sampling methods

Bioaerosols have been studied in a variety of work environments, including farm buildings,[3-5] sawmills,[6, 7]dentists' offices,[8] peat moss processing plants[9] and machining plants.[10, 11] However, the traditional culture-based sampling techniques used in these studies do not allow a full description of airborne microorganisms, as most are non-culturable.[12] It is now possible to use several different types of samplers together with molecular biology techniques to fully describe airborne microbial biodiversity. Today, with impingers, such as the Coriolis (Bertin Technologies, Montigny-le-Bretonneux, France)[13] or the BioSampler (SKC Inc.), two-stage cyclones, such as the BC 251 (NIOSH),[14] or filter samplers, such as the IOM cassette (Institute of Occupational Medicine), all airborne microorganisms (culturable as well as non-culturable) can be captured and subsequently identified using molecular approaches.

1.3 Archaea: a major component of bioaerosols

1.3.1 Description

Archaea are one of the three primary domains of life, along with eukarya and bacteria.[15] Archaea are divided into several phyla, the two main ones being the *Euryarchaeota* and the *Crenarchaeota*. The *Euryarchaeota* include all methanogenic and halophilic species as well as a number of thermaphilic and psychrophilic microorganisms. The *Crenarchaeota* include the hyperthermophilic archaea.[16-18] In addition to the *Korarchaeota* phylum, which to date includes only a single cultivated species, Brochier-Arman et al. (2008) suggest a fourth archaeal phylum, the *Thaumarchaeota*, encompassing the mesophilic *Crenarchaeota*.[16]

Archaea share some characteristics with bacteria and others with eukarya. Their information processing system (DNA replication, transcription, translation and repair) is similar to that of eukaryotes, but they have a bacteria-like metabolism.[17-19] Archaea also have properties that are all their own, such as the ability of many to grow in extreme environments, resistance to a number of antibiotics, a cell wall that lacks peptidoglycan and a unique lipid membrane. In fact, the lipids found in archaea (mainly archaeols and caldarchaeols) are not found in any other forms of life. These are polar lipids, in which the carbon chain is generally saturated and bound to glycerol by ether linkages.[20, 21] Caldarchaeols characteristically form a lipid monolayer in the archaeal membrane, unlike archaeols, which form a lipid bilayer. [22-24] The lipid composition of archaeal membranes varies greatly depending on the species,[25, 26] and Choquet et al. have

demonstrated that a higher proportion of caldarchaeols in the membrane means greater archaeobacterial resistance to hydrolysis and oxidation.[26]

1.3.2 Archaea in bioaerosols

For a long time, scientists believed that archaea were only present in extreme environments, such as acidic or hot springs, super-salty pools or the depths of the sea. As archaeal growth and cultivation requirements are very difficult to meet, these microorganisms have been and still are little studied by microbiologists.

However, with today's innovative molecular biology techniques, archaea have been detected in environments much less hostile to human beings. Methanogens, for example, have been found in swine manure,[27, 28] cow manure[29] and human feces.[30] These archaea are susceptible to aerosolization and can appear in large numbers in the bioaerosols in many work environments—on farms, for example (in swine confinement buildings, dairy barns and poultry houses) or in wastewater treatment plants. In fact, in 2009 Nehmé et al. demonstrated the presence of up to 10^8 archaea/m^3 of air in swine confinement buildings.[31] The bioaerosols studied were composed mainly of two methanogenic archaeal species: *Methanobrevibacter smithii* (MBS) and *Methanosphaera stadtmanae* (MSS). This was the first study to show presence of airborne archaea and it was made possible thanks to molecular approaches. In fact, methanogens cannot be detected with traditional culture techniques as they are extremely sensitive to oxygen and thus greatly affected by aerosolization.

1.4 Immunogenic potential of archaea

Archaea are composed of potentially immunogenic molecules, such as lipids and membrane proteins. The properties of archaeal lipids differ substantially from those of bacterial or eukaryotic cell lipids. Archaeal lipids are stable and their uptake by phagocytic cells is greater than that of conventional liposomes.[26, 32] The immunogenic potential of archaeal lipids, also called archaeosomes, and their use as adjuvants in vaccines, are being studied. In addition, it has been demonstrated that the lipids of some archaea induce an immune response characterized by recruitment and activation of macrophages and dendritic cells at the injection site as well as development of a humoral and cell-mediated mixed T_H1/T_H2 immune response. [33-37] Krishnan et al. have demonstrated that MBS lipids induce a stronger immune response than MSS lipids.[36]

Recently, Yamabe et al. identified the first antigenic archaeal protein capable of being recognized by the human immune system.[38] These researchers demonstrated that Group II chaperonin, the heat-shock protein from *Methanobrevibacter oralis*, an archaea that may be implicated in periodontitis, induces production of specific antibodies in the serum of patients with the disease. Group I chaperonins, also called HSP60, are found in bacteria, whereas Group II chaperonins are associated with archaea (thermosomes) and eukarya (CCT-TRiC).[38, 39] HSP60 is known to be a potent antigen in bacteria and may be one of the main immunogenic molecules during an infection. In fact, during bacterial stress, this protein accumulates on the cell surface, increasing its immunogenicity.[40, 41]

1.5 Respiratory diseases associated with bioaerosols

Bioaerosols, also known as organic dust, are found in many work environments, including farms, peat moss processing plants, sawmills and machining plants, and they cause respiratory problems in workers.[10, 42-45] The aerodynamic diameter of the particles that bioaerosols contain is less than 200 µm, and when particles under 5 µm in diameter are inhaled, they can reach the bronchi and the alveoli, where they can have a toxic and/or inflammatory effect.

People who work in environments that are highly contaminated by bioaerosols, such as swine confinement buildings and dairy barns, may inhale a very large quantity of biological particles. For example, as a worker in a swine confinement building inhales up to 42 m^3 of air in a work day and bioaerosols contain an average 10^8 bacteria per m^3, this worker inhales up to 10^{10} bacteria in a work day.[46, 47] Inhalation of organic dust can cause infectious diseases if certain pathogenic microorganisms are present in the air. Exposure to bioaerosols can also trigger toxic reactions and cause chronic bronchitis and organic dust toxic syndrome. Last, exposure to bioaerosols can be associated with hypersensitivity reactions and respiratory disorders such as allergic asthma, rhinitis and hypersensitivity pneumonitis. Dairy barns, for example, are linked to respiratory diseases such as Farmer's Lung, a form of hypersensitivity pneumonitis, whereas swine confinement buildings are more likely to be associated with the development of chronic bronchitis. The inflammatory responses of these diseases fall into two groups: T_H1 and T_H2. T_H1-mediated diseases are characterized by a cellular immune response involving IFNγ, IL-12 and IL-6. T_H2-mediated diseases potentiate a humoral response and involve the cytokines IL-4, IL-5 and IL-13.[48] Hypersensitivity pneumonitis is a T_H1 disease characterized by accumulation of large numbers of lymphocytes in the lungs as well as fibrosis and granulomas. Allergic asthma is a T_H2-mediated disease characterized by dense infiltration of granulocytes into the lungs, where tissue remodelling and bronchial hyperreactivity may occur.[49-51]

1.6 Rationale

There have been a number of studies of bioaerosols to demonstrate the effect of some of their components on workers' health. In fact, certain pathologies, including organic dust toxic syndrome and chronic bronchitis, have been attributed to the presence of airborne endotoxins (a component of the cell wall of gram-negative bacteria) in farm buildings.[52-54] *Saccharopolyspora rectivirgula* (SR), a bacteria of the actinomycetes group, has been described as the etiological agent in hypersensitivity pneumonitis (Farmer's Lung).[55] However, the etiology of many respiratory diseases in workers caused by bioaerosols remains to be determined. Thanks to new molecular biology techniques, surprising discoveries have been made about the composition of swine manure and associated bioaerosols. Nehmé et al. demonstrated in 2008 that the majority of airborne bacteria in swine confinement facilities are gram positive and are from swine manure.[47] This discovery undermined the importance of endotoxins in the etiology of respiratory diseases associated with bioaerosols. With the discovery in 2009 of a very large number of airborne archaea in swine confinement buildings,[31] the role of bioaerosol components till then unknown took on importance. This 2009 study by Nehmé et al. was the first to demonstrate the presence of airborne archaea and made it possible to consider involvement of archaea and non-culturable components of bioaerosols in the development of occupational respiratory diseases. No studies to date have looked at the impact of archaea on respiratory health.

1.7 Hypothesis

The project described herein investigated the hypothesis that archaea are a major component of the bioaerosols found in a number of work environments and that they play a role in respiratory diseases caused by bioaerosols.

2. SPECIFIC OBJECTIVES

Three specific objectives were set in order to verify the hypothesis of this research project:

1. Characterize bioaerosol bacteria and archaea present in dairy barns, wastewater treatment plants and poultry houses:

    a. Develop effective molecular detection techniques for all species of archaea.

    b. Compare the efficacy of three air samplers in dairy barns.

2. Determine presence of IgG specific to *Methanobrevibacter smithii*, *Methanosphaera stadtmanae* and *Saccharopolyspora rectivirgula* in the serum of workers in swine confinement buildings, dairy barns and wastewater treatment plants.

3. Characterize the immunogenicity of *Methanobrevibacter smithii* and *Methanosphaera stadtmanae* in a mouse model of chronic airway exposure.

