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BOARD-INVITED REVIEW: Fate and transport of bioaerosols associated with livestock operations and manures

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ABSTRACT: Airborne microorganisms and microbial by-products from intensive livestock and manure management systems are a potential health risk to workers and individuals in nearby communities. This report presents information on zoonotic pathogens in animal wastes and the generation, fate, and transport of bioaerosols associated with animal feeding operations and land applied manures. Though many bioaerosol studies have been conducted at animal production facilities, few have investigated the transport of bioaerosols during the land application of animal manures. As communities in rural areas converge with land application sites, concerns over bioaerosol exposure will certainly increase. Although most studies at animal operations and wastewater spray irrigation sites suggest a decreased risk of bioaerosol exposure with increasing distance

from the source, many challenges remain in evaluating the health effects of aerosolized pathogens and allergens in outdoor environments. To improve our ability to understand the off-site transport and diffusion of human and livestock diseases, various dispersion models have been utilized. Most studies investigating the transport of bioaerosols during land application events have used a modified Gaussian plume model. Because of the disparity among collection and analytical techniques utilized in outdoor studies, it is often difficult to evaluate health effects associated with aerosolized pathogens and allergens. Invaluable improvements in assessing the health effects from intensive livestock practices could be made if standardized bioaerosol collection and analytical techniques, as well as the use of specific target microorganisms, were adopted.

Key words: animal feeding operation, bioaerosol, dispersion, land application, manure, pathogen

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INTRODUCTION

Animal feeding operations (AFO) generate vast quantities of manure (feces and urine) and wastewater that must be treated, stockpiled, or beneficially used. In the United States there are approximately 238,000 AFO producing an estimated 500 million wet tons of manure annually. Of particular concern is the intensification of animal production, which has led to the creation of concentrated AFO (CAFO) that make up about 15% of all AFO. The major producers of manure are cattle (beef and dairy), poultry (chicken and turkey), and swine operations (Wright et al., 1998). Depending upon the animal production facility, the solid and liquid manures are typically stored in piles or holding ponds, mechanically dewatered, composted, anaerobically digested for biogas production, or a

combination of the above. Animal manures applied as solids, semi-solids, and liquids have traditionally been used as soil conditioners and as a source of nutrients for crop production (Power and Dick, 2000; Risse et al., 2006). When improperly managed, however, manures can pollute surface and ground waters with nutrients and pathogenic microorganisms (Ritter, 2000).

Because commercial livestock carry an increased microbial load in their gastrointestinal system, they are often reservoirs of zoonotic pathogens (temporarily or permanently), which can be transmitted to the environment in untreated manures (Gerba and Smith, 2005; Venglovsky et al., 2009). An area of growing interest is airborne pathogens and microbial by-products generated at AFO and during the land application of manures (Chang et al., 2001b; Wilson et al., 2002; Cole et al., 2008; Chinivasagam et al., 2009; Dungan and Leytem, 2009a; Millner, 2009), which can potentially affect the health of livestock, farm workers, and individuals in nearby residences (Heederik et al., 2007). Land application of untreated solid and semi-solid manures and use of pressurized irrigation systems to apply liquid manures and wastewaters increase the chances

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Table 1. List of important zoonotic bacterial pathogens associated with animals¹

Bacterium	Animal hosts	Transmission routes	Disease	Present in manure	Nonfecal sources
Bacillus anthracis	Cattle, goats, sheep, horses, pigs	Skin wounds, food, inhalation	Cutaneous, pulmonary, or gastrointestinal anthrax	Yes	Soil
Brucella spp.	Cattle	Direct contact, food, inhalation	Brucellosis	Yes (rare)	No
$Campylobacter\ jejuni$	Poultry and wild birds	Food, water, direct contact	Campylobacterioses	Yes	Maybe
Clostridium botulinum	Many	Food	Botulism	Maybe	Soil, sediments
Clostridium perfringens	Many	Food, wounds	Gastroenteritis, gas gangrene	Yes	Soil, sediments
Coxiella burneti	Cattle, sheep, goats, others	Inhalation (infected dust), direct contact	Q fever	Yes	Milk, urine, semen
Enterohemorrhagic Escherichia coli	Cattle, sheep, goats, pigs	Food, water	Hemorrhagic colitis, hemolytic uremic syndrome	Yes	No
Leptospira spp.	Cattle, many others	Direct contact, skin lesions	Leptospirosis	Yes	Urine, stagnant water
$Listeria\ monocytogenes$	Cattle, sheep, pigs	Food, water, inhalation	Listerosis	Yes	Soil, poorly ripened silage
Mycobacterium bovis and tuberculosis	Cattle, some others	Inhalation, undercooked food, skin wounds	Tuberculosis	Yes	Sputum, milk, urine
Salmonella spp. (nontyphoidal)	Calves, pigs, poultry	Food, fomites, water	Salmonellosis, acute gastroenteritis, Guillain-Barré syndrome	Yes	No
Yersinia enterocolitica and pseudotuberculosis	Pigs, others	Food, direct contact, water	Yersiniosis	Yes	Maybe

¹Krauss et al. (2003) and Sobsey et al. (2006).

that microorganisms will become aerosolized (Teltsch et al., 1980a; Brooks et al., 2004; Hardy et al., 2006; Peccia and Paez-Rubio, 2007). Despite the potential for bioaerosol formation during these activities, very few research papers have addressed the risk of human exposure to pathogens during the land application of animal wastes (Boutin et al., 1988; Murayama et al., 2010). To date, much of the research in this area has been conducted with municipal wastewaters (US EPA 1980, 1982; Tanner et al., 2005; Peccia and Paez-Rubio, 2007) and biosolids (Dowd et al., 2000; Brooks et al., 2005a,b; Tanner et al., 2008).

Considering the fact that the number of CAFO continues to grow (USDA National Agricultural Statistics Service, 2009), along with a growing farm worker and encroaching civilian population, an increased understanding of the fate and transport of airborne microorganisms is required to ensure public health is not compromised. The purpose of this review is to highlight the current knowledge of bioaerosol fate and transport, with a specific focus on bioaerosols generated at AFO and during the land application of animal manures. Readers seeking more information on bioaerosol collection and analytical methodologies should refer to a recent review by Dungan and Leytem (2009b). Additional emphasis is placed on dispersion models as a means to assess the transport of bioaerosols and subsequent risk of exposure to individuals in the downwind plume.

