Measuring Airborne Microorganisms and Dust from Livestock Houses

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ABSTRACT

Airborne transmission has been suspected to be responsible for epidemics of highly infectious disease in livestock production. In such transmission, the pathogenic microorganisms may associate with dust particles. However, the extent to which airborne transmission plays a role in the spread of diseases between farms, and the relationship between microorganisms and dust remain unclear. In order to better understand airborne transmission and to set up effective control techniques, this study investigated the performance of multi-stage air scrubbers on the reduction of emissions of microorganisms and dust from pig houses, and to evaluate the effectiveness and efficiency of different sampling devices for collecting microorganisms and dust.

In winter, multi-stage scrubbers reduced emissions of airborne total bacteria by between 46% and 85%, PM_{10} by between 61% and 93%, $PM_{2.5}$ by between 47% and 90%, and ammonia by between 70% and 100%. The EU reference dust sampler with an impaction pre-separator, which was designed for sampling dust in ambient air, could not be used to sample $PM_{2.5}$ in livestock houses where dust concentrations were high, because overloading occurred. A sampler with a cyclone pre-separator was more tolerant of dust loads in livestock houses and was validated as a reference equivalent sampler. The method for evaluating the efficiency of bioaerosol samplers for airborne microorganisms was appropriate. It calculated the physical and biological sampling efficiencies separately, by excluding the viability losses in the non-sampling processes. The Andersen six-stage impactor, the All Glass Impinger (AGI-30) and the MD-8 had higher physical efficiencies than the OMNI-3000. The Andersen impactor and the AGI-30 had high (100%) biological efficiencies on sampling all five aerosolized microbial species (*Enterococcus faecalis*, *Escherichia coli*, *Campylobacter jejuni*, *Mycoplasma synoviae* and Gumboro vaccine virus). *C. jejuni* and Gumboro vaccine virus were inactivated by the OMNI-3000 during sampling, whereas *E. coli* and *C. jejuni* were inactivated by MD8. As a result, these two bioaerosol samplers had lower biological efficiencies. Although recipient broilers became infected, no culturable airborne *Campylobacter* were detected by the Andersen impactor, the AGI-30 and the OMNI-3000 in an airborne transmission of *Campylobacter* in broilers.

From this study we concluded that installing multi-stage scrubbers at the air exhausts makes it possible to appreciably reduce aerial pollutant emissions from livestock production systems. As PM samplers with cyclone pre-separators are less vulnerable to high dust loadings, it is recommended that they are used to sample dust in livestock production systems. The efficiency of the bioaerosol samplers varies according to the microbial species sampled. Suitable samplers can be selected on the basis of their efficiencies and detection limits. Low aerial concentrations of specific microbial species are difficult to detect with current bioaerosol samplers. Knowledge gaps still exist throughout the process of long-distance airborne transmission of microorganisms, from suspension and transportation to deposition and infection.

IN DEDICATION OF MY PARENTS AND MY WIFE

摘 要

在畜禽生产中，病原菌以空气为载体在畜禽场间传播的方式被认为是动物流行病传播机制之一。在此传播过程中，病原菌通常附着在粉尘上。然而，与其他一些较为熟知的传播机制相比（例如：粪口传播，媒介传播等），人们对空气传播在疾病场间扩散中的重要程度以及病原菌与所附着粉尘之间的关系知之甚少。为了更好的了解空气传播的机理，并以此建立有效的防控体系，本文开展了多项基础性研究，其中包括：研究多层空气过滤系统对猪舍废气中微生物和粉尘的减排效果，评价针对畜禽舍内粉尘采样设备的有效性，测试不同微生物采样器采样效率，现场应用微生物采样器进行空气中细菌采集。

