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“Microbiological Characterization and Mitigation of Bioaerosols in CAFOs”
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NIOSH Final Report

ABSTRACT:

Title: “Microbiological Characterization and Mitigation of Bioaerosols in CAFOs”

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Rationale: Respiratory tract infections are common among workers in confined animal feed operations (CAFOs) and little is known about the kinds of aerosol microbes workers are exposed to that might predicate diseases. The goal of the project was to use molecular technology to characterize microorganisms in swine and dairy CAFOs, and to develop and test potential mitigation methods.

Progress: Overall considerable progress was made. The basic goal of documenting the nature of CAFO aerosol microbiology was achieved. Methods were developed for the harvest of large air volumes (typically ~2 m³ per sample) using fluid impingement (Sceptor Industries Omni, heavily customized). DNA was isolated and ribosomal RNA genes were obtained by PCR and cloning, and sequenced to identify microbes. CAFO aerosols are dominated by animal fecal microbiology. We additionally showed that our methods are suitable for the harvest and analysis of viruses, using Porcine Circovirus 2 as a model. PCV2 was detected in all swine facilities but not dairy facilities. Our methods are suitable for virus surveillance in animal facilities, a critical regulatory issue in monitoring spread of zoonotic disease.

In parallel, we studied the use of weakly ionizing electric field for aerosol disinfection. Viable cells, but not spores, are subject to inactivation, suggesting that weak electric fields applied to air filtration equipment may be an effective engineering technology for rapid inactivation of vegetative cells on low-pressure drop filter media. Additionally, we determined that PCR of rRNA genes is not useful as a marker for inactivation of microbes by UV light; UV lesions are too rare to restrict PCR.

SECTION 1

Key Findings:

Project Aims:

1. Identify and quantify airborne microorganisms in CAFOs using culture independent technology.
2. Develop and validate PCR-based assays applicable to detection and quantification of selected bioaerosol pathogens.
3. Evaluate UV irradiation and weakly ionizing electric fields for bioaerosol disinfection potential.
4. Determine the impact of UV irradiation and weakly ionizing electric field on total and viable microbial loads in CAFO area.)

Significant progress has been made in all these areas as follows:

1. Identify and quantify airborne microorganisms in CAFOs using culture independent technology.

We characterized the identity, distribution, and abundance of airborne microorganisms present in CAFOs using direct microscopy, broad-range rRNA PCR, and DNA sequence analysis of air samples collected from within and nearby swine and cattle operations in the western United States. DNA was purified from aerosol samples and used in PCR to obtain ribosomal RNA genes, which then were sequenced to determine the identities of resident microbes. In both kinds of facilities the major kinds of organisms were characteristic of the kind of CAFO (swine vs. bovines) and the kinds of organisms associated with the corresponding fecal microbiota. No potential pathogens were detected. This work is described in a PhD thesis (Rodriguez, 2009) and recently was submitted for publication (Rodriguez *et al.*, submitted, 2011).

As a “control” for the CAFO environment, we conducted parallel surveys in human-occupied environments (several offices, meeting rooms, hotel lobbies and other sites). The indoor concentrations of microbes in aerosol typically are ~10x the outdoor concentrations. The dominant aerosol microbes were found to correspond mainly to human origin. (This might seem surprising, but take note that we are 37°C in a ~22°C environment, so we continuously emit a convective plume carrying our resident microbes!) This work is under preparation for publication (Feazel *et al.*).

As a minor project in another work environment with considerable concern about potential pathogens, we analyzed aerosol microbiology associated with pumping operations in New Orleans. Remarkably, aerosols associated with large-scale pump-spray operations barely penetrated the resident microbiology. No conspicuous potential pathogens above background were detected. This work has been described (Rodriguez *et al.*, 2010)