ZOONOTIC PATHOGENS IN LIVESTOCK WASTES

Domesticated livestock harbor a variety of bacterial, viral, and protozoal pathogens, some of which pose a risk to other animals and humans. Infectious diseases that are transmissible from animals to humans and vice versa are known as zoonoses. These diseases can be transmitted to humans through direct contact (skin wounds, mucous membranes), fecal-oral route, ingestion of contaminated food and water, or aerogenic route (e.g., droplets, dust). Tables 1, 2, and 3 present a list of important bacterial, viral, and protozoal zoonotic pathogens associated with animals and their wastes, respectively. Many of these pathogens are endemic in commercial livestock and, therefore, are difficult to eradicate from both the animals and production facilities. Some well-recognized zoonotic pathogens are Escherichia coli O157:H7, Salmonella spp., Campylobacter jejuni, Apthovirus that causes foot-and-mouth disease (FMD), and protozoal parasites such as Cryptosporidium parvum and Giardia lamblia. This section is not meant to be an exhaustive review of zoonotic pathogens; more detailed information on zoonoses can be found in Krauss et al. (2003) and Sobsey et al. (2006).

Escherichia coli are native inhabitants of the gastrointestinal tract of mammals, but a subset of diarrhetic E. coli, known as enterohemorrhagic, enteropathogenic,

Table 2. List of important zoonotic viral pathogens associated with animals¹

Virus	Family/genus	Animal hosts	Transmission routes	Disease	Present in manure
Hepatitis E virus	Hepeviridae/ Hepevirus	Pigs, chicken, rats, maybe others	Fecal-oral, food or water, possible direct contact	Hepatitis	Yes
Picornaviruses	$Picornaviridae/\ Apthovirus$	Cattle, sheep, goats, pigs, other cloven- hoofed animals	Direct contact, fomites, inhalation, water	Foot-and-mouth	Yes
H1N1 virus	$Orthomyxoviridae/\ Influenzavirus\ A$	Pigs	Direct contact, inhalation	Swine influenza	Maybe
SARS coronavirus	Coronaviridae/ Coronovirus	Pigs, chickens, other animals	Inhalation	Severe acute respiratory syndrome	Yes
Rabies virus	$Rhabdoviridae/\ Lyssavirus$	Wild and domestic carnivores	Saliva (broken skin and mucous membranes)	Rabies	Maybe
Vesicular stomatitis virus	$Rhabdoviridae/\ Vesiculovirus$	Cattle, horses, mules, pigs	Insect vectors	Vesicular stomatitis	Maybe

¹Krauss et al. (2003) and Sobsey et al. (2006).

and enterotoxigenic, are associated only with animals and humans. Enterohemorrhagic E. coli (e.g., serovar O157:H7) causes intestinal infections in humans, and complications range from mild diarrhea to severe hemorrhagic colitis or hemolytic-uremic syndrome (Krauss et al., 2003). Salmonella occur in cattle, pigs, poultry, wild birds, pets, rodents, and other animals; however, only nontyphoidal Salmonella (e.g., S. enterica serovar Enteritidis) occurs in both humans and animals. Human infection generally occurs through the ingestion of contaminated foodstuffs or excretions from sick or infected animals, resulting in acute gastroenteritis. Campylobacter jejuni is among the most common causes of diarrheal disease in the United States, and this is attributed to the relatively low infectious dose (<500 organisms). The main reservoirs of *C. jejuni* are wild birds and poultry, although among farm animals pigs are important carriers. Infection in humans occurs by ingestions of contaminated food (raw or undercooked poultry meat, pork, or milk) or water or by direct contact with contaminated feces.

Foot-and-mouth disease is a highly contagious and sometimes fatal viral disease of cloven-hoofed animals (domestic and wild). Human infections with the FMD virus are rare and infections can usually be traced to

direct handling of infected animals or contact during slaughter. Cryptosporidium parvum is a protozoal parasite that is widespread in mammals and is increasingly recognized as a major cause of human diarrhea. In animals, clinical signs are most commonly observed in newborn calves. Infected animals shed the organism in their feces, and human infection occurs though the ingestion of contaminated food and water. Giardiasis, caused by various Giardia spp. (e.g., G. lamblia), is considered one of the most prevalent parasitic infections in the world, especially in developing nations with poor sanitary practices. Animal hosts of Giardia spp. include cattle, sheep, pigs, cats, rodents, and other mammals, which are direct or indirect sources of human infection. Transmission commonly occurs through the ingestion of food or water contaminated with feces.

Although the common route of transmission for many zoonotic pathogens is direct ingestion or contact, the inhalation of infectious particles should also be considered. It is well documented that communicable and noncommunicable human diseases are transmitted through airborne routes; however, the airborne transmission of some of the above-mentioned zoonotic pathogens is unknown and quite controversial. Zoonotic pathogens, such as *Mycobacterium tuberculosis* and *Hantavirus*, are

Table 3. List of important zoonotic protozoal pathogens associated with animals¹

Protozoan	Animal hosts	Transmission routes	Disease	Present in manure
Balantidiasis coli	Pigs, wild animals	Food, water	Balantidiasis	Yes
Cryptosporidium parvum	Calves, lambs, many mammals	Direct contact, food, water, inhalation	Cryptosporidiosis	Yes
Giardia lamblia	Cattle, sheep, pigs, goats, many others	Food, water	Giardiasis	Yes
Microsporidia (many genera)	Pigs, cattle, goats, others	Possible ingestion of dirty water, inhalation	Microsporidosis	Yes
Toxoplasmosis gondii	Domestic cats, pigs, many mammals	Fecal-oral, water, undercooked meat	Toxoplasmosis	Yes

¹Krauss et al. (2003) and Sobsey et al. (2006).

known to be transmitted through aerogenic routes and are capable of causing severe disease in infected individuals (Sobsey et al., 2006). However, some enteric pathogens (e.g., Salmonella spp.) are not typically associated with aerogenic routes of exposure, but based on studies with animals there is evidence suggesting that airborne transmission is possible (Wathes et al., 1988; Harbaugh et al., 2006; Oliveira et al., 2006). Furthermore, there is much uncertainty associated with the dose-response of airborne pathogens and biological agents because many relationships have not been established to date (Pillai and Ricke, 2002; Douwes et al., 2003; Hermann et al., 2009).