实验结果表明，多层空气过滤系统使冬季猪舍废气中总细菌浓度降低了 46-85%，PM_{10}（空气动力学直径小于 10 μm 的粉尘）浓度降低了 61-93%，$PM_{2.5}$（空气动力学直径小于 2.5 μm 的粉尘）浓度降低了 47-90%，氨气浓度降低了 70-100%。欧盟标准撞击式（impaction） $PM_{2.5}$ 粉尘采样器由于过载原因不能在粉尘浓度较高的畜禽舍内使用。旋风式（cyclone）粉尘采样器有较强的抗过载能力，可以更有效地应用于畜禽舍中的 $PM_{2.5}$ 浓度测量。本文中所采用实验室模拟方法可以有效评价微生物采样器的物理采样效率和生物采样效率。相比 OMNI-3000，Andersen 六级采样器、AGI-30 和 MD-8 具有更高的物理采样效率。对于本实验中涉及的五种空气微生物（粪肠球菌"*Enterococcus faecalis*"，大肠杆菌"*Escherichia coli*"，弯曲杆菌"*Campylobacter jejuni*"，鸡滑液囊支原体"*Mycoplasma synoviae*"和法氏囊病毒疫苗"Gumboro vaccine virus"），Andersen 和 AGI-30 采样器的生物采样效率均达 100%。由于受到应激，空气中的弯曲杆菌和法氏囊病毒疫苗在被 OMNI-3000 采集时显著失活。MD8 在采集大肠杆菌和弯曲杆菌时，也使这些细菌失活。所以这两种采样器对相应空气微生物的生物采样效率较低。在弯曲杆菌空气传播试验中，虽然健康鸡被感染，然而 Andersen、AGI-30 和 OMNI-3000 采样器都未能从空气中采集到存活的弯曲杆菌。

本文证明，在畜禽舍排风口处安装多层空气过滤系统可以有效降低废气中污染物浓度，从而达到减排效果。旋风式粉尘采样器比欧盟标准撞击式采样器更适合于畜禽舍的粉尘浓度测量。不同微生物采样器的物理及生物采样效率不同。在实地采样前，应测评欲使用采样器的采样效率，以选取最优采样器进行下一步应用。空气传播在空气中的病原菌浓度较低时亦可发生，然而，当病原菌浓度低于微生物采样器的最低检测限时，采样器将不能检测到这些病原菌。有关致病菌在畜禽场间的远距离空气传播的知识相对缺乏，相关研究应逐步开展。

献给我的父母和我的爱人

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

Livestock production has intensified tremendously in some areas in the world during the last 50 years. For example, in 2009, there were approximately 3900 farms with 12 million pigs and 1300 farms with 97 million poultry in the Netherlands, an area of only about 41 848 km^2 (CBS 2010). In China, the contribution of intensive production (farms with 500 head of pigs or more) to total pig production almost doubled from 1999 to 2004, and the same trend was also seen in poultry production (Chen et al. 2008). Intensive livestock production creates high yields (e.g. milk, meat and eggs) per unit of labor, feed input and housing area. However, it is associated with high concentrations and emissions of aerial pollutants, which may create nuisance and health problems to farmers and people living in the vicinity, and have detrimental effects (eutrophication, acidification and global warming) on the local, regional or global environment (Wing and Wolf 2000; Wilson 2004). Two of these air pollutants are of special interests in this study. They are airborne microorganisms and dust.

The concentrations of airborne microorganisms and dust in livestock houses are generally much higher than those in the ambient air. The concentrations of microorganisms in urban areas are around 3 $\log_{10}$ colony forming unit (CFU) m^{-3} (Fang et al. 2008; Wang et al. 2010). The figures for microbial concentrations in livestock houses can be as high as 6 $\log_{10}$ CFU m^{-3} and may even reach 9 $\log_{10}$ CFU m^{-3} (Seedorf et al. 1998). Research on airborne dust concentration is increasingly focused on the fractions with small particle sizes, e.g. PM$_{10}$ (particulate matter smaller than 10 µm) and PM$_{2.5}$ (particulate matter smaller than 2.5 µm). These fractions do not readily settle on surfaces and are reported to be more harmful to human health because they can be deposited deeper in the respiratory tract than the larger particle fractions. The monitoring of PM$_{10}$ and PM$_{2.5}$ in EU urban areas has revealed that their concentrations are around the annual average limits permissible under EU legislation (Pérez et al. 2008; Rodríguez et al. 2008), i.e. 40 µg m^{-3} for PM$_{10}$ and 25 µg m^{-3} for PM$_{2.5}$ (European Commission 1999; European Commission 2005). The concentrations of PM$_{10}$ and PM$_{2.5}$ in livestock houses can be around 4400 and 170 µg m^{-3}, respectively (Zhao et al. 2009). Although no clear relationship has been demonstrated between the microbial and dust concentrations and human health, a bunch of epidemiological studies have shown that people working with livestock suffer respiratory diseases due to inhalation of the highly concentrated aerial pollutants from livestock houses (Schiffman 1998; Von Essen and Romberger 2003; von Essen and Auvermann 2006).