2. Develop and validate PCR-based assays applicable to detection and quantification of selected bioaerosol pathogens.

Bolstered by the success of the sampling campaign, we developed quantitative PCR assays for potential pathogens in the CAFO settings. One assay was for *Mycobacterium avium paratuberculosis* (MAP), the causative agent of bovine Johne’s Disease, the other assay for porcine circoviruses. Although highly sensitive in control experiments, the MAP assay did not detect MAP in the facilities we studied, which had occasionally had Johne’s animals. (This remains a viable approach for surveillance of animal facilities, but needs further work.) On the other hand, we readily detected porcine circovirus 2 in swine but not bovine facilities. These (and similar) assays of aerosol

microbiology could be valuable disease screening tools for surveillance for zoonotic or other animal diseases, both bacterial and viral, based on bulk aerosol samples rather than the usual swabs. This work is submitted (Rodriguez et al., 2011)

3. Evaluate UV irradiation and weakly ionizing electric fields for bioaerosol disinfection potential.

Weak electric fields have been shown to significantly enhance the capture efficiencies (approaching those of HEPA filters) of airborne particulate matter through coarse fibrous material of HVAC filters, without the associated pressure demands of HEPA filters. The low current electric fields used in these filters may also have a biocidal effect on airborne microorganisms. The mechanism(s) responsible for microbial inactivation by electric fields has not been elucidated, and the disinfection potential has not been optimized for indoor air quality applications. In this study surrogates that are commonly used to model the behavior of pathogenic bioaerosols were immobilized on ASHRAE rated filter media, placed in defined electric fields and their response was observed. Several filter media were tested and experiments were carried out under static and air flow through conditions. Exposure of *Bacillus subtilis* (vegetative), *Mycobacterium parafortuitum*, and *Pseudomonas aeruginosa* resulted in up to 5 log inactivation. There was no obvious response of *B. subtilis* spores to the fields employed in this study. The results of this study showed no apparent effect of airflow on inactivation of surrogate pathogens. Results suggest that weak electric fields applied to air filtration equipment may be an effective engineering technology for rapid inactivation of vegetative cells on low-pressure drop filter media, which provide considerable energy savings over high pressure filters. This work has been described (Lachendro, 2009; Ryan *et al.*, 2010).

4. Determine the impact of UV irradiation and weakly ionizing electric field on total and viable microbial loads in CAFO area.)

This minor component of the project has made no progress because of difficulties in identifying a host facility. In-lab studies (above) provide, however, a basis for suggesting the probable successful application.

Translation of Findings:

Analysis of aerosol microbiology associated with CAFOs indicates clearly these are environments that are expected to predispose workers to respiratory disease. Adequate ventilation, a first line of facility remediation, was observed in no facility that we analyzed. OSHA regulations apparently are not adequate for these facilities, or are not enforced, but should be. The methods that we developed for aerosol harvest and molecular analysis for both bacteria and viruses work well and could be immediately deployed in surveillance programs for particular agents, for instance H1N1 influenza in

poultry facilities. Future improvements in the technology and methods could easily be realized.

The discovery that weak electric fields applied to air filtration equipment may be an effective engineering technology for rapid inactivation of bacteria on low-pressure drop filter media deserves further research and development. The method could provide considerable energy savings over high-pressure filters.

Outcomes/Impact:

1. Potential outcomes:

a. Requirement for high-volume ventilation in CAFOs is indicated by the microbiology findings.

b. The air sampling and molecular analytical methods are immediately applicable for further study or surveillance of microbial or viral agents.

c. The finding that weakly ionizing electric fields coupled with low-flow filters can be significantly sterilizing opens an arena for development of novel air control systems for any indoor environment.

2. Immediate outcomes:

a. Training of graduate student students and postdoctoral fellows

b. Development of credible aerosol analytical technology with immediate potential applications e.g. for surveillance for zoonotic diseases.

c. The finding that weak electric fields applied to low pressure air filtration equipment could provide novel (and less expensive) ways to disinfect airflow begs further development.

3. End outcomes:

a. This work is too new to have 'end outcomes', but future development looks promising.