LAND APPLICATION OF MANURES

Although the land application of manures is often utilized as a means to dispose of a waste by-product, rather than from a beneficial use perspective, manures are an excellent source of major plant nutrients such as nitrogen, phosphorus, and potassium, as well as some secondary nutrients. The application of manure not only improves soil nutrient status, but also has a significant effect on physical and biological properties (Sommerfeldt and Chang, 1985; Khaleel et al., 1991; Peacock et al., 2001). Manure applications increase the OM content in soils, which in turn promotes the formation of water-stable soil aggregates and improves water infiltration, water-holding capacity, microbial activity, and overall productivity.

To distribute the livestock manures and wastewaters to agricultural fields a variety of techniques are often utilized (Pfost et al., 2001). Manures with a low moisture content, such as chicken litter or dewatered feces, can be land-applied using a manure slinger or spreader. Wastes that have a very low solids content, such as wastewater from flush systems, holding ponds, or lagoons, can be land applied via furrow irrigation, directly injected (e.g., drag-hose), or sprayed using a tanker or pressurized irrigation systems (e.g., spray gun, center-pivot). Application methods that launch liquid and solid manures into the air create a potentially hazardous situation as pathogens may become aerosolized and transported to downwind receptors (Sorber and Guter, 1975; Brooks et al., 2004). The aerosolized pathogens could potentially be directly inhaled or ingested after they land on fomites, water sources, or food crops.

AEROSOLIZATION AND BIOAEROSOLS

Aerosolization is a process where fine droplets evaporate completely or to near dryness; thus, microorganisms in these droplets are transformed into solid or semi-solid particles (i.e., bioaerosols). During spray irrigation events of liquid manures and wastewaters, the water stream is broken up into droplets of various sizes. The size of the droplets is related to the sprinkler head configuration and operating pressure of the irrigation system. Fine droplets, $<100~\mu m$ in diameter, evapo-

rate relatively quickly, whereas those >200 μ m do not evaporate appreciably (Hardy et al., 2006). However, the evaporation rate of water droplets increases with decreasing humidity and increasing temperature. In a study conducted with low pressure sprinklers, total evaporation losses ranged from 0.5 to 1.4% for smooth spray plate and 0.4 to 0.6% for coarse serrated sprinklers (Kohl et al., 1987). In a US EPA report (1980), the aerosolization efficiency (E) ranged from 0.08 to 2.7%, with a median value of 0.33% over 17 spray irrigation events using rotating impact-sprinklers. Aerosolization efficiency is the fraction of the total water sprayed that leaves the vicinity of the irrigation system as an aerosol, rather than as droplets.

Bioaerosols are viable and nonviable biological particles, such as bacteria, virus, fungal spores, and pollen grains and their fragments and by-products (e.g., endotoxins, mycotoxins), that are suspended in the air (Grinshpun et al., 2007). Airborne microorganisms and their components are generated as a mixture of droplets or particles, having different aerodynamic diameters ranging from 0.5 to 100 μm (Lighthart, 1994; Cox and Wathes, 1995). The generation of bioaerosols from water sources occurs during bubble bursting or splash, and wave action and microorganisms (single cells or groups) are usually surrounded by a thin layer of water (Stetzenbach, 2007). Aside from natural activities, land spreading of slurries, pressurized spray irrigation events, and aeration basins at wastewater treatment plants are a few ways microorganisms become aerosolized. Bioaerosols generated directly from relatively dry surfaces (e.g., feedlots, soils, plants) or during the land application of dry manures can be released as individual or groups of cells or associated with inorganic or organic particulate matter (Cambra-López et al., 2010). Aerosol particles 1 to 5 µm in diameter are of the greatest concern because they are readily inhaled or swallowed, but the greatest retention in the lung alveoli occurs with the 1- to 2-µm particles (Salem and Gardner, 1994).

FACTORS AFFECTING AIRBORNE MICROORGANISMS

Unlike microorganisms in soils, waters, and manures, aerosolized or airborne microorganisms are very susceptible to a variety of meteorological factors (Cox and Wathes, 1995). The most significant factors that affect viability are relative humidity, temperature, and solar irradiance (Table 4). In general, laboratory and field studies have shown that microorganism viability decreases with decreases in relative humidity and increases in temperature and solar irradiance (Poon, 1966; Dimmock, 1967; Ehrlich et al., 1970b; Goff et al., 1973; Marthi et al., 1990; Theunissen et al., 1993; Lighthart and Shaffer, 1994). As relative humidity decreases, there is less water available to the microorganisms, which causes dehydration and subsequent inactivation of many microorganisms. However, because

Table 4. Studies testing the stability of aerosolized microorganisms under various stress conditions

Organisms	Variables tested	References
Pasteurella tularensis	Relative humidity, solar radiation, temperature	Beebe, 1959; Cox and Goldberg, 1972; Ehrlich and Miller, 1973
Adenovirus 2, Coxsackie B1, Influenza A, Sindbis, Vaccinia	UV radiation	Jensen, 1964
Escherichia coli	Temperature, relative humidity, oxygen, aerosol suspensions	Poon, 1966; Cox and Baldwin, 1967
Pasteurella pestis, Serratia marcescens	Relative humidity	Hatch and Dimmick, 1966
Columbia SK viruses	Temperature, relative humidity	Akers et al., 1966
Newcastle virus, bovine rhinotracheitis virus, vesicular stomatitis virus, $E.\ coli$ B	Relative humidity	Songer, 1967
T3 bacteriophage		
Serratia marcescens, Sarcina lutea, Escherichia coli, spores of Bacillus subtilis var. niger	Carbon monoxide concentration, relative humidity, temperature	Ehrlich et al., 1970b; Lighthart, 1973
Flavobacterium	Relative humidity, temperature	Ehrlich et al., 1970a
Serratia marcescens	Oxygen concentration, relative humidity, UV radiation	Riley and Kaufman, 1972; Cox et al., 1974; Ko et al., 2000
Simian virus 40	Relative humidity	Akers et al., 1973
Various strains of $E.\ coli$ and Semliki forest virus	Relative humidity, aerosol suspensions, preaerosolization stresses	Cox, 1976
Reovirus	Relative humidity	Adams et al., 1982
$Enterobacter\ cloacae,\ Erwinia\ herbicola,$	Relative humidity, temperature, droplet size	Marthi et al., 1990
$Klebsiella\ planticola,\ Pseudomonas\ syringae$		
Pseudomonas syringae, Erwinia herbicola	Temperature, relative humidity	Walter et al., 1990
Chlamydia pneumoniae	Relative humidity, temperature	Theunissen et al., 1993
Mycobacterium bovis	UV radiation, relative humidity	Ko et al., 2000

temperature influences relative humidity, it is often difficult to separate their effects (Mohr, 2007). Targets of relative humidity- and temperature-induced inactivation of airborne microorganisms appear to be proteins and membrane phospholipids (Cox and Wathes, 1995). Viruses with structural lipids are stable at low relative humidities, whereas those without lipids are more stable at high relative humidities.