Airborne microorganisms in livestock production systems can be emitted to the ambient air through the ventilation exhausts. Whether and to what extent that emitted pathogenic microorganisms are transmitted to other nearby livestock units and/or to humans through the airborne route remains unclear. Lab-scale experiments have confirmed the airborne transmission of some microorganisms from animal to animal: it was found that healthy animals kept physically but not aerially separated from the infected animals became infected (Berthelot-Herault et al. 2001; Brockmeier and Lager 2002). Also, some infectious microorganisms were collected kilometers away from the source farm

(Otake et al. 2010). However, to date these findings have not been incontrovertibly linked to underlying physical and biological processes. So, knowledge gaps exist in the whole process of airborne transmission of microorganisms, from generation and transportation through inhalation and finally to infection (Stark 1999).

It is assumed that microorganisms are somehow associated with dust, which may act as a carrier of microorganisms in the air. However, the relation between microorganisms and dust in the whole process of airborne transmission is unclear. Knowledge about this relation may be helpful for developing and implementing effective control technologies for airborne microorganisms and dust from livestock houses. It is therefore important to carry out investigations in areas such as the identification of their sources, suspension in the air, physical and biological decay in their airborne transportation, deposition in respiratory tracts, as well as the available abatement and sampling technologies.

Various abatement techniques have been implemented to reduce the concentrations and emissions of airborne microorganisms and dust from livestock houses (Pearson and Sharples 1995; Gustafsson 1999; Cambra-Lopez et al. 2009). The performance of these techniques varies when they are applied under different housing systems and to different animal species. The best approach to reduce microorganisms and dust from livestock houses is thought to be to control them at source, because this approach not only reduces the emissions but also improves the indoor air quality. However, the demand for a better aerial environment forced the authorities to impose stricter legal limits on exposure to air pollutants. To comply with these regulations, more and more end-of-pipe abatement techniques, i.e. acid and biological scrubbers, are being applied to the air exhausts of livestock houses. The acid scrubbers have been originally designed for reducing ammonia emission, and are not very effective at reducing odor. The biological scrubbers are more effective at reducing odor emission, but vary considerably in their reductions of airborne microorganisms (Seedorf and Hartung 1999; Aarnink et al. 2005). Recently, multi-stage scrubbers (combined systems with acid scrubbers and biological scrubbers) have been developed, to achieve higher removal efficiencies for the major air pollutants, e.g. microorganisms, dust, ammonia and odor.

Sampling of airborne microorganisms and dust is important for evaluating the performance of control techniques and for assessing the bio-security of the aerial environment. Researchers and manufacturers are still improving the available sampling techniques and looking for new techniques with high efficiencies and accuracies. Samplers for collecting airborne microorganisms apply different principles, including impaction, impingement, cyclone forces and filtration. The efficiencies of these samplers are generally known to be imperfect because airborne microorganisms may either miss being collected by so-called non-isokinetic sampling or are killed by various sources of stresses during sampling. To date, the efficiencies of the samplers for collecting different microbial species have not been well established. Notably, there is no standard protocol for sampling airborne microorganisms that specifies the requirements for hardware and also the procedures immediately prior, during and

after the sampling. The lack of a protocol makes it difficult to interpret and compare the results of different studies. The sampling protocol for collecting PM_{10} and $PM_{2.5}$ in ambient air has been legislated by the EU commission and US EPA (European Commission 1998; European Commission 2005; US EPA 2010). These sampling techniques cannot be used to sample dust in livestock houses because the concentrations and particle sizes of dust in livestock houses are profoundly different from those in the ambient air, and this may compromise the efficiency and accuracy of the sampling. There is therefore an urgent need to develop a technique and eventually a protocol suitable for sampling dust in livestock houses.