3. METHODS

3.1 Sampling

3.1.1 Dairy barns

Bioaerosols were sampled in 13 dairy barns in eastern Québec. Air samples were collected from areas where the animals are kept and cared for. The sampling took place in winter, at the time of maximum confinement. The air samplers were positioned about a metre from the ground and operated for about four to five hours during the morning or evening milking period. Three different models were used: the NIOSH BC 251, flow rate 10 L/min, plugged into an AirCon-2 pump (Gilian); three IOM cassettes (SKC, Ancaster, Ontario, Canada), flow rate 2 L/min, loaded with 25-mm gelatin membranes and plugged into a Gilair-5 (Levitt-Safety Ltd. Dorval, Québec, Canada); and the Coriolis µ sampler (Bertin Technologies, Montigny-le-Bretonneux, France), operating at 100 L/min. Bacterial and archaeal capture capabilities of the three samplers were compared.

3.1.2 Wastewater treatment plants

The protocol used in the dairy barns was also employed at wastewater treatment plants, but only one type of sampler was used, the IOM cassettes. Eight sites at two different water treatment plants were sampled in winter. At each plant, four different sites were studied: the screening site, the detritis tank, the stilling basin and the biofiltration site.

3.1.3 Poultry houses

Samples were collected in Saskatchewan at 15 establishments where animals are raised in multi-stacked cages and 15 establishments where the animals are floor-housed. Area as well as personal samples were collected. Marple cascade impactors (Thermo Electron Corp., Waltham, MA, USA) with 5-µm filters were used, flow rate 2 L/min.[56] Stages 3, 4 and 5 (>3.5µm) were used for the analyses.

3.2 Sample processing

3.2.1 IOM

The three gelatin filters from the IOM cassettes were dissolved in 15 mL of phosphate buffer. The solution was divided into aliquot fractions of 1.5 mL and centrifuged for 10 min at 21,000 g. The cell pellets thus obtained were stored at -20^{o}C until DNA extraction.

3.2.2 NIOSH 251

The NIOSH sampler filters (stage 3) were transferred to sterile plastic tubes containing 2 mL of a solution of NaCl (0.9%) and Tween 20 (0.05%). This solution had been added to the first stage (2 mL) as well as the second stage (1 mL) of the sampler. The suspensions obtained from the

three stages were homogenized by vortexing for 15 minutes then divided into aliquot fractions and centrifuged. The pellets were stored at -20°C until DNA extraction.

3.2.3 Coriolis µ

The liquid samples were divided into 1.5-mL aliquot fractions and centrifuged for 10 min at 21,000 g. The pellets thus obtained were stored at -20°C until DNA extraction.

3.3 DNA extraction

A QIAamp DNA extraction kit (Qiagen, Mississauga, Ontario, Canada) was used for total DNA extraction of microorganisms in the samples. The manufacturer's protocol for isolating bacterial DNA was followed, including a 1.5-hour proteinase K digestion step.

3.4 Quantitative PCR (qPCR)

All real-time qPCR assays were performed on a DNA Engine Opticon 2 (Bio-Rad, Mississauga, Ontario, Canada). iQ SYBR green Supermix and iQ Supermix kits (Bio-Rad Laboratories, Hercules, CA) were used to quantify the samples. Table 1 lists primers, probes and references as well as the thermal protocols used.

Table 1: Primers and probes used for PCR and qPCR assays in this study

Primer/probe	Target	Nucleotide sequence (5'-3')	Reference
A751F	Sense primer quantification of total archaea	CCG ACG GTG AGR GRY GAA	57
A976R	Antisense primer quantification of total archaea	YCC GGC GTT GAM TCC AAT T	58
EUB F	Sense primer quantification of total bacteria	GGT AGT CYA YGC MST AAA CG	59
EUB R	Antisense primer quantification of total bacteria	GAC ARC CAT GCA SCA CCT G	59
EUB probe	Fluorescent probe quantification of total bacteria	FAM-TKC GCG TTG CDT CGA ATT AAW CCA C-TAMRA	59
Sac-86F	Sense primer quantification of SR	TGT GGT GGG GTG GAT GAG T	60
Sac-183R	Sense primer quantification of SR	ACC ATG CGG CAG AAT GTC CT	60
A333F	Sense primer archaeal DGGE	TCC AGG CCC TAC GGG	58
A751R (GC)	Antisense primer archaeal DGGE	TTC RYC YCT CAC CGT CG	57
GC archaea	GC clamp antisense primer archaeal DGGE	CGC CCG CCG CGC CCC GCG CCC GGC CCG CCG CCC CCG CCC C	61
341F	Sense primer bacterial DGGE	CCT ACG GGA GGC AGC AG	61
907R	Antisense primer bacterial DGGE	CCG TCA ATT CCT TTG AGT TT	62
GC *bacteria*	GC clamp sense primer bacterial DGGE	CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG G	61
Mnif 202 F	Sense primer MBS	GAA AGC GGA GGT CCT GAA	63
Mnif 353R	Antisense primer MBS	ACT GAA AAA CCT CCG CAA AC	63
Mnif Probe	Fluorescent probe MBS	FAM- CCG GAC GTG GTG TAA CAG TAG CTA –BHQ-1	63
MSS 122F	Sense primer MSS	CTA ACA TCA AAG TAG CTC C	64
MSS 414R	Antisense primer MSS	TCC TCT AAG ACC GTT T	64

3.5 PCR-DGGE

For bacterial analysis, the variable regions V3 to V5 of the gene coding for 16S rRNA were amplified by PCR (586 bp) using primers GC-341f and 907r (Table 1) and the PCR conditions described by Yu and Morrisson.[62] Archaeal biodiversity was determined using primers A333F and GC-A751R, resulting in 418-bp band under the PCR conditions described by Blais Lecours et al.[65] Amplicons were visualized on 1.5% agarose gel, and the amount of DNA generated was estimated with the help of an EZ Load Precision Molecular Mass Ruler (Bio-Rad) and the software Gene Tools (SynGen).

DGGE profiles were generated with the method described by Muyzer et al.[61] and a DCode system (Bio-Rad). Hence 100 ng of PCR products were loaded on a 0.5X TAE polyacrylamide gel with a denaturing gradient between 30% and 55% (100% denaturant = 7 mol/L urea and 40% v/v formamide). Electrophoresis was performed in 0.5X TAE buffer for 16 hours at 60 V and a temperature of 60°C. The DNA fragments were stained for 15 minutes with the fluorochrome SYBR Gold (Invitrogen) and visualized with a ChemiGenius 2Xe imager (SynGene). The DNA bands present were excised and reamplified by PCR before being sent for sequencing.

The software GelCompar II, version 6.05 (AppliedMaths, Belgium), was used to standardize and compare all profiles obtained. To do this, a molecular standard was employed, with 1% tolerance in band position. A Pearson correlation coefficient was used to calculate profile similarity, and UPGMA (unweighted pair-group method using arithmetic averages) was used for clustering.

The sequence of each DGGE band excised from the gel was compared with sequences in the GenBank database using the BLASTN[66] similarity search tool distributed by the National Center for Biotechnology Information (NCBI) [www.ncbi.nlm.nih.gov/BLAST/]. Isolate affiliation was determined based on sequence similarity.

3.6 Specific IgG detection for worker archaeal and SR exposure assessment

3.6.1 Methanogen cultivation

Antigens typical of those inhaled by workers were obtained by cultivating the methanogens *Methanosphaera stadtmanae* (MSS) and *Methanobrevibacter smithii* (MBS), these two species constituting the majority of airborne archaea in swine confinement buildings. The Hungate technique for cultivating anaerobic microorganisms was used together with knowledge acquired during training in the laboratory of Robert Forster of Agriculture Canada.[67] Pellets obtained were washed, frozen and lyophilized for subsequent use.

3.6.2 IgG blood levels in workers

Workers in dairy barns and wastewater treatment plants were recruited for blood draws. Thanks to the Regroupement stratégique Bioaerosols et santé respiratoire, blood serum drawn for an earlier project from workers in swine confinement facilities was made available. This stage of the project involving human subjects was approved by the research ethics committee of the

Québec Heart and Lung Institute (IUCPQ - CER-20508). The ELISA technique was used to determine exposure of workers in dairy barns and wastewater treatment plants to MSS, MBS and *Saccharopolyspora rectivirgula* (SR). Workers who inhale these microbial species present in bioaerosols on a daily basis will develop IgG antibodies specific to them. An ELISA assay was thus used to detect such antibodies, with the help of a protocol developed by the team of Dr. Duchaine.[44, 68] The limit of detection was determined based on the response of control subjects who had had no contact with dairy farms or wastewater treatment plants. However, the controls could have had contact with MSS and/or MBS and/or SR in some other way (contaminated workplace, frequent contact with animal feces, etc.), which meant their serum might contain MSS-, MBS- or SR-specific IgG antibodies—and this could affect the results. To address this problem, the number of control subjects in the study was increased, and the frequency of positive results in each group was compared.

3.7 Immunogenicity of MBS and MSS in airways in a mouse model

A mouse model of chronic lung exposure studied extensively because the mice develop symptoms like those of hypersensitivity pneumonitis was used to characterize the pulmonary inflammatory response to archaea.[69-71] Groups of six C57BL/6 mice were intranasally instilled with saline, MBS or MSS three times a week for three consecutive weeks and then euthanized four days after the last instillation (Fig. 1). Bronchoalveolar lavages (BAL) were performed on each mouse (three 1-mL lavages of saline solution) to collect cells and cell mediators produced in the airways. Blood was also collected from the jugular veins of the mice to check for antibodies produced on exposure to MBS or MSS. BAL fluid total and differential cell counts were performed. One lung was removed from each mouse for histopathologic testing.