Oxygen concentration is also known to affect bacterial survival because it is involved in the inactivation of bioaerosols through the production of free radicals of oxygen (Cox and Baldwin, 1967; Cox et al., 1974). Because bacteria are much more complex, biochemically and structurally, than viruses, viruses tend to be more resistant to the effects of oxygen and temperature-induced inactivation, except in the case of spore-forming bacteria such as *Clostridium* spp. (Mohr, 2007). Inactivation of bioaerosols by solar irradiance is highly dependent upon wavelength and is exacerbated by dehydration and oxygen (Beebe, 1959; Riley and Kaufman, 1972; Cox and Wathes, 1995; Ko et al., 2000). Short-wavelength ionizing radiation (e.g., x-rays, gamma rays, UV) induces free-radical-mediated reactions that cause damage to biopolymers, such as nucleic acids and proteins. Another factor, known as the open-air factor, is based on the fact that the survival of many outdoor airborne microorganisms is generally poorer than in inside air under similar conditions (Cox and Wathes, 1995). This effect was attributed to ozoneolefin reaction products in the outdoors. Whereas the above-mentioned factors influence viability, microbial factors such as the type, genus, species, and strain of an organism also affect its airborne survival (Songer, 1967; Ehrlich et al., 1970b).

TRANSPORT OF BIOAEROSOLS

Microorganisms associated with droplets that evaporate to dryness or near-dryness before impacting the ground or vegetation are transported in air currents. When bioaerosols are released from a source, they can be transported short or long distances and are eventually deposited in terrestrial and aquatic environments (Brown and Hovmøller, 2002; Jones and Harrison, 2004; Griffin, 2007). The transport, behavior, and deposition of bioaerosols are affected by their physical properties (i.e., size, shape, and density) and meteorological factors they encounter while airborne. Because most bioaerosols are not perfectly spherical, the most useful size definition is aerodynamic diameter, which is the major factor controlling their airborne behavior (Kowalski, 2006). Aerodynamic diameter is defined as the diameter of a spherical particle of water (a unit density sphere) with which a bioaerosol or microorganism has the same settling velocity in air. Meteorological factors such as wind velocity, relative humidity, temperature, and precipitation affect the transport of bioaerosols, with atmospheric stability being a major factor (Lighthart and Mohr, 1987; Lighthart, 2000; Jones and Harrison, 2004). Relative humidity not only affects microorganism viability as discussed above, but also affects settling velocity because it directly influences the density and aerodynamic diameter of the bioaerosol unit (Ko et al., 2000; Mohr, 2007). The deposition of bio-

Table 5. Microorganisms identified in aerosol samples from various livestock operations

Operation	Organisms identified	Reference
Swine barns	Alternaria, Aspergillus, Monilia, Mucor, Penicillium, Rhizopus	Scarpino and Quinn, 1998
Cattle, swine, and poultry barns	Acinetobacter spp., Chryseomonas luteola, Citrobacter freundii, Escherichia coli, Enterobacter agglomerans, Klebsiella spp., Oligella urethralis, Moraxella spp., Pseudomonas spp., Xanthamonas maltophilia, Shewanella putrefaciens	Zucker et al., 2000
Swine barns	Actinomycetes, Alternaria, Aspergillus, Aureobasidium, Botrytis, Candida, Cephalosporium, Cladosporium, Curvularia, Diplococcus, Drechslera, Fusarium, Geotrichum, Monilia, Oidium, Paecilomyces, Penicillium, Sclerotium, Stemphyllium, Trichoderma, Ulocladium, Zygomyces	Chang et al., 2001b
Swine barns	Bacillus, Enterococcus, Lactobacillus, Listeria, Nocardia, Penicillium, Pseudomonas, Staphylococcus	Predicala et al., 2002
Cattle feedlot	Bacillus spp., Chrysobacterium sp., Corynebacterium spp., Helcococcus sp., Micrococcus sp., Paenibacillus sp., Alternaria sp., Bipolaris sp., Chryosporium sp., Cladosporium sp., Penicillium sp.	Wilson et al., 2002
Cattle shed	Absidia, Alternaria, Aspergillus, Choanephora, Cladosporium, Corynespora, Curvularia, Drechslera, Ganoderma, Leptosphaeria, Memnoniella, Mucor, Nigrospora, Penicillium, Periconia, Rhizopus, Torula, Syncephalastrum	Adhikari et al., 2004
Swine concentrated animal feeding operations	Coliforms, Staphylococcus aureus	Green et al., 2006
Duck fattening unit	Enterobacteriaceae, Pseudomonadaceae, Vibrionaceae, Legionellaceae	Zucker et al., 2006
Swine barns	Aerococcus spp., Anaerococcus spp., Clostridium spp., Lactobacillus spp., Streptococcus spp.	Nehme et al., 2008
Poultry and duck facilities	Salmonella	Fallschissel et al., 2009
Swine barns	$Methanosphaera\ stadtmanae,$ other Methanobacteriales, and Methanosarcinales	Nehme et al., 2009

aerosols occurs through gravitational settling, impaction, diffusion onto surfaces, and wash-out by raindrops (Muilenberg, 1995). For particles with an aerodynamic diameter $>5~\mu m$, gravitational settling and impaction are the leading causes of particle loss during transport (Mohr, 2007). For larger airborne particles ($>25~\mu m$), removal by raindrops is quite efficient.

Assessment of bioaerosol transport is generally accomplished by setting liquid impingement or solid impaction systems at an upwind location (background) and various downwind distances from the source (Dungan and Leytem, 2009b). In brief, the aerosol samplers are usually set at 1.5 m above the ground, which corresponds to the average breathing height for humans. Air is then pulled through the samplers at a specified flow rate (e.g., 12.5 L·min⁻¹ for glass impingers) for several minutes to hours using a vacuum pump. Samples are then analyzed via culture-dependent or molecularbased (e.g., PCR) assays or microscopically to calculate a microorganism concentration per cubic meter of air. In the case of airborne endotoxins, samples are typically collected on filters, subsequently extracted using a weak Tween solution, and analyzed using the kinetic Limulus amebocyte lysate assay (Schulze et al., 2006; Dungan and Leytem, 2009c). The most prevalent microorganisms identified in bioaerosol samples from AFO are presented in Table 5.