OBJECTIVES AND OUTLINE OF THIS THESIS
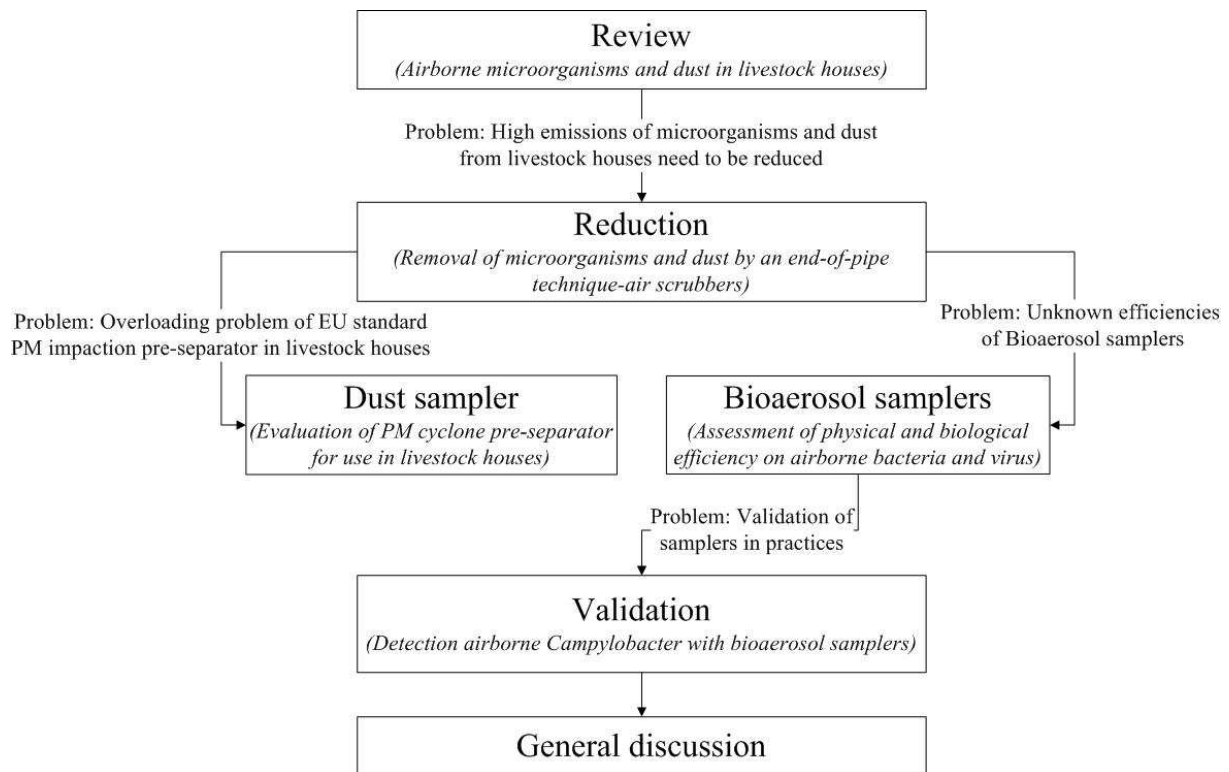
Figure 1. Overview of the structure of this thesis.

The objective of this thesis was to gain knowledge about airborne microorganisms in livestock production systems and their relation to dust throughout the process of airborne transmission. Figure 1 gives an overview of the structure of this thesis. The detailed objectives were:

▪ to review the current knowledge on airborne microorganisms and their relation to dust in livestock houses with respect to their sources, concentrations, physical and biological decay in transmission, deposition in the respiratory tracts of human and animal, and sampling and mitigation techniques. (Chapter 2).

▪ to evaluate the effects of three multi-stage air scrubbers installed in three different pig houses in reducing emissions of airborne microorganisms, dust, ammonia and CO_2. (Chapter 3).

- to investigate the overloading problem of the EU reference PM samplers (with impaction pre-separators) when used in livestock houses, and to evaluate alternative PM samplers (with cyclone pre-separators) in terms of their suitability for use in livestock houses. (Chapter 4).
- to evaluate the physical and biological efficiencies of the Andersen six-stage impactor, the AGI-30, the OMNI-3000 and the MD8 for collecting four aerosolized bacterial species (*Enterococcus faecalis*, *Escherichia coli*, *Campylobacter jejuni* and *Mycoplasma synoviae*) and one viral species (Gumboro vaccine virus). (Chapters 5, 6 and 7).
- to detect airborne *Campylobacter* with three bioaerosol samplers (Andersen impactor, AGI-30 and OMNI-3000) in a transmission experiment with broilers. (Chapter 8).