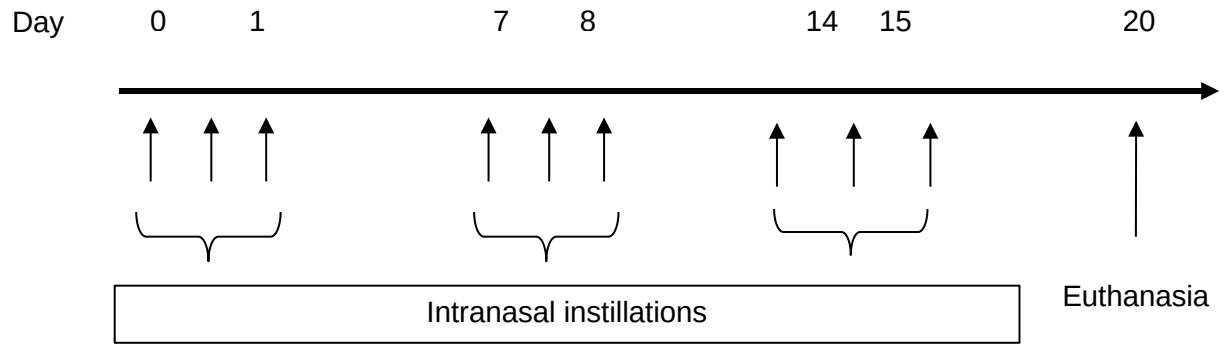

Figure 1: Mouse model instillation protocol for immunogenicity testing

3.8 Statistical analyses

3.8.1 Dairy barn data

Quantitative data were expressed using the mean and standard error of the mean (SEM). Total archaeal and total bacterial data were analyzed using a mixed analysis of variance (ANOVA) with two experimental factors: a fixed factor associated with comparison of the different

samplers; and a block variable analyzed as a random effect associated with the different farms sampled. The same statistical approach was used to compare data from the different NIOSH stages. Data from workers and controls were analyzed using Student's t-test. For some variables, values were $\log_{10}$-transformed to stabilize variance. The normality assumption was verified using Shapiro-Wilk tests after a Cholesky factorization. Brown and Forsythe's variation of the Levene test was used to verify the homogeneity of variance. Relationships between variables were expressed using Pearson's correlation coefficient. Results with p values ≤ 0.05 were considered significant. All analyses were conducted using the statistical package SAS (SAS Institute Inc., Cary, NC).

3.8.2 Poultry house data

Archaeal concentrations are expressed as raw data. However log transformations of these data were required for the statistical analysis. The unpaired Student's t-test was used to compare the different environments.

3.8.3 Mouse model data

Quantitative data are expressed using the mean and standard error of the mean (SEM), or the median and the interquartile range (continuous variables). For continuous data, a one-way ANOVA was used to compare the groups. For some variables, values were $\log_{10}$-transformed to stabilize variance. The normality assumption was verified using Shapiro-Wilk tests after a Cholesky factorization. Brown and Forsythe's variation of the Levene test was used to verify the homogeneity of variance. The histopathologic data were processed using a one-way ANOVA with a Poisson distribution. Results with p values ≤ 0.05 were considered significant. All analyses were conducted using the statistical package SAS (SAS Institute Inc., Cary, NC). The letters on the tables and figures indicate significant differences between the groups compared.

4. RESULTS

4.1 Airborne archaea and bacteria in the study environments

4.1.1 Dairy farms

4.1.1.1 Quantification of total airborne archaea and bacteria in dairy barns

First we demonstrated by qPCR that the bioaerosols from the dairy barns contained up to 10^6 archaea and 10^8 bacteria/m^3 air and that the results obtained from the three types of samplers were comparable (Fig. 2). Comparison of the performance of the IOM, NIOSH and Coriolis samplers (n = 13 for each type of sampler) showed not statistical differences (archaea: $p = 0.3$; bacteria: $p = 0.5$). Limits of detection were $4x10^2$ 16S rRNA genes for archaea and $2x10^3$ 16S rRNA genes for bacteria.

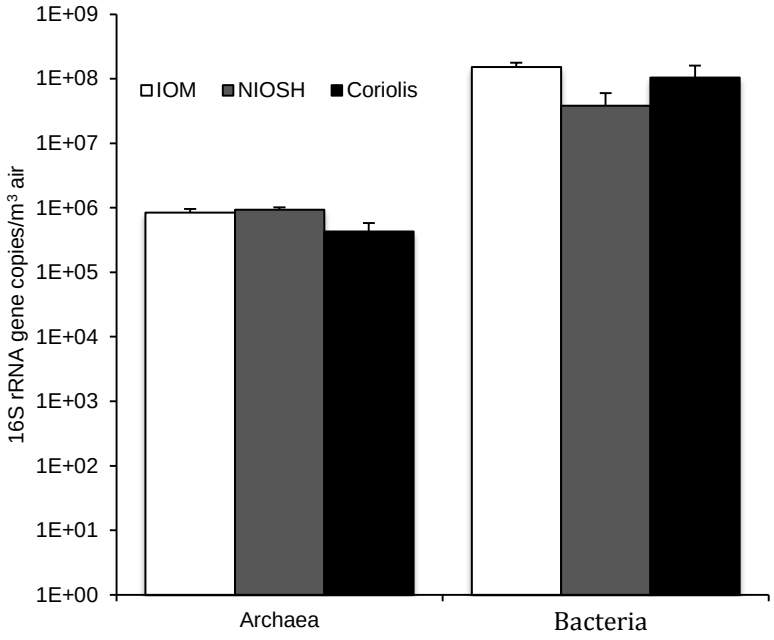

Figure 2: Archaeal and bacterial 16S rRNA gene concentrations in dairy barn air samples collected with three different samplers (mean ± SEM)

In addition, in the dairy barns there was no correlation between airborne archaeal and airborne bacterial concentrations (Fig. 3) with any of the three air samplers tested: the IOM (r = 0.262, $p = 0.4$), the NIOSH (r = 0.083, $p = 0.8$) and the Coriolis (r = 0.046, $p = 0.9$) (n=13 for each sampler type). Data in Fig. 3 are colour-coded by farm.

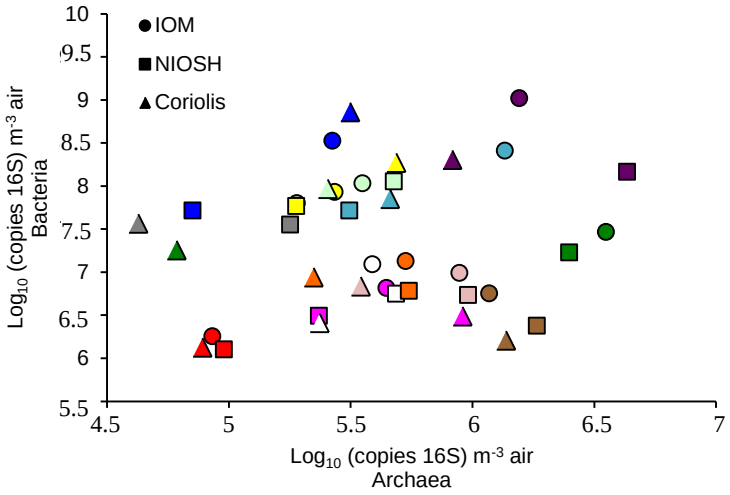

Figure 3: Lack of correlation between archaeal and bacterial 16S rRNA gene concentrations in dairy barn air samples

4.1.1.2 Quantification of airborne SR in dairy barns

Airborne *S. rectivirgula* (SR) was also quantified, as this microorganism is often found in dairy farms and can cause the lung disease known as Farmer's Lung. We thus demonstrated through qPCR that there was great variation in airborne concentrations of this microorganism in the dairy barn samples (n=13) (Fig. 4). An average of $1.4x10^6$ 16S rRNA genes/m^3 air were detected, but concentrations of airborne SR in some barns were below the limit of detection ($2x10^3$ genes/m^3 air), whereas concentrations as high as $1.3x10^7$ 16S rRNA genes/m^3 air were detected in others (Fig. 4).

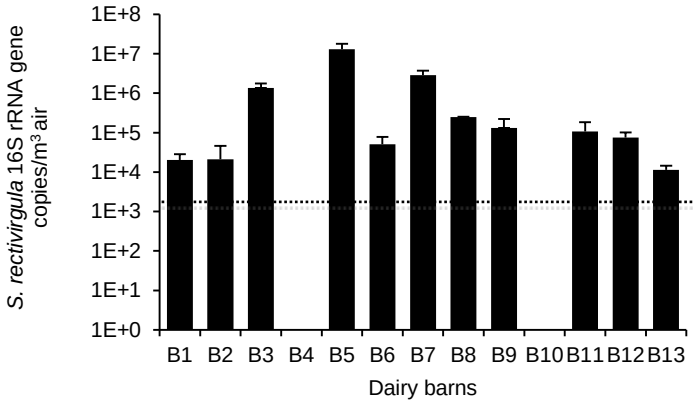

Figure 4: SR 16S rRNA gene concentrations in dairy barn air samples obtained with the IOM sampler (mean ± SEM). The dotted line at $2x10^3$ genes/m^3 air indicates PCR limit of detection.

4.1.1.3 Particle size distribution of total airborne archaea and bacteria in dairy barns

As Fig. 5 shows, most of the archaea and bacteria in the dairy barn air samples were collected in stage 1 of the NIOSH sampler; that is, the aerodynamic diameter of the particles was greater than 2.1 µm. Stage 2 collected particles no smaller than 0.41 µm, and stage 3 filtered out the smallest particles. Quantitative PCR was used to compare quantities of archaea and bacteria in the three stages of the NIOSH air samplers (n=13). The limit of detection was $1x10^2$ 16S rRNA genes for archaea and $6x10^2$ 16S rRNA genes for bacteria.