With most bioaerosol studies, whether conducted at AFO, composting facilities, wastewater treatment plants, biosolids application sites, or wastewater spray irrigations sites, the general trend observed is that the airborne microorganism concentrations decrease with distance from the source (Goff et al., 1973; Katzenelson and Teltch, 1976; Boutin et al., 1988; Taha et al., 2005;

Green et al., 2006; Low et al., 2007). In a study at a swine operation, the average bacterial concentrations within the barns were 1.8×10^4 cfu·m⁻³, and although the outside air concentration decreased with distance from the facility, at 150 m downwind the bacterial concentration was still 2.5-fold greater (208 cfu·m⁻³) than at the upwind location (Green et al., 2006). In a recent study by Matković et al. (2009), airborne concentrations of fungi inside a dairy barn were about 6×10^4 cfu·m⁻³ throughout the day (morning, noon, and night) and downwind concentrations approached background levels $(2.0 \text{ to } 6.2 \times 10^3 \text{ cfu} \cdot \text{m}^{-3})$ at distances as close as 5 to 50 m from the barn. At an open-lot dairy, the average endotoxin concentration at a background site was 24 endotoxin units (EU)·m⁻³, whereas at the edge of the lot and 200 and 1,390 m further downwind, the average concentrations were 338, 168, and 49 EU·m⁻³, respectively (Dungan and Leytem, 2009a). Table 6 presents airborne concentrations for microorganisms and endotoxins within and downwind of various livestock operations.

Boutin et al. (1988) investigated bioaerosol emissions associated with the land application of swine and cattle slurries by way of tractor-pulled tanker and fixed high-pressure spray guns. Near the source, total bacterial counts were about 2,000 cfu·m⁻³, regardless of the land application method. The bacterial counts steadily decreased with distance from the application site and pathogenic bacteria such as Salmonella, Staphylococcus, and Klebsiella pneumoniae were not detected. However, compared with tank spreading, which sprays closer to the ground, airborne bacterial concentrations were greater at greater distances from the spray guns, which is likely related to the upward discharge of slurry into

Table 6. Airborne concentrations of microorganisms and endotoxin at livestock operations

Operation	Microbe or agent	Sample location	$Concentration^1$	Reference
Landspreading of cattle and swine waste	Total culturable bacteria	Upwind 20 to 200 m downwind	$10^{1} \text{ cfu·m}^{-3}$ $10^{1} \text{ to } 10^{3} \text{ cfu·m}^{-3}$	Boutin et al., 1988
Cattle, swine, and poultry houses	Inhalable endotoxin Respirable endotoxin	Inside houses	3 to 64,347 $\mathrm{EU \cdot m^{-3}}$ 0.1 to 260 $\mathrm{EU \cdot m^{-3}}$	Seedorf et al., 1998
Cow and calf houses	Total endotoxin Gram-negative bacteria	Inside houses	$36 \text{ and } 761 \text{ EU} \cdot \text{m}^{-3}$ 0 to $10^3 \text{ cfu} \cdot \text{m}^{-3}$	Zucker and Müller, 1998
Swine house	Total endotoxin Respirable endotoxin	Inside houses	14 to 818 $EU \cdot m^{-3}$ 0.02 to 1,643 $EU \cdot m^{-3}$	Chang et al., 2001a
Swine barn	Total culturable bacteria	Upwind Inside barn 150 m downwind	$10^{1} ext{ cfu·m}^{-3}$ $10^{3} ext{ cfu·m}^{-3}$ $10^{2} ext{ cfu·m}^{-3}$	Green et al., 2006
Cattle, swine, and poultry houses	Gram-negative bacteria	Inside houses	10^0 to 10^2 cfu·m ⁻³	Zucker et al., 2000
Open-air swine house	Total culturable bacteria Gram-negative bacteria Total culturable fungi	Inside house	10^{3} to 10^{6} cfu·m ⁻³ 10^{0} to 10^{3} cfu·m ⁻³ 10^{2} to 10^{4} cfu·m ⁻³	Chang et al., 2001b
Dairy shed	Total cultural fungi	Inside shed	$10^2 \text{ to } 10^3 \text{ cfu} \cdot \text{m}^{-3}$	Adhikari et al., 2004
Broiler shed	Escherichia coli Salmonella	Inside and outside of shed	$10^2 \text{ to } 10^4 \text{ cfu} \cdot \text{m}^{-3}$ 0.7 to 2.3 MPN·m ⁻³	Chinivasagam et al., 2009
Swine shed	Total culturable bacteria E. coli	Inside shed	$10^5 { m cfu \cdot m}^{-3} \ 10^1 { m cfu \cdot m}^{-3}$	Chinivasagam and Blackall, 2005
Various animal operations	Inhalable endotoxin	Personal samplers	$2 \text{ to } 8,120 \text{ EU} \cdot \text{m}^{-3}$	Spaan et al., 2006
Cattle, swine, and poultry houses	Inhalable endotoxin Respirable endotoxin	Inside houses	3 to 21,933 $EU \cdot m^{-3}$ 0.3 to 12,282 $EU \cdot m^{-3}$	Schierl et al., 2007
Duck fattening	Salmonella	Inside unit	10^1 to 10^6 targets·m ⁻³	Fallschissel et al., 2009
Dairy	Total culturable fungi	Upwind Inside barn 5 to 50 m downwind	$10^{3} \text{ cfu·m}^{-3}$ $10^{3} \text{ to } 10^{5} \text{ cfu·m}^{-3}$ $10^{2} \text{ to } 10^{4} \text{ cfu·m}^{-3}$	Matković et al., 2009
Open-lot dairy	Total endotoxin	Upwind 5 m downwind 200 m downwind	1 to 88 $EU \cdot m^{-3}$ 3 to 849 $EU \cdot m^{-3}$ 2 to 261 $EU \cdot m^{-3}$	Dungan et al., 2010a
Open-lot dairy	Total culturable bacteria	Upwind 5 m downwind 200 m downwind	$10^{3} \text{ to } 10^{4} \text{ cfu} \cdot \text{m}^{-3}$ $10^{4} \text{ to } 10^{7} \text{ cfu} \cdot \text{m}^{-3}$ $10^{3} \text{ to } 10^{5} \text{ cfu} \cdot \text{m}$	Dungan et al., 2010b