The findings from these seven studies are synthesized in Chapter 9. That chapter ends with the main conclusions from this thesis on airborne microorganisms and dust from livestock houses.

AIRBORNE MICROORGANISMS FROM LIVESTOCK PRODUCTION SYSTEMS AND THEIR RELATION TO DUST

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ABSTRACT. High concentrations of airborne microorganisms are present in livestock production systems. These microorganisms can be emitted outside through ventilation exhausts, and the pathogenic ones are suspected to pose a risk of airborne infection to humans in vicinity and to animals on other farms. However, the extent to which airborne transmission may play a role in the epidemic is not yet fully understood. Furthermore, how dust acts as a carrier of microorganisms in an airborne transmission is unknown. This review paper presents the current knowledge of the entire process of airborne transmission of microorganisms - from suspension, transportation until deposition and infection - and their relation to dust. Descriptions of the sources, species, size distributions and concentrations of airborne microorganisms and dust in livestock production systems have been generalized. Their physical and biological decay during airborne transmission are discussed. The deposition of microorganisms and dust in human and animal respiratory tracts and their infection dose are reviewed. Finally, the sampling strategies, sampling devices and the mitigation techniques of airborne microorganisms and dust in livestock production systems are introduced. It is concluded that much less is known about the complexity in the processes of the airborne transmission of microorganisms in relation to dust. In order to fill the gaps, there needs to be multidisciplinary collaborative research involving engineers, veterinarians, microbiologists and animal scientists.

Keywords. Airborne transmission, Agriculture, Animal, Production, Microorganisms.

INTRODUCTION

Pathogenic microorganisms in livestock production systems are important because of their ability to cause diseases in animals and/or in humans. Many of these microbial species occur in high concentrations in the air inside the livestock houses and they are also emitted to the environment surrounding the houses (Seedorf et al., 1998). The extent to which these pathogenic microorganisms are transmitted to other livestock and to humans through the airborne route remains unclear. For example, a large proportion of the indirect transmission, i.e. transmission between animal houses and between herds, of important livestock diseases such as foot-and-mouth disease (FMD) is not attributable to known routes (Elbers et al., 2001; Gloster et al., 2003). Airborne transmission is therefore often assumed to be one cause of this indirect transmission, but attempts to link transmission of FMD virus or other pathogenic microorganisms to prevalent wind directions have been unsuccessful.

Given the lack of knowledge on indirect transmission it is important to study airborne transmission better in order to understand what role it can play. Here we define airborne transmission as "a mechanism that causes animals and humans to become infected by inhaling aerial pathogenic microorganisms through respiratory tracts". The airborne transmission of certain pathogenic microorganisms from animal to animal has been demonstrated in lab-scale experiments in which healthy animals separated physically but not aerially from infected animals became infected (Berthelot-Herault et al., 2001; Brockmeier and Lager, 2002). Furthermore, some microorganisms collected kilometers away from the source farm were found to be capable of infecting healthy animals intramuscularly or intratracheally (Otake et al., 2010). However, there is still uncertainty, because of the incomplete knowledge about the entire process of airborne transmission of microorganisms, from generation and transportation through inhalation and finally to infection (Stark, 1999).

Dust probably plays a role as the carrier of the microorganisms in the air, because it seems the microorganisms are associated with particle sizes larger than individual microorganisms (Zhao et al., 2011a). In 1987, the importance of the relationship between airborne microorganisms and dust from livestock production systems was reviewed by Muller and Wieser (1987). The authors separately described the indoor properties (source, concentration and constitute) of airborne microorganisms and dust, and the dispersion in ambient air outdoors. Since then, much research has been done on specific processes involved in the transmission of the airborne microorganisms and dust. However, we lack an integrated overview of and insight into all the processes involved in the airborne transmission of microorganisms in association with dust.