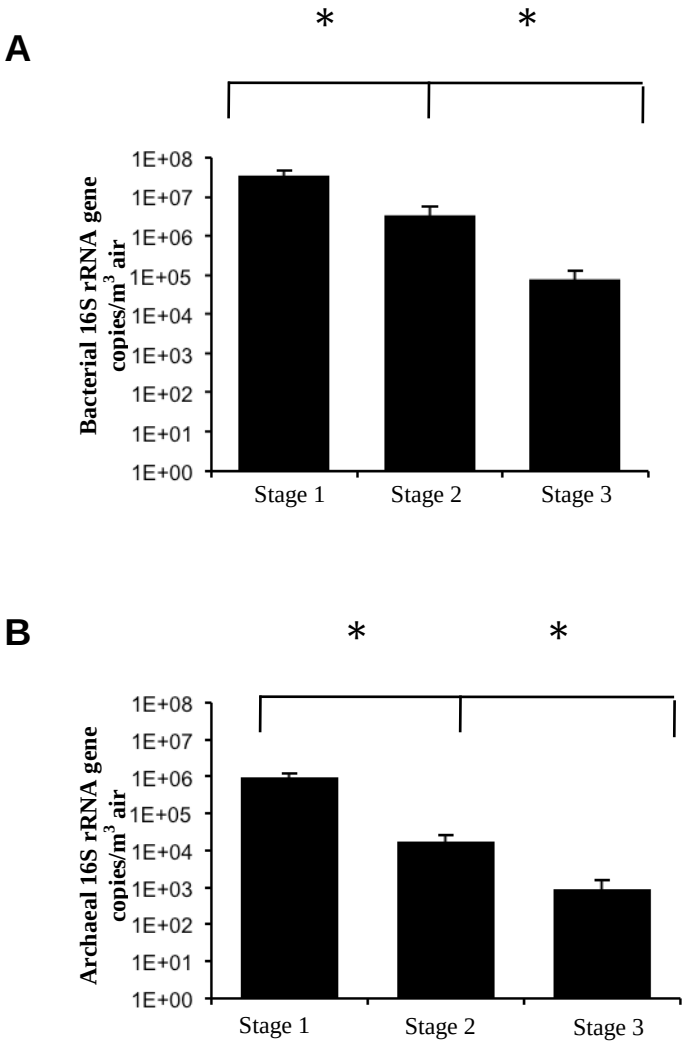

Figure 5: Airborne particle size distribution in dairy barns: archaeal (A) and bacterial (B) 16S rRNA gene concentrations in dairy barn air samples (mean ± SEM). *: $p < 0.005$

4.1.1.4 Main airborne archaeal and bacterial phylotypes detected in dairy barns using PCR DGGE

Methanogenic archaea as well as several species of bacteria are aerosolized in dairy barns. The PCR-DGGE method was used to identify the different species of archaea and bacteria in the air samples collected from the 13 dairy barns sampled (Table 2).

Table 2: Sequence matches for bands from DGGE gels containing dairy barn archaeal and bacterial DNA

Type of DNA	Band No.	Frequency (Number of positive samples)[α]	Most similar sequence	Length (bp)	% similarity
Archaeal					
	1	5	*Methanobrevibacter sp.* JQ267743	302	97
	2	2	*Methanobrevibacter smithii* JQ267744	313	97
	3	9	*Methanobrevibacter sp.* JQ267745	300	95
	4	12	*Methanobrevibacter sp.* JQ267746	305	95
	5	13	*Methanobrevibacter ruminantium* JQ267747	316	98
Bacterial					
	1	13	*Staphylococcus gallinarun* JQ267748	570	99
	1	13	*Crocebacterium ilecola* JQ267749	549	99
	2	12	*Oxalobacter sp.* JQ267750	507	97
	3	13	*Agrobacterium tumefaciens* JQ267751	543	99
	4	12	*Clostridium quinii* JQ267752	542	99
	4	12	*Staphylococcus sp.* JQ267753	564	99
	5	2	*Agrobacterium sp.* JQ267754	435	94
	6	12	*Corynebacterium variabile* JQ267755	550	98
	7	13	*Corynebacterium xerosis* JQ267756	484	98
	8	13	*Corynebacterium sp.* JQ267757	482	95

[α] Of a total of 13 dairy barns

4.1.1.5 qPCR quantification of airborne MSS and MBS in dairy barns

Two methanogens are frequently found in animal guts, *Methanosphaera stadtmanae* (MSS) and *Methanobrevibacter smithii* (MBS). Quantitative PCR (qPCR) was used to determine concentrations of these microorganisms in air samples collected from 13 barns with the Coriolis sampler (Fig. 6). MSS was detected in the samples from five dairy barns at concentrations ranging from $3.4x10^2$ to $2.0x10^3$ microorganism/m^3 air, whereas MBS was present at concentrations of $1.3x10^3$ to $7.9x10^3$ microorganism/m^3 air in four of the 13 dairy barns sampled. The limit of detection was $3.3x10^2$ genes specific to each of the microorganisms per m^3 of air.

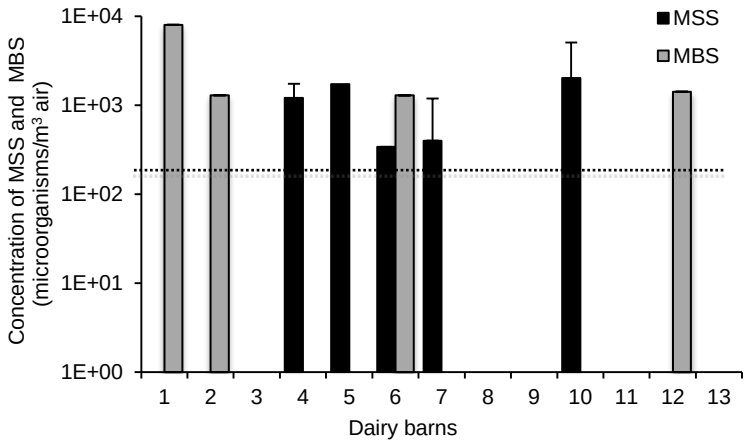

Figure 6: MSS and MBS concentrations in dairy barn air samples (mean ± SEM). The dotted line indicates the PCR limit of detection.

4.1.2 Poultry houses

4.1.2.1 Quantification of total airborne archaea in poultry facilities

Higher concentrations of airborne archaea were found in cage-housed than in floor-housed poultry facilities. This was true with both types of air sampling tested: area sampling of the poultry facility and personal sampling of air to which a worker is exposed (Fig. 7). The symbols along the X axis indicate facilities where no airborne archaea were detected.

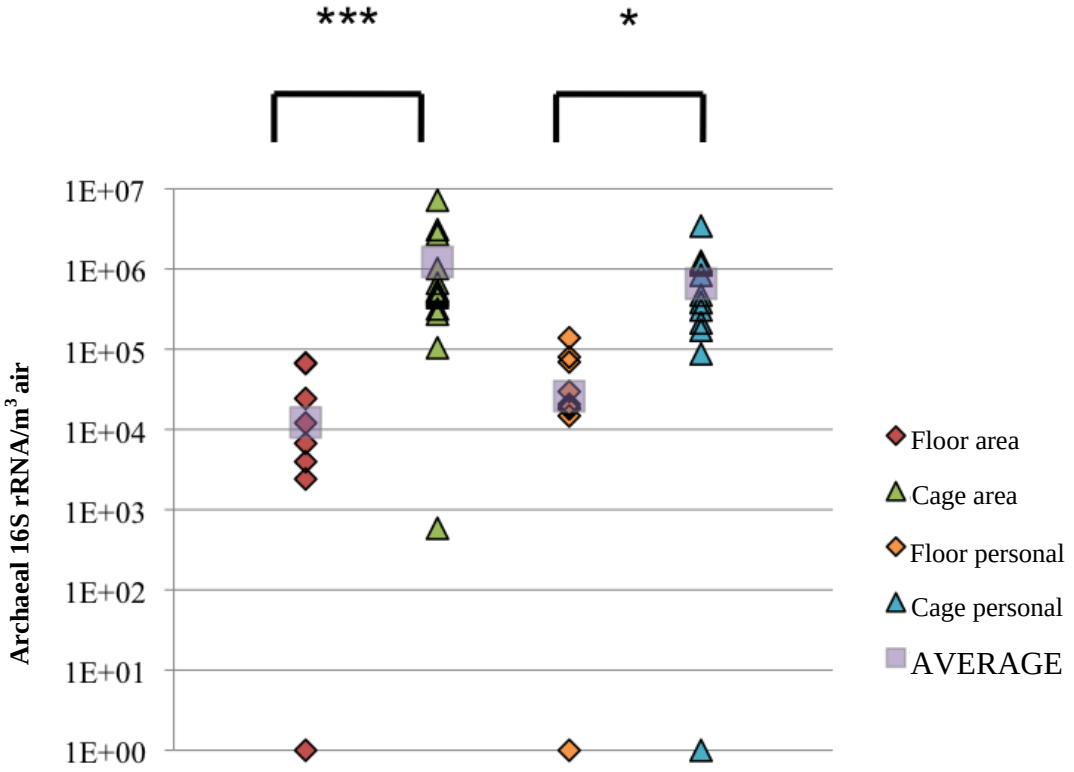

Figure 7: qPCR quantification of airborne archaea in cage-housed and floor-housed poultry facilities. Each point on the graph represents data from a single facility, with a grey square indicating the average for all facilities. The data were log-transformed prior to statistical analysis (*p<0.05 ***p<0.001).