¹EU = endotoxin units; MPN = most probable number.

the air that enhances droplet size reduction and drift. To our knowledge, the Boutin et al. (1988) study is the only peer-reviewed report that addresses bioaerosol transport during spray irrigation of livestock manures, whereas most other reports address spray irrigation of industrial and municipal wastes (Katzenelson and Teltch, 1976; Parker et al., 1977; Camann et al., 1988; Brooks et al., 2005a; Tanner et al., 2005). In a preliminary pilot-scale field study conducted by Kim et al. (2007), swine manure was land-applied through a center pivot irrigation system and bioaerosol samples were collected upwind and 8, 14, and 23 m downwind. Total airborne coliform concentrations were found to decrease with distance, from about 10⁸ most probable number (MPN)·m⁻³ at 8 m to near background concentrations at 10⁶ MPN·m⁻³ at 23 m downwind.

Although the focus of this review is on bioaerosols associated with animal operations and manures, one could reasonably expect microorganisms in industrial and municipal wastewaters to behave similarly once aerosolized. Differences in survivability may occur though, depending upon the concentration and type of OM in the wastes because some organic substances are known to act as osmoprotectants (Cox, 1966; Marthi and Lighthart, 1990) and may provide some degree of

physical protection against UV radiation and drying (Sobsev and Meschke, 2003; Aller et al., 2005). Parker et al. (1977) investigated the transport of aerosolized bacteria during the spray irrigation of potato processing wastewater. As with other similar studies, there was a decrease in the airborne microorganism concentration with distance from the irrigation system. These authors reported detection of coliforms at distances as far as 1.0 to 1.5 km from the source; however, there was no way to verify if they were above background concentrations because that information was not provided in the report. During the land application of liquid and dewatered domestic sewage sludge (biosolids) via spray tanker and spreader/slinger, respectively, indicator organisms (coliforms, Clostridium perfringens, E. coli) were not detected at distances greater than 30 m (Brooks et al., 2005b). In most of the above-mentioned bioaerosol transport studies, fecal contamination indicator organisms were targeted. Fecal indicator organisms are generally chosen because they are more abundant and easily identified in the aerosols (Teltsch and Katzenelson, 1978; Bausum et al., 1982; Brenner et al., 1988), although they may behave differently from pathogens (Dowd et al., 1997; Carducci et al., 1999). Alternatively, to improve upon estimates of off-site transport of bio-

aerosols, some researchers have used molecular-based approaches to track microorganisms from swine houses (Duan et al., 2009) or during the land application of class B biosolids (Low et al., 2007) and domestic wastewater (Paez-Rubio et al., 2005). This approach is called microbial source tracking and has only recently been applied to aerosol samples.

Although emission rates for bioaerosols during the land application of livestock wastes are not currently available, emission rates have been calculated for the application of dewatered and liquid class B biosolids onto agricultural land. Emission rate is a useful variable for understanding the impact of waste application, and similarities between application of municipal and livestock wastes can be made because the same spreading equipment is often used. During the land application of dewatered biosolids using a slinger, average emission rates for total bacteria, heterotrophic bacteria, total coliforms, sulfite-reducing clostridia, and endotoxin were reported to be 2.0×10^9 cfu·s⁻¹, 9.0×10^7 cfu·s⁻¹, 4.9×10^3 cfu·s⁻¹, 6.8×10^3 cfu·s⁻¹, and 2.1×10^7 10⁴ EU⋅s⁻¹, respectively (Paez-Rubio et al., 2007). In a study conducted by Tanner et al. (2005), ground water seeded with $E.\ coli$ was sprayed using a spray-tanker, and emission rates were reported to range from 2.0 to $3.9 \times 10^3 \text{ cfu} \cdot \text{s}^{-1}$. Interestingly, when studies were conducted using liquid biosolids, neither coliform bacteria nor coliphage were detected in air 2 m downwind, although these microorganisms were detected in the biosolids. Although no reason was given for the latter outcome, the direct measurement of bioaerosols does provide necessary information required for calculating emission rates. A bioaerosol emission rate is a required input variable for all aerosol fate and transport models that predict absolute concentration at a specified distance from the source (Paez-Rubio et al., 2007).

DISPERSION MODELING

Atmospheric dispersion modeling is a mathematical simulation used to predict the concentration of an air contaminant at various distances from a source. In an effort to assess the transport and diffusion of airborne microorganisms associated with human and livestock diseases, dispersion modeling has been utilized (Sørensen et al., 2001; Garten et al., 2003; Pedersen and Hansen, 2008). In Australia, atmospheric dispersion models have been developed as part of preparedness programs to manage potential outbreaks of foot-and-mouth disease (Cannon and Garner, 1999; Garner et al., 2006). In early bioaerosol transport studies, models were based upon a modified version of the inert particle dispersion model developed by Pasquill (1961). Although some of the inert particle model assumptions will not be met at a typical AFO, the model assumes 1) Gaussian distribution of particles in the crosswind and vertical planes; 2) particles are emitted at a constant rate; 3) diffusion in the direction of transport is negligible; 4) particles are <20 µm in diameter (i.e., gravitational effects are negligible); 5) particles are reflected from the ground (i.e., no deposition or reactions at surface); 6) wind velocity and direction are constant; and 7) terrain is flat. The original form of the inert particle dispersion model is

$$\chi(x, \gamma, z) = \frac{Q \exp(-\gamma^2 / 2\sigma_y^2)}{2\pi\sigma_y \sigma_z \overline{u}}$$

$$\left\{ \exp\left[-\frac{(z - H)^2}{2\sigma_z^2}\right] + \exp\left[-\frac{(z + H)^2}{2\sigma_z^2}\right] \right\},$$
[1]

where χ is the number of particles per cubic meter of air at a downwind location x, γ , and z (i.e., alongwind, crosswind, and vertical coordinates, respectively); Q is the number of particles emitted per second; \bar{u} is the mean wind speed in meters per second; σ_y and σ_z are the SD of the crosswind and vertical displacements of particles at distance x downwind, respectively; and H is the height of the source including plume rise. If ground-level and centerline concentrations are to be determined, then z and γ are set to zero. For a ground-level source H is also set to zero, the simplified equation then becomes

$$\chi(x,0,0) = \frac{Q}{2\pi\sigma_y\sigma_z\overline{u}}.$$
 [2]