The objective of this paper is to review current knowledge on airborne microorganisms from production systems for typical livestock species (swine, poultry and cattle), and their relation to dust. Specifically, we identified the source, species, size distribution, and concentration of airborne microorganisms and dust from livestock production systems, as well as the factors affecting their

concentrations in "AIRBORNE MICROORGANISMS AND DUST IN LIVESTOCK PRODUCTION SYSTEMS". The physical and biological decay of airborne microorganisms and dust during transmission will be described in "DECAY OF MICROORGANISMS AND DUST IN THE AIR". The deposition of airborne microorganisms and dust in respiratory tracts, and the infective dose of pathogenic microorganisms to animals will be introduced in "DEPOSITION AND INFECTIVE DOSE". The strategy and techniques for sampling microorganisms and dust in livestock production systems are proposed in "SAMPLING OF AIRBORNE MICROORGANISMS AND DUST". The mitigation techniques will be described in "MITIGATION TECHNIQUES FOR AIRBORNE MICROORGANISMS AND DUST".

AIRBORNE MICROORGANISMS AND DUST IN LIVESTOCK PRODUCTION SYSTEMS

Identification the sources of airborne microorganisms and dust

Identifying the source of microorganisms and dust in livestock production systems helps to elucidate how airborne transmission is generated, and ultimately can help to develop and implement strategies that prevent such transmission from being generated (Bull et al., 2006; Cambra-Lopez, 2010). Sources of dust in livestock production systems have been identified and assessed qualitatively and quantitatively (Aarnink et al., 1999; Donham and Gustafson, 1982). It is generally accepted that all dust sources are also sources of airborne microorganisms because these source materials somehow contain certain microbial species that may be generated together with dust. However, the source identification of airborne microorganisms has not yet been extensively investigated, and it is thought to be more complicated than the source identification of dust in at least two ways. The first way is associated with the complex of microbial species in a source. A source material always contains a microbial flora composed of many different microbial species. Identifying the source of each microbial species is much more laborious than identifying the sources of dust. The second way is associated with the dynamic viability of microorganisms in the generation process (Milne et al., 1989); microorganisms may either decay or multiply in the source material. Thus the source identification for microorganisms should also be dynamic.

Source of airborne microorganisms

Airborne microorganisms in livestock production systems originate from the animals, organic materials, farm personnel and visitors, and ambient air.

Animals shed microorganisms by means of excretion. Their feces contain many microbial species, some of which are highly concentrated (Letellier et al., 1999; Pell, 1997). Consider two common zoonotic bacterial species, *Salmonella* and *E. coli*. Both have been found in feces: *Salmonella* at a concentration of 2-7 log cfu g^{-1} feces (Gray and Fedorka-Cray, 2001; Himathongkham et al., 1999) and *E. coli* at 2-6 log cfu g^{-1} feces (McGee et al., 2001; Omisakin et al., 2003). Furthermore, feces are an important pathway for virus shedding from infected animals (Fouchier et al., 2003). A list of viral species that may be excreted by cattle was proposed by Pell (1997). Many other viruses have been

recovered from animal feces, such as avian influenza A virus (Webster et al., 1978) and Newcastle disease virus (Spradbrow et al., 1988) in poultry, swine fever virus (Van Oirschot, 1979), hepatitis E virus (De Deus et al., 2007) and porcine reproductive and respiratory syndrome virus (PRRSV) (Yoon et al., 1993) in pigs. The microorganisms in feces can go into suspension in the air when they are disturbed by air flow or animal activity. Some studies have managed to identify feces as the source of airborne microorganisms by polymerase chain reaction (PCR) technology. A study by Duan et al. (2009) found the airborne *E. coli* strains inside and downwind from the pig houses were closely associated with those isolated from pig feces. Water content binds particles in feces and prevents their suspension, so, the microorganisms in dry feces that have low water content might become airborne more easily than microorganisms in fresh feces. The water content of fresh feces is about 85-92% (Derikx et al., 1994). The water takes days to evaporate to less than 10% - the water content of the airborne dust in livestock production systems (Aarnink et al., 1999). This means the microorganisms must undergo a latent period between the moment they are excreted in the feces and the moment they go into suspension in the air.