4.1.2.2 Airborne archaea, bacteria, endotoxins and dust in personal and area samples: comparison of cage-housed and floor-housed poultry operations

Concentrations of airborne archaea, bacteria, endotoxins and dust in the area samples (Fig. 8A) and the personal samples (Fig. 8B) from the two types of poultry operations (cage-housed and floor-housed) were compared. Archaeal concentrations alone proved higher in the cage-housed operations—in both area and personal samples. As for the other parameters (bacteria, endotoxins and dust), values were always higher in the floor-housed operations.

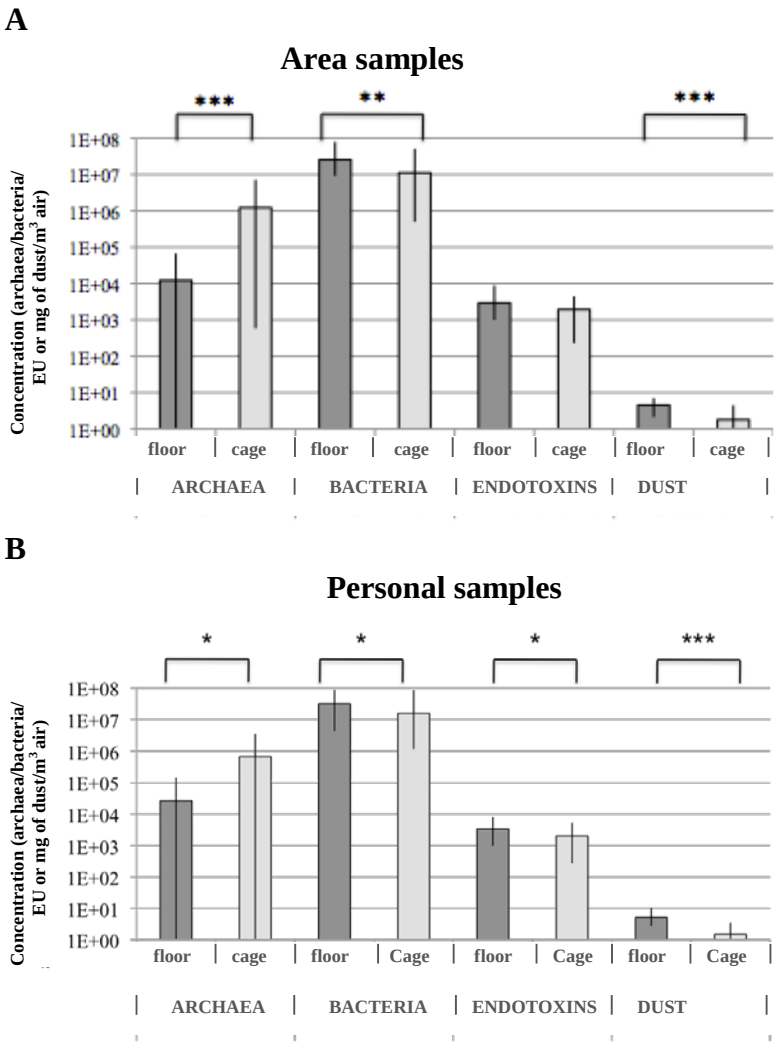

Figure 8: Average concentrations of archaea, bacteria, endotoxin and dust in bioaerosols from cage-housed and floor-housed poultry operations. The data were log-transformed prior to statistical analysis and are expressed in quantities of microorganisms (archaea and bacteria), endotoxin units or mg of dust per m^3 of air (*p<0.05 **p<0.01 ***p<0.001).

4.1.2.3 Principal airborne archaeal phylotypes detected in poultry houses by PCR DGGE

Two airborne methanogenic archaeal species and airborne halophilic archaeal species were detected in the poultry facilities (Table 3). PCR DGGE was used to identify the different airborne archaeal species in the poultry houses sampled. Neither of the two airborne methanogenic archaeal species (MBS and MSS) found in the dairy barns was detected in the air samples from the poultry operations.

Table 3: Sequence matches for bands from DGGE gels containing archaeal DNA from bioaerosols in cage-housed poultry operations

Band no.	Affiliation	Length (bp)	% similarity
1	*Methanobrevibacter woesei* (DQ445724.1)	321	100
2	*Methanosarcina mazei* (JN413085.1)	321	100
3	*Haloquadratum walsbyi* (FR746099.1)	217	100

4.1.3 Wastewater treatment plants

4.1.3.1 qPCR quantification of total airborne archaea and bacteria in wastewater treatment plants

Up to 10^4 archaea/m^3 air were detected at two of four sampling sites in the first plant only (Fig. 9). Limits of detection for this quantification were $4x10^2$ 16S rRNA genes for archaea and $2x10^3$ 16S rRNA genes for bacteria.

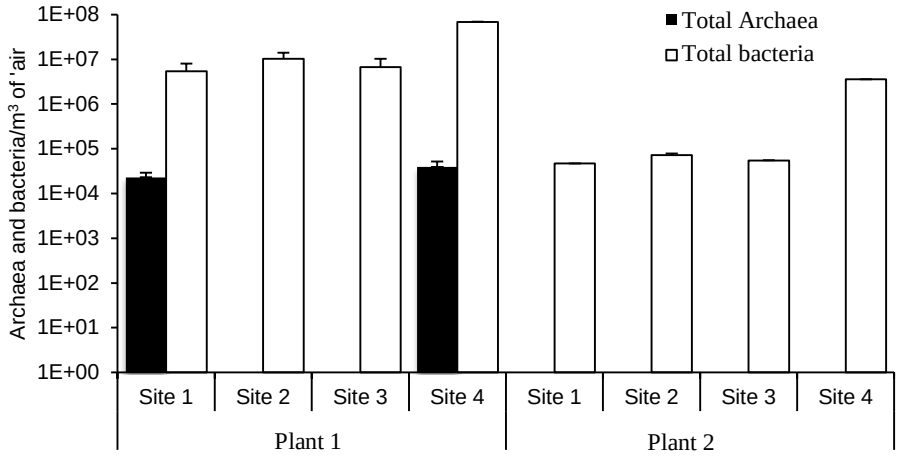

Figure 9: Archaeal and bacterial 16S rRNA gene concentrations in air samples from two wastewater treatment plants (mean ± SEM)

4.1.3.2 Main airborne archaeal phylotypes detected in wastewater treatment plants using PCR DGGE

Two methanogenic archaeal species and one halophilic archaeal species were detected in air samples from one of two wastewater treatment plants sampled (Table 4). The PCR-DGGE method was used to identify the archaeal species in air samples collected from the two wastewater treatment plants.

Table 4: Sequence matches for bands from DGGE gels containing archaeal DNA from a wastewater treatment plant

Band No.	Most similar sequence	Length (bp)	% similarity
1	*Methanospirillum sp.*	228	94
2	*Methanocorpusculum labreanum*	285	98
3	*Haloarcula sp.*	248	96

1 = Plant 1, site 4, NIOSH stage 1
2 = Plant 1, site 1, IOM
3 = Plant 1, site 4, NIOSH stage 3

4.1.3.3 qPCR quantification of airborne MSS and MBS in wastewater treatment plants

Airborne MSS and MBS were detected at one of the two wastewater treatment plants sampled. MSS was detected with the IOM sampler at site 1 of plant 1 at a concentration of $8.2x10^3$ MSS/m^3 air, whereas MBS was detected with this same sampler at a concentration of $1{,}59x10^4$ MBS/m^3 air at site 4 of plant 1. The limit of detection for these two microorganisms was $4x10^2$ microorganisms/m^3 air.

4.2 MBS-, MSS- and SR-specific IgG in serum of workers in swine confinement buildings, dairy barns and wastewater treatment plants

No significant results were obtained for presence of MBS- or MSS-specific IgG in the plasma of workers compared to control subjects at any of the work environments studied. Percentage positive plasma was not higher in the workers than in the controls. However, we did demonstrate higher concentrations of SR-specific IgG in the workers than in the control group (Table 5).

Table 5: SR-specific IgG in plasma of dairy farm workers compared to controls*

		Intensity of immune response		
		Negative	**Positive (total)**	**Strongly positive**
Control subjects (n=35)	n	30	5	1
	%	85.7	14.3	2,9
Workers (n=29)	n	18	11	3
	%	62.1	37.9	10.3

*Significant difference between workers and controls in distribution frequency ($p<0.05$)

4.3 Immunogenicity of MBS and MSS in a mouse model

4.3.1 Histology and lung damage

Haematoxylin- and eosin-stained lung sections were obtained from mice instilled with saline, MBS and MSS three times a week for three weeks (Fig. 10). MSS strongly induces the formation of tertiary lymphoid structures (arrows) compared to MBS. Specimens shown are representative of six individual observations per group.

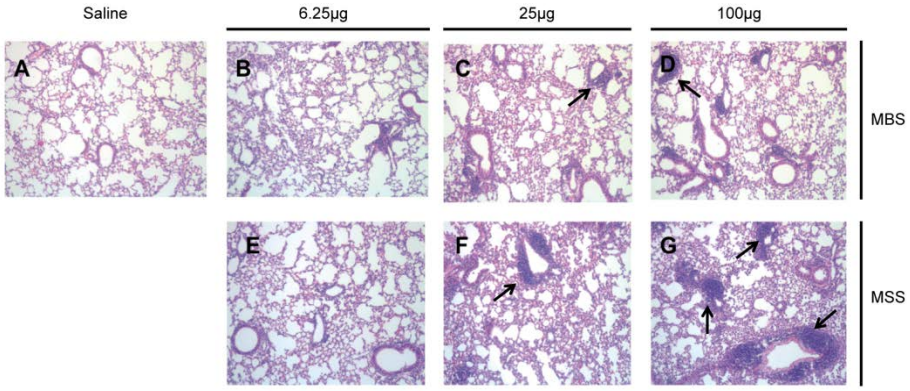

Figure 10: MBS- and MSS-induced histopathologic lung alterations in mice instilled three times a week for three weeks with (A) saline; (B) MBS 6.25 µg; (C) MBS 25 µg; (D) MBS 100 µg; (E) MSS 6.25 µg; (F) MSS 25 µg; or (G) MSS 100 µg. Arrows indicate tertiary lymphoid structures.