Because the Pasquill dispersion model is based on inert particles, Lighthart and Frisch (1976) added a biological decay term as follows:

$$\chi(x, \gamma, z)_{BD} = \chi(x, \gamma, z) \exp(-\lambda t),$$
 [3]

where λ is the microbial death rate (per second) and t is approximated by x/\bar{u} . Subsequent researchers utilized the biological decay term, along with the dispersion model, to assess bioaerosol transport from point sources (Peterson and Lighthart, 1977; Teltsch et al., 1980b; US EPA, 1982; Lighthart and Mohr, 1987). When only part of the material released into the atmosphere becomes an aerosol, as occurs during sprinkler irrigation, Eq. [3] becomes

$$\chi (x, \gamma, z)_{BD} = \chi (x, \gamma, z) E \exp(-\lambda t),$$
 [4]

where E is the aerosolization efficiency factor (Teltsch et al., 1980b). The microbial death and inactivation rates are generally derived from empirical laboratory data under static atmospheric conditions using pure cultures (Hatch and Dimmick, 1966). Therefore, it is imperative when developing microbial death rates to conduct the experiments with numerous microbial types and under varying environmental conditions (Peterson and Lighthart, 1977). In laboratory studies, microbial death rates for Sarcina lutea at 15°C were 4.6×10^{-2} and 5.8×10^{-4} s⁻¹ at around 2 and 90% relative humidity, whereas

death rates for Pasturella tularensis at 27°C were 7.1 \times 10⁻² and 2.4 \times 10⁻³ s⁻¹ at similar relative humidities, respectively (Cox and Goldberg, 1972; Lighthart, 1973). Whereas these microbes are non-spore formers, one would expect spore-forming bacteria to survive longer under changing atmospheric conditions as a result of their ability to tolerate greater temperature and radiation (Madigan and Martinko, 2006). As mentioned previously, the viability of airborne microorganisms will vary greatly depending upon growth media used and microbial genus and species being tested. In field trials conducted at Pleasanton, CA, microbial death rates during the spray irrigation of municipal wastewater were determined under a variety of environmental conditions (US EPA, 1980). The median death rate constants for total coliform, fecal coliform, and coliphage were 3.2, 2.3, and 1.1×10^{-2} s⁻¹, respectively. Death rate constants for E. coli, prepared in sterilized municipal wastewater, were reported to range from 8.8×10^{-3} s^{-1} in the morning to 6.6×10^{-2} s⁻¹ in the afternoon (Teltsch et al., 1980b).

Parker et al. (1977) modified Pasquill's inert particle dispersion model to predict the transport of bioaerosols from an area source (i.e., sprinkler irrigation of potato processing wastewater). Even though the model contained a biological decay term, the authors did not model decay or loss of viability of microorganisms due to a lack of experimental data. Dowd et al. (2000) later used the same area-source model with microbial death rates from the literature to predict bioaerosol transport during the land application of dewatered domestic sewage sludge (biosolids). Based upon model predictions at a high wind speed of 10 m·s⁻¹, bacterial concentrations would be 69 and 6.5 m^{-3} of air at 100 and 10,000 m, respectively. To assess the risk of infection to workers and nearby populations, a Beta-Poisson model as described by Haas (1983) was utilized. Using dose-response data for Salmonella Typhimurium, the predicted risk of infection at 100 m with a 10 m·s⁻¹ wind speed and 8 h exposure period was 13\%, whereas at 1,000 and 10,000 m it decreased to 8.7 and 1.6%, respectively. Risk of infection for Coxsackievirus B3 was also determined; however, an incorrect dose-response value was used in the single-hit exponential model, and predicted risk of infection should have actually been about 3 orders of magnitude less than their published values. Overall, their model predictions suggest that bioaerosols from land-applied biosolids can increase the risk of viral and bacterial infection to onsite workers, but there was little or no risk to population centers >10 km from the application site under low-wind conditions ($\leq 5 \text{ m} \cdot \text{s}^{-1}$). The results from such studies should be used cautiously because the results were not empirically derived and, as outlined by Pillai and Ricke (2002), there is uncertainty associated with the dose-response of different organisms and hosts.

In a 1982 US EPA report, microorganism concentrations in aerosols from spray irrigation events of municipal wastewater were predicted using an atmospheric diffusion model. The diffusion model consisted of 4 principal components:

$$C_d = D_d \ Q_a \ M_d + B, \tag{5}$$

where C_d is the concentration of microorganisms per cubic meter of air; D_d is the atmospheric diffusion factor at distance d from the source (s·m⁻³); Q_a is the aerosol source strength (microorganism s⁻¹); M_d is microorganism die-off factor (not to be confused with microbial death rate, λ) as described in Eq. [3] (i.e., number of organisms that are viable at distance d); and B is the background concentration (microorganisms m⁻³). D_d is calculated using the inert particle dispersion model as shown in Eq. [1], but Q was set to unity. For a wastewater irrigation event, the aerosol source strength was further defined as

$$Q_a = W F E I, ag{6}$$

where W is the microorganism concentration in the was tewater (organisms L^{-1}); F is the flow rate of the irrigation was tewater (L·s⁻¹); E is the aerosolization efficiency factor $(0 < E \le 1)$; and I is the microorganism impact factor (i.e., aggregate effect of all of factors affecting microorganism survivability; I > 0). Using input data from a US EPA (1980) report, total coliform concentrations were determined 770 m from the centerline of 240-m-long linear source under stable (summer night) and unstable (summer midday) atmospheric conditions. The wastewater flow rate during the irrigation event was set at 70 $\text{L}\cdot\text{s}^{-1}$, with a total coliform concentration of 1.0×10^7 cfu·L⁻¹ and respective night and midday wind speeds of 2 and 4 m·s⁻¹, E of $3.3 \times$ 10^{-3} and 1.6×10^{-2} , I of 0.48 and 0.27, λ of 0.02 and 0.05 s^{-1} , and aerosol age (a_d) of 385 and 193 s. The Q_a for total coliforms during night and midday was determined to be 1.1×10^6 and 3.0×10^6 cfu·s⁻¹, respectively. When background coliform concentrations were subtracted, the respective total airborne concentrations at 770 m downwind were predicted to be only 0.1 and $4.4 \times 10^{-3} \text{ cfu} \cdot \text{m}^{-3}$. During midday conditions, fecal streptococci concentrations at 770 m downwind were predicted to be 2-fold greater than total coliforms, even though the source concentration was 2-fold less. This is owing to the fact that fecal streptococci had a microorganism impact factor of 5.7 and death rate of zero.