SECTION 2

Background for project:

Respiratory tract infections are common among workers in confined animal feed operations (CAFOs) and little is known about the kinds of aerosol microbes workers are

exposed-to that might predicate diseases. The main goal of the project was to use molecular technology to characterize microorganisms in swine and dairy CAFOs, and to develop and test potential mitigation methods. Secondary goals were to explore the feasibility of using weakly ionizing electrically charged high flow-rate filters for remediation of microbe-charged air.

Specific aims:

1. Identify and quantify airborne microorganisms in CAFOs using culture independent technology.
2. Develop and validate PCR-based assays applicable to detection and quantification of selected bioaerosol pathogens.
3. Evaluate UV irradiation and weakly ionizing electric fields for bioaerosol disinfection potential.
4. Determine the impact of UV irradiation and weakly ionizing electric field on total and viable microbial loads in CAFO area.)

Methodology:

Significant progress was made in each of Aims 1-3 and much of the work is now published. Engineering of an adequate device for high-volume air collection and scrubbing of microorganism-sized particles was challenging because of the necessity for rigorous cleaning of internal pump and tubing conduits. The most mechanically useful fluid impinger for our use, the Sceptor Industries Omni 3000, could not be cleaned internally and biofilm formation in the fluid-handling system contributed unacceptable contamination. This problem was resolved by developing a semi-automatic fluid-handling system based on replaceable tubing and peristaltic pumps rather than metal tubes and butterfly valves. The device and handling have been described (Rodriguez, 2009). Standard ribosomal RNA and other gene PCRs, cloning, sequencing and data processing are described (Rodriguez 2009, Rodriguez et al., 2010).

Results and Discussion:

1. Identify and quantify airborne microorganisms in CAFOs using culture independent technology.

We characterized the identity, distribution, and abundance of airborne microorganisms present in CAFOs using direct microscopy, broad-range rRNA PCR, and DNA sequence analysis of air samples collected from within and nearby swine and cattle operations in the western United States. DNA was purified from aerosol samples and used in PCR to obtain ribosomal RNA genes, which then were sequenced to determine the identities of resident microbes. In both kinds of facilities the major kinds of organisms were characteristic of the kind of CAFO (swine vs. bovines) and the kinds of organisms associated with the corresponding fecal microbiota. Several potential

opportunistic pathogens were detected. The methods that we developed for aerosol harvest and molecular analysis of bacteria are applicable to viruses, which we showed by detecting and analyzing porcine circovirus 2 in swine facilities. The techniques need be modified only slightly to be immediately deployed in surveillance programs for particular agents, for instance H1N1 influenza in poultry facilities. Future improvements in the technology and methods could easily be realized. This work is described in a PhD thesis (Rodriguez, 2009) and recently was submitted for publication (Rodriguez *et al.*, submitted, 2011).

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Publications:

Lachendro, E.J. (2009), Inactivation of surrogate bioaerosols on clean filter media by low current electric fields, MS Thesis, Department of Civil, Environmental and Architectural Engineering, University of Colorado at Boulder.

Rodriguez, M. (2009), The application of molecular based tools for bioaerosol source tracking and disinfection assessment, PhD Dissertation, Department of Civil, Environmental and Architectural Engineering, University of Colorado at Boulder.

Ryan, H, K.M. McCabe, N. Clements L. Erickson, M. Hernandez, and S. Miller (2010) Bioaerosol inactivation using ultraviolet germicidal irradiation in flow-through control devices, *Aerosol Science and Technology*, 44:541–550.

Rodriguez, M., J. Walker, N.R. Pace and M. Hernandez, M. (2010) Molecular source tracking of bioaerosols in the quarantined Katrina flood zone. *Aerosol Science and Technology*. 44:230–239.

Rodriguez, M., D.N. Frank, P. Koll, L.K. Baumgartner, M.T. Hernandez and N.R. Pace. Culture-independent analysis of bioaerosols in concentrated animal feeding operations (CAFOs). Manuscript submitted (Aerosol Science and Technology).

Feazel, L., Koll, P., Baumgartner, L., Peterson, K., Frank, D.N., M. Hernandez and N.R. Pace. Microbiology of the human-occupied indoor environment. Manuscript in preparation.