Table 6 shows average histopathologic scores for five criteria in each group of mice tested. No significant difference between MBS and MSS, regardless of the dose, was noted for perivascular infiltration of mononuclear cells and macrophage accumulation. However, alterations in these two respects were significantly more severe when the lungs were exposed to a 100-µg dose of MBS or MSS compared to lower doses of the same archaeal species. Regarding peribronchial infiltration of monocular cells and thickening of the alveolar septa, the severity of the alteration differed significantly depending on whether exposure was to MBS or MSS at all doses, but there

was no difference between doses of the same species. Last, there was no significant alteration in perivascular infiltration of granulocytes when mice lungs were exposed to MBS or MSS, no matter what the dose.

Table 6: Histopathological lung alterations in mice exposed to MBS or MSS

	Saline	MBS (µg)			MSS (µg)		
Criterion		**6.25**	**25**	**100**	**6.25**	**25**	**100**
Perivascular infiltration of mononuclear cell	0	1	2	3[a]	2	3	4[a]
Perivascular infiltration of granulocytes	0	0	0	0	1.5	0	0
Peribronchial infiltration of mononuclear cell	0	0[a]	1[a]	0[a]	1[b]	1[b]	1[b]
Thickening of alveolar septa	0	1[a]	1[a]	2[a]	2[b]	2[b]	3[b]
Macrophage accumulation	0	0.5	1.5	2[a]	1.5	1	2[a]

Results are expressed as median scores for each group, graded on a scale from 0 (no alteration) to 5 (severe alteration). Letters *a* and *b* indicate statistical differences between different doses of MBS and MSS with respect to the same criterion. Six mice per group were analyzed. $p<0.05$.

4.3.2 Leukocytes in bronchoalveolar lavage fluid (BALF)

The different types of leukocytes in the bronchoalveolar lavage fluid (BALF) were identified for each group of mice tested. For both archaeal species, BALF cell subtypes were mainly composed of macrophages and lymphocytes (Fig. 11). MSS also induced a significant accumulation of eosinophils and neutrophils. Results from two pooled experiments with similar results are indicated: 14 to 22 mice per group were analyzed.

4.3.3 Lymphocytes in bronchoalveolar lavage fluid (BALF)

Numbers of $CD4^+$ T cells, $CD8^+$ T cells and $CD19^+$ B cells were analyzed in BALF of mice exposed for three weeks to specific doses of MBS and MSS (Fig. 12). Compared to MBS, MSS induced strong $CD4^+$ T and $CD19^+$ B cell responses in airways, which plateaued at the lowest dose instilled.

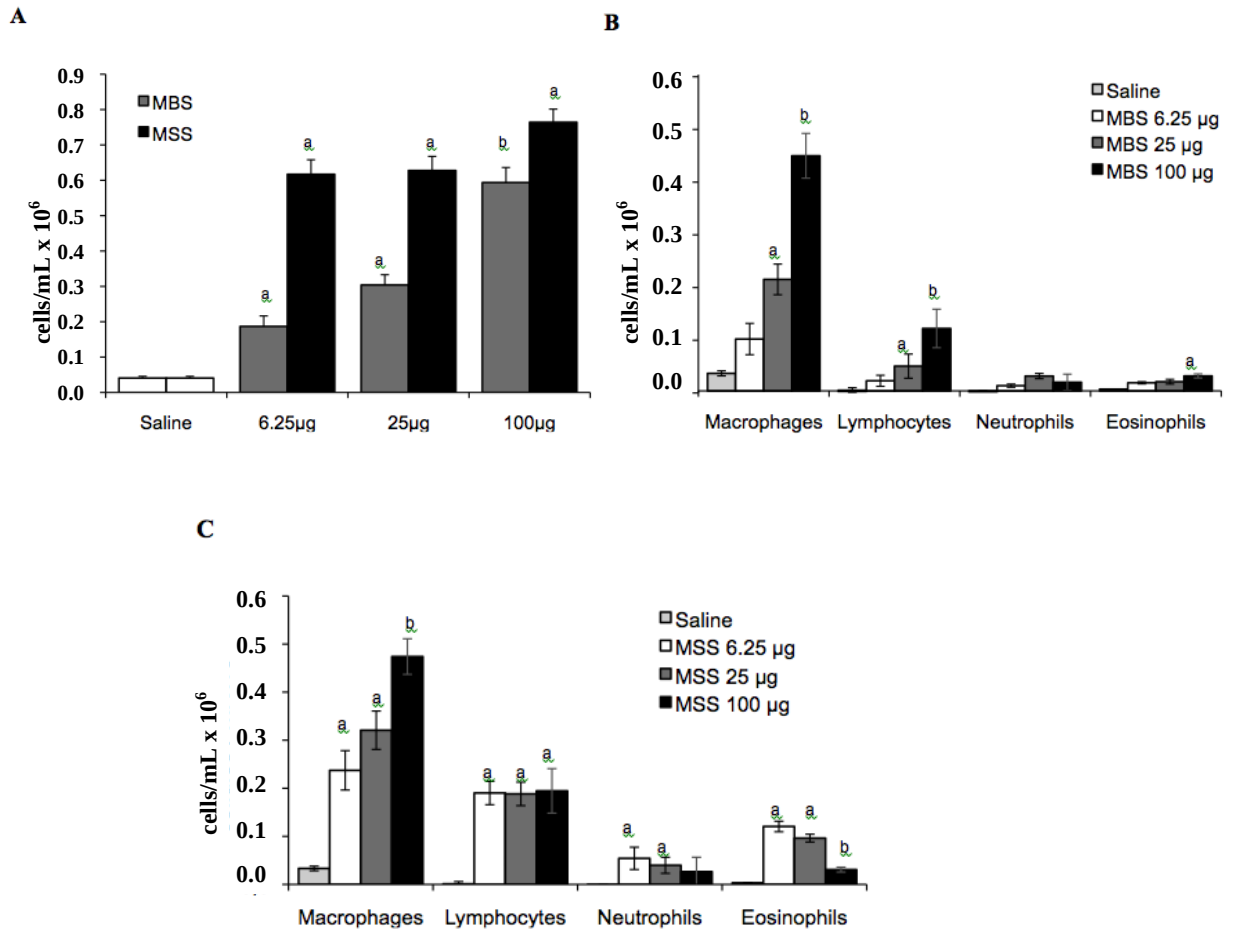

Figure 11: Quantification of leukocytes in BALF from mice instilled with increasing doses of two archaeal species over three weeks: (A) total number of immune cells; (B) BALF leukocyte counts with MBS exposure; and (C) BALF leukocyte counts with MSS exposure. Results are expressed as mean ± SEM. Letters *a* and *b* indicate statistical differences between treatments of the same cell type ($p<0.05$)