Lighthart and Mohr (1987) modified a version of the Gaussian plume model used by Peterson and Lighthart (1977) to include an airborne microbial survival term that was a best-fit function of temperature, relative humidity, and solar radiation. The model included an algorithm using microbial source strength and local hourly mean weather data to drive the model through a typical summer or overcast and windy winter day. At high wind speeds or short travel times, the model predicted greater viable near-source concentrations because the microorganisms did not have time to become inactivated. As travel times were increased, due to slow

wind speeds or longer distances, inactivation of microorganisms became more prevalent.

Lighthart and Kim (1989) used a simulation model to describe the dispersion of individual droplets of water containing viable microbes. The droplet dispersion model was separated into 5 submodels: 1) aerosol generation, 2) evaporation, 3) dispersion, 4) deposition, and 5) microbial death. The position of each droplet, at each time step in the trajectory, was located in a 3-dimensional coordinate system. When the modeling process was repeated for many droplets, a simulation of a cloud of droplets then occurred. The effect of evaporation was determined to be an important factor when simulated in the model, as aerosols were carried further downwind. Whereas the model takes into account the physical, chemical, and measured meteorological parameters for each water droplet, potential shortcomings revolved around the ability of the model to predict nearsource survival dynamics of airborne microorganisms (e.g., effect of microorganisms on water evaporation, critical water content of microbes). Also, the droplet dispersion model does not take into account rapidly changing wind conditions (e.g., gusts) and, therefore, use of average wind velocities will lead to an oversimplification of meteorological conditions and microbial dispersion. When the model was compared with a release of Pseudomonas syringae, deposition rates were found to be similar within 30 m of the source. The simulation model was later used by Ganio et al. (1995) to model a field spray event of Bacillus subtilis var. niger spores. Using the same meteorological conditions as the spray event, the model produced a bioaerosol deposition pattern somewhat similar to that obtained in the field (r² = 0.66).

A variety of short- and long-range dispersion models have been developed to understand and manage the airborne spread of epidemics such as foot-and-mouth disease (Gloster et al., 1982; Sørensen, 1998; Cannon and Garner, 1999; Sørensen et al., 2000; Rubel and Fuchs, 2005; Garner et al., 2006; Mayer et al., 2008). In a recent paper by Gloster et al. (2010), a historic outbreak of FMD in 1967 (Hampshire, UK) was modeled using 6 internationally recognized dispersion model systems. Whereas one-half of the models [Nuclear Accident Model (NAME), Veterinary Meteorological decision-support system (VetMet), Plume Dispersion Emergency Modeling System (PDEMS)] were run using observational data provided, the other one-half [Australian Integrated Windspread Model (AIWM), Modéle Lagrangien Courte Distance (MLCD), National Atmospheric Release Advisory Center (NRAC)] used numerically derived meteorological data, and comparisons between outputs were made. Using the same virus emission data, the models produced very similar 24 h integrated concentrations along the major axis of the plume at 1, 5, 10, 15, and 20 km. Although there were differences between the estimates, as a result of model assumptions with respect to upward diffusion rates for surface material and choice of input weather data, most

estimates were within one order of magnitude. These models also predicted similar directions for livestock at risk; however, additional model assumptions such as microbial fate and susceptibility to airborne infection can substantially modify the size and location of the downwind risk area.

SUMMARY AND FUTURE IMPLICATIONS

Based on information presented in this review, it is evident that animal feeding operations and manure application practices contribute to the formation of bioaerosols at greater concentrations than found in background environments. As population centers grow and converge on such operations, there will be an increasing potential for exposure to airborne pathogens and microbial by-products that are transported off site. Exposure to airborne bacteria, virus, fungi, and microbial by-products is not limited to inhalation routes because deposition on fomites, food crops, and water bodies and subsequent ingestion also represent transmission routes of concern. The ability to accurately quantify airborne microorganisms within and downwind from a source is important when evaluating health risks to exposed humans and animals. However, the actual risk of exposure from airborne pathogens has not been fully recognized for a variety of reasons including choice of bioaerosol collection technique, analytical methodology, target microorganism, and dispersion and infectivity model inputs.

To date, most bioaerosol transport studies have targeted fecal indicator organisms because they are generally more abundant and easily detected. Pathogens on the other hand are often at concentrations that are several orders of magnitude less than indicator organisms, making their detection difficult in highly diluted aerosol samples. Because the survivability of aerosolized fecal indicator organisms is likely different from that of pathogens, a first step to improve future bioaerosol studies should include the selection of organisms that better represent targeted pathogens, along with standardized methods for their collection in outdoor environments. As molecular-based approaches improve with respect to sensitivity and rapidity, it may be appropriate to standardize and use such technologies to directly detect pathogens of interest in aerosol samples, avoiding the need for indicator organisms. Standardization of target microorganisms and collection and analytical methodologies will improve the ability of researchers to compare results, refine dispersion models, and develop unified risk estimates.

Although animal operations and manure management practices are not currently regulated with respect to bioaerosol emissions, the possibility that control measures will someday be implemented is quite realistic. Without standardized methodologies, regulatory agencies will have to base decisions on inconsistent data sets, and the effectiveness of mitigation strategies to control

bioaerosol emissions will not be properly determined. Because land application of manures will remain a viable nutrient utilization and disposal option into the foreseeable future, emphasis must be placed on research addressing the airborne transport of pathogens because there is a lack of information on this topic. Furthermore, there is a surprising lack of information concerning the infectivity of aerosolized pathogens, especially enteric pathogens. Clearly, a critical component of a risk determination is not only understanding bioaerosol dispersion and transport, but also the dose-response of zoonotic pathogens. To advance our understanding of risks associated with airborne pathogens from animal feeding operations, it will be necessary for a variety of scientists, including but not limited to aerobiologists, clinical microbiologists, epidemiologists, animal scientists, and risk modelers, to convene under a common setting to address these issues in more detail and work toward a common goal of standardizing of variety of bioaerosol collection and analytical methodologies.

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