During inhalation and exhalation the surface of the mucus in the respiratory tract is destabilized through an interplay between surface tension and viscous forces (Edwards et al., 2004) and this can result in microorganisms in the mucus going into suspension. These microorganisms suspended in the air in the respiratory tract can be expelled from the body via coughing, sneezing and breathing, and can thus become airborne in ambient air. This source of airborne microorganisms is widely accepted in the human model of disease transmission, and pathogenic microorganisms have been frequently recovered from the exhaled aerosols (Fabian et al., 2008; Weber and Stilianakis, 2008). Only a few studies have been carried out to directly detect the microorganisms in air exhaled by animals. By sampling exhaled air in masks placed over the heads of infected pigs, Cho et al. (2006) recovered PRRSV and Hermann et al. (2008) recovered two bacterial species, *Mycoplasma hyopneumoniae* and *Bordetella bronchiseptica*. Neither PRRSV nor *M. hyopneumoniae* could be proved to be infective, however, because only their genes were detected in the air samples. Furthermore, none of the four viral species (PRRSV, Porcine circovirus 2, swine influenza virus, and Porcine respiratory coronavirus) investigated by Hermann et al. (2008) were present in the exhaled air, although they were found in oral and nasal swabs taken from the infected pigs. These results indicate that some microorganisms in animal respiratory tracts might not readily become suspended in the air or be expelled out of the body, which implies that this is not an important source of microorganisms in ambient air in livestock production systems. However, the reason the other microorganisms were not detected could also be because the quantities of exhaled microorganisms were below the detection limit of the sampling devices. Animal respiratory tracts can only be excluded as a source of airborne microorganisms until it has been incontrovertibly established that every single microorganism is detectable.

Studies done for the safety assurance of food, sterilization and the international trade in hides have investigated and identified microorganisms in animal skins (Baird-Parker, 1962; Gailiunas and Cottral,

1966; Kloos et al., 1976). No information is currently available on the release of microorganisms from animal skins to the air, however, this release is assumed to be possible, given that microorganisms could be conveyed from deeper in the skin to the skin surface via sweat (Baxby and Woodroffe, 1965) and be aerosolized by desquamation (Aarnink et al., 1999). Animal products, e.g. eggs (De Reu et al., 2008; Doyle, 1984) and milk (Donaldson et al., 1983; Doyle and Roman, 1982), contain microorganisms. When eggs are broken or milk is splashed, the microorganisms might become airborne.

Organic materials such as feed and litter, which are brought into livestock production systems, may serve as carriers for variety of microorganisms. The microorganisms originate from the soil and are transferred to standing crops by wind, rain, mechanical agitation, or insects (Maciorowski et al., 2007). Both non-pathogenic and pathogenic microorganisms have been recovered from feed; analysis has revealed concentrations of gram-negative bacteria in feed as high as 5 $\log_{10}$ CFU g^{-1} (Hofacre et al., 2001). These microorganisms can be disseminated together with feed particles during feeding (Andersson et al., 1999; Chang et al., 2001); the extent of dissemination depends greatly on how the feed is given (Pearson and Sharples, 1995).

Litter is a mixture of bedding materials (e.g. wood shavings, chopped straw, sawdust, and rice hulls etc.) animal feces, dander and feed (Torok et al., 2009). The provision of litter in livestock production systems may improve animal welfare by increasing the incidence of natural behaviors (Appleby and Hughes, 1991), which, however, may result in more microorganisms being present in the air than in housing systems without litter (Madelin and Wathes, 1989; Vucemilo et al., 2007). The microorganisms arrive in litter during the harvesting and processing of the bedding material and feed, and through animal excretion and secretion. The concentrations of aerobic bacteria in poultry litter range from 3 to 9 $\log_{10}$ CFU g^{-1} (Lu et al., 2003; Martin et al., 1998). Most of the bacteria in the poultry litter are gram-positive. Gram-negative bacteria and mold account for a fraction of the total microbial count, but due to the high concentration of the total microorganisms, their numbers can still be high in some cases (Martin et al., 1998). Surprisingly, some pathogenic bacteria which are commonly recovered from animal feces (for instance *E. coli*, *Salmonella* and *Campylobacter*) are not detectable in litter (Lu et al., 2003). The reason is not clear, but suggested explanations are the absence of bacterial prevalence in the sampled flocks, and bacterial concentrations in litter being below the detection limit of the analysis technology. Other microbial species which may be involved in degradation of wood and cycling of nitrogen and sulfur have been identified in poultry litter