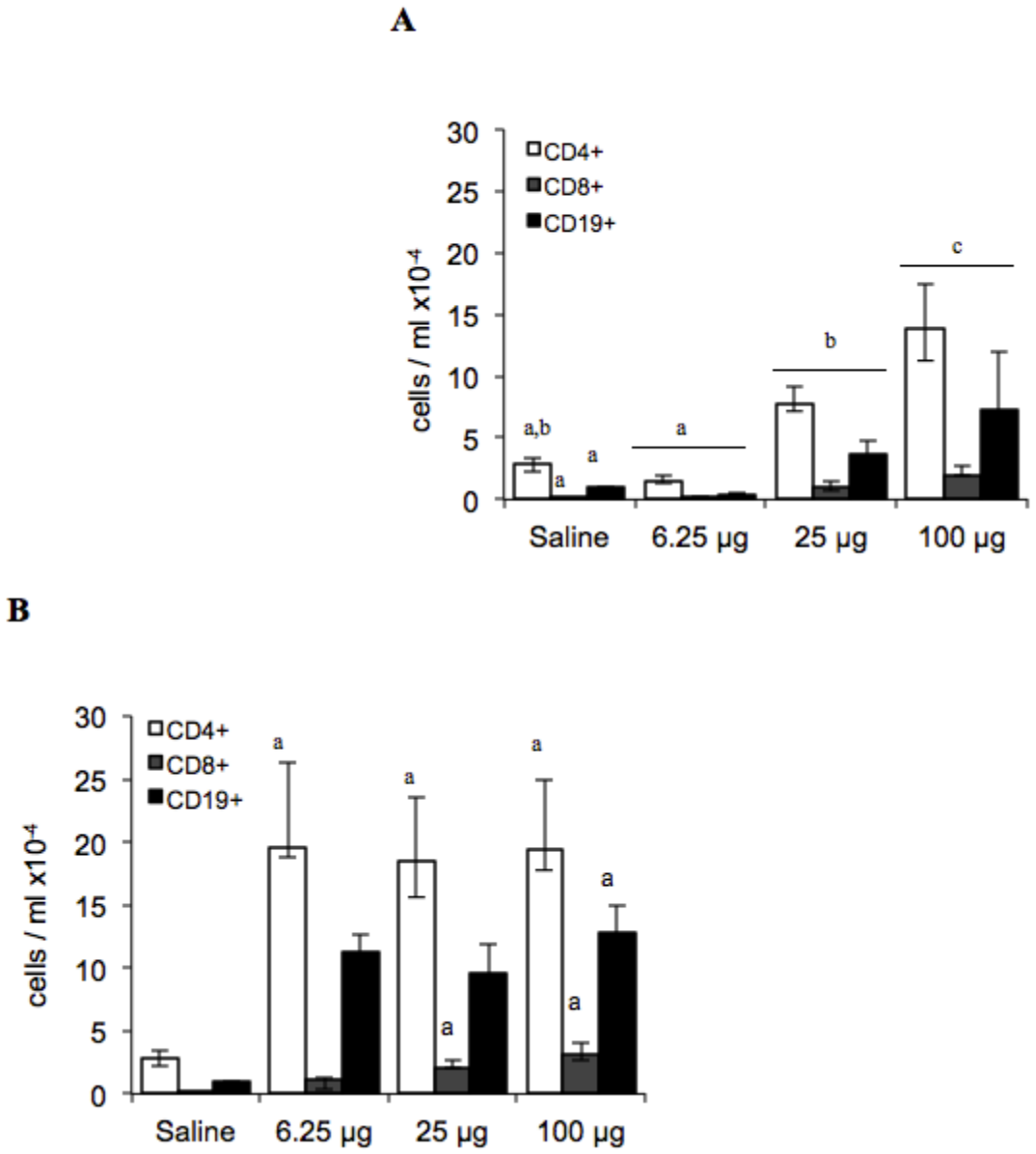

Figure 12: Dominance of $CD4^+$ T lymphocytes and $CD19^+$ B lymphocytes in BALF of mice instilled with (A) MBS and (B) MSS. Results are expressed as median ± interquartile range. Letters *a*, *b* and *c* indicate statistical difference between treatments of the same cell type. Six mice per group were analyzed ($p<0.05$).

4.3.4 Quantification of specific antibodies (IgG) in plasma

Titers of antigen-specific IgG were measured by ELISA in plasma of mice exposed to specific doses of MBS and MSS (Fig. 13). Both archaeal species induced a dose-responsive generation of archaea-specific antibodies

4.3.5 Response of myeloid dendritic cells

Flow cytometry analyses were performed on BALF cells of mice exposed to MBS and MSS for three weeks (Fig. 14). Compared to saline-treated mice, MBS and MSS induced significant accumulation of myeloid dendritic cells in the airways (p<0.05). Myeloid dendritic cell response, however, tended to be greater with MSS (p = 0.08).

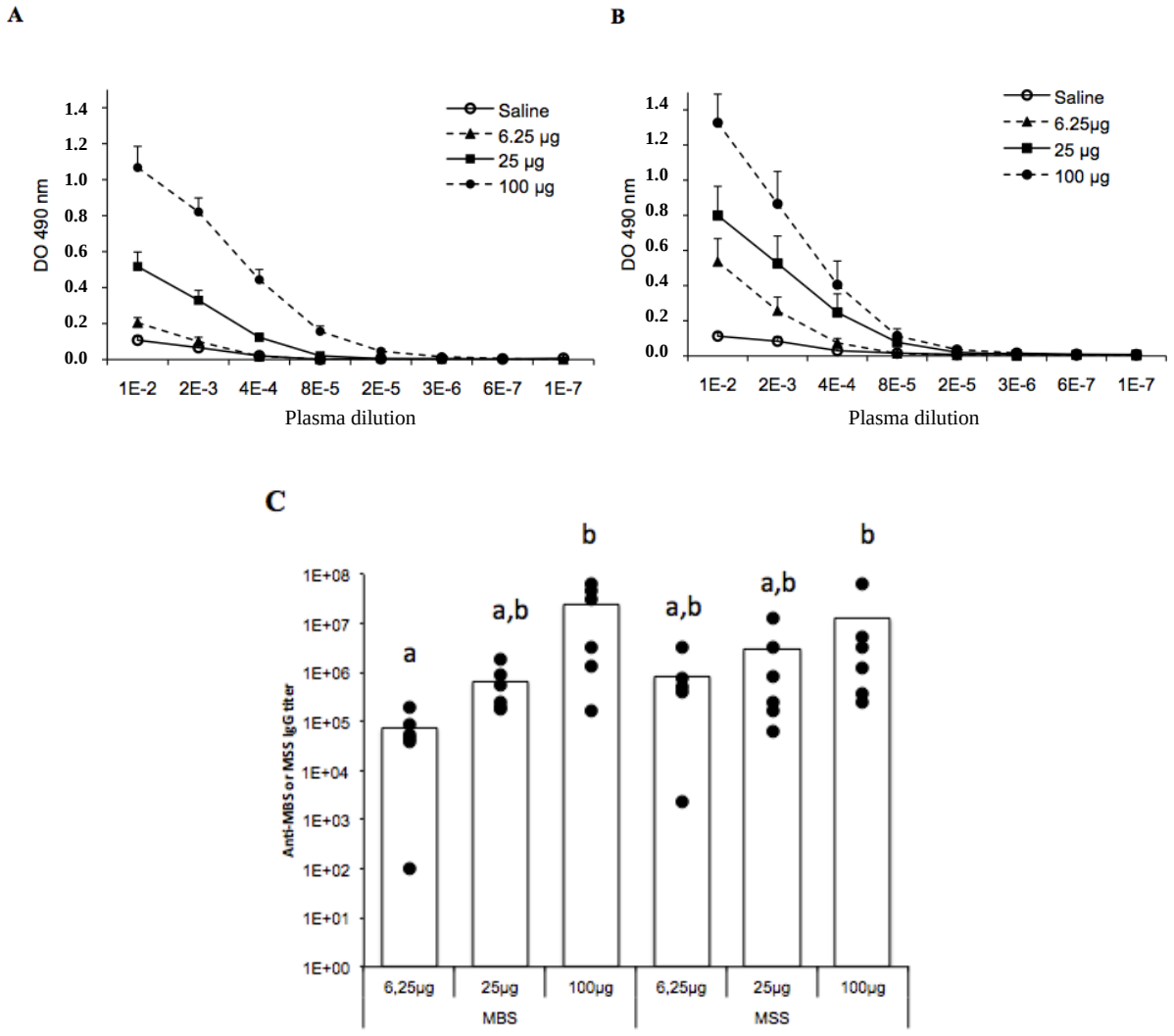

Figure 13: Induction of antigen-specific IgG in plasma of mice exposed to (A) MBS and (B) MSS. (C) Titers are expressed as inverse log of plasma dilution. Results are expressed as mean ± SEM. Letters *a* and *b* indicate statistical differences between treatment regimens. Six mice per group were analyzed (p<0.05).

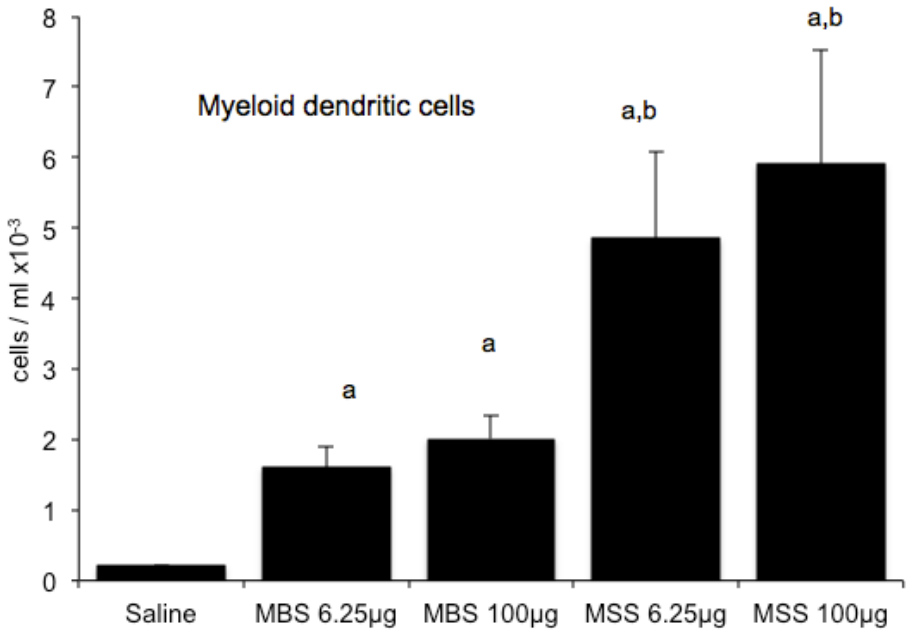

Figure 14: Myeloid dendritic cell response in airways of mice exposed to MBS and MSS. Results are expressed as an absolute number of cells (mean ± SEM). Six mice per group were analyzed. Letter *a* indicates a statistically significant difference compared to saline ($p \leq 0.05$). Letter *b* indicates a non statistically significant difference between MSS and MBS ($p = 0.08$)

5. DISCUSSION

5.1 Airborne archaea in facilities sampled

A molecular biology-based protocol for general detection of archaea was developed in this study. Unlike similar protocols described in the literature, the method developed in this study and described herein does not favour one specific group of archaea over others and thus eliminates bias in the quantification.

Airborne archaea were found in high concentrations in dairy barns and poultry facilities and in smaller quantities in wastewater treatment plants. These microorganisms are thus present in the air not only in farm buildings but also in certain industrial facilities. In other words, they are a major component of the bioaerosols in many work environments.

In poultry facilities, airborne archaea were found in higher concentrations in cage-housed than in floor-housed operations. On the other hand, concentrations of other bioaerosol components studied (bacteria, endotoxins and dust) were higher in floor-housed operations. As workers in facilities with cage housing report more respiratory symptoms (chronic phlegm),[72] there may be reason to associate airborne archaea with these symptoms.

The nature and concentration of the bioaerosols from the wastewater treatment plants sampled may have been affected by the composition of plant effluents. Such effluents vary from one day to the next and may thus contain more or less organic matter—and this would have an impact on the organic particles aerosolized. The results described herein were obtained from spot samples. In addition, of the two wastewater plants sampled, the effluents at plant 1 were mainly of domestic origin, whereas those at plant 2 were of industrial origin, and this may explain the differences between in bacterial and archaeal concentrations measured in the two plants.

5.2 Correlation of airborne archaeal and bacterial concentrations

There was no correlation between airborne archaeal and bacterial concentrations in the dairy barns sampled, unlike what has been noted in swine confinement facilities.[31] In fact, airborne archaeal concentrations were similar in all farm facilities tested, whereas airborne bacterial concentrations varied substantially from one farm to the next. This may be because archaeal communities are relatively constant and less variable than bacterial communities in cattle rumen.[73] In addition, there are more sources of bacteria (manure, water, hay, straw) than of archaea (manure) in dairy farms, and this could affect airborne concentrations of the two microorganisms. The differences noted between the swine confinement facilities and the dairy farms with respect to correlation of quantities of airborne bacteria and archaea may be attributable to the different intestinal microflora of swine and cattle. In fact, quantities of archaea in cattle rumen are very stable compared to those in the pig gastrointestinal tract.[28, 73]

5.3 Comparison of samplers

Three air samplers operating on different principles of sample collection were used for all dairy barn sampling to determine their efficacy in environments highly contaminated by bioaerosols. Compared were an impinger that uses a liquid collection medium (Coriolis), a three-stage

impactor that uses a solid medium (NIOSH) and gelatin filters (IOM cassettes). The particle collection efficacy of the three samplers in environments highly contaminated by bioaerosols proved comparable. This result is not surprising, as we demonstrated with the NIOSH sampler that the aerodynamic diameter of most particles sampled was greater than 2.1 µm, that is, within the particle collection range of the three samplers tested.

5.4 Biodiversity of airborne archaea in facilities tested

The biodiversity of the airborne archaea found in the three industries studied (dairy farming, poultry farming and wastewater treatment) was assessed. Our findings show that methanogens are the most common airborne archaeal species in these work environments; methanogens are generally found in anaerobic environments such as the digestive systems of animals and humans. In fact, the methanogens *Methanobrevibacter smithii,*[74, 75] *Methanosphaera stadtmanae,*[74, 75] *Methanobrevibacter ruminantium*[74] and *Methanobrevibacter woesei* [76] are all archaebacterial species found in the digestive systems of cattle, poultry and humans, and this may explain their presence in the air of the facilities tested—all of which contain human and animal feces. Regarding the airborne archaea detected at the wastewater treatment plants, *Methanospirillum hungatei* and *Methanocorpusculum labreanum* are known to be found in bioreactors such as the biofilters at sites 4 of the wastewater treatment plants sampled.[77, 78]

5.5 Airborne MBS and MSS in facilities sampled

By using contemporary techniques, such as qPCR, we were able to detect airborne MBS and MMS in dairy barns and wastewater treatment plants, which would have been impossible using culture methods. These microorganisms are sensitive to oxygen, and are thus non-culturable after aerosolization. However, though qPCR made it possible to detect MSS, no association with a DNA band was found with the PCR-DGGE technique. This is probably because DNA specificity and amplification are better when a particular microorganism is targeted with species-specific PCR primers rather than the universal PCR primers that are used to determine the microbial biodiversity of a sample.

5.6 Airborne SR in dairy barns

Recommendations are frequently made to dairy farmers about not storing wet hay in barns.[79-82] These warm environments promote development and subsequent aerosolization of *Saccharopolyspora rectivirgula* (SR), the actinomycete responsible for Farmer's Lung. Our study demonstrated smaller quantities of airborne SR in dairy barns than previously detected by culture methods.[3] However, despite the recommendations to farmers, the air in some farms still contains high concentrations of SR.

5.7 MBS-, MSS- and SR-specific IgG in the blood of workers

As airborne MBS, MSS and SR were detected in the facilities sampled, determination of worker exposure to these microorganisms in the workplace was in order. An exposure marker in the blood (IgG specific to the microorganism studied) is used for this purpose.[83, 84] However, no difference in quantity of MBS- or MSS-specific IgG in dairy farm or wastewater treatment plant workers compared to controls was demonstrated. This may be due to a number of factors,

including immune tolerance induced by constant exposure of the workers to archaea in bioaerosols. In addition, these two methanogenic species are present in normal human intestinal flora, which might explain the small quantity of antibodies specific to these microorganisms in the blood of the study subjects. Furthermore, personal samples were not collected, only area samples, which makes it difficult to interpret results regarding worker exposure; area samples are suitable for determining air quality but not for measuring exposure.

As the air in dairy barns still contains high concentrations of SR, more SR-specific IgG antibodies were produced in the plasma of dairy farm workers than in that of control subjects. Since these antibodies are used in diagnosing Farmer's Lung, it can be confirmed that dairy farm workers are still at risk of developing this respiratory disease.

5.8 Inflammatory potential of archaea and possible impact on respiratory health

We have demonstrated that archaea are a major component of bioaerosols in many work environments. Bioaerosols can be the source of a number of respiratory diseases, but the bioaerosol components responsible for these diseases are not all known. With the mouse model used in this study, we were able to demonstrate that two archaeal species commonly found airborne in work environments, MSS and MBS, have immunogenic potential. In fact, though these microorganisms are extremely sensitive to oxygen and hence non-viable and non-infectious in bioaerosols, they can stimulate the immune system and trigger a full immune response leading to generation of antigen-specific antibodies. This study demonstrated that MSS and MBS have different immunogenic properties and hence can modulate the nature and type of immune response differently: MSS, but not MBS, induces an inflammatory response characterized by granulocytes in the bronchoalveolar lavage fluid (BALF). The MSS-specific immune response was also more severe than the response induced by MBS. The potent immunogenicity of MSS was confirmed by much greater accumulation of myeloid dendritic cells (crucial to acquired immunity) compared to MBS.

The results obtained in mice exposed to archaea can be compared to those produced in an identical mouse model exposed to SR, which causes Farmer's Lung and, according to our results, is still present airborne in dairy barns. Studies of the impact of this microorganism on the airways demonstrate that it triggers severe lymphocytosis in the lungs[55, 70] but induces very few granulocytes—the latter being characteristic of the inflammatory response triggered by MSS. It seems, then, that the pulmonary immune response triggered by exposure to different species of archaea is agent-specific.

The studies described in this report demonstrate the immunogenic potential of two methanogen species, but the impact of these microorganisms on respiratory health is still not well understood. The results obtained show that archaea must be investigated when characterizing bioaerosols, as they constitute a key fraction of airborne agents and can have an impact on the respiratory health of humans exposed to them. However, the role of archaea in the etiology of respiratory illnesses remains to be determined.

6. CONCLUSION

The work described in this report made it possible to suggest a new immunogenic agent in bioaerosols. The characterization of archaea in bioaerosols demonstrated their substantial presence in the air in certain environments. In addition, the study of archaea in pulmonary inflammation in mice provided the first evidence of the pathogenic potential of archaea in humans. Last, the project was a springboard to a new field of research: the role of archaea in human inflammation. We are just starting to explore the presence of archaea in our environment and our response to these poorly understood agents. Their role as protective agents, immunostimulators, proinflammatories or tolerated agents merits further investigation in future studies.

This project also made it possible to demonstrate that the different air samplers used for dairy farm sampling (Coriolis, IOM and NIOSH) yield similar results. This is crucial, as there is no standardized sampling method and we were able to demonstrate the robustness of the results.

7. APPLICABILITY OF RESULTS

Though the results of this research are fundamental, it nonetheless appears clear that the composition of bioaerosols in the environments studied is more complex than was anticipated. With this research project, our team confirmed its leading role in the study of human exposure to archaea. We were the first to describe this phenomenon (in swine confinement facilities) and this project confirmed that it appears in other facilities as well (poultry farms, wastewater treatment plants and dairy farms).

Today's technologies make it possible to shed light on bioaerosol components, which, until now, had never been described in the environments studied. The main application of the results of this research stems from demonstration that other compounds, such as certain archaea, can act on the lung immune system. In investigations carried out to characterize these environments, endotoxins have traditionally been selected as the measure to illustrate immunogenic and inflammatory potential. However, archaea may act in synergy with or in the absence of endotoxins or other bioaerosol components.

Another application of the results of this research lies in the selection of air samplers to collect bioaerosols for analysis with molecular methods. Our research showed that in the environments studied, the type of air sampler used had no impact on quantitative results. However, sampling efficacy must be optimal for particles of inhalable size, given the results obtained with the NIOSH sampler (aerodynamic diameter ≥ 2.1 µm).

8. SPINOFFS

The results of this study made it possible to formulate new hypotheses for rapid measurement of immune response induction potential in workers exposed to bioaerosols using a cellular model, leading thus to collaboration between Dr. Caroline Duchaine and Dr. David Marsolais of the research centre of the Québec Heart and Lung Institute (CRIUCPQ).

In addition, we participated in a study of samples from a variety of farming facilities in Denmark (mink farm, dairy farms and swine confinement facilities). We will also have an opportunity to characterize samples of international origin using the methods developed in this project (European asthma/agriculture studies). All of this was made possible thanks to the expertise developed in this project.

In studies designed to characterize bioaerosols in work environments, we suggest that parameters other than quantification of endotoxins and total bacteria be added, such as qPCR quantification of archaea.

Though we have demonstrated that archaea have a strong immunogenic potential in mice, archaea are nonetheless only one component of a complex mix of bioaerosols found in the environments studied. It may be that other components, such as endotoxins, can have a synergic effect when combined with archaea and pose respiratory health risks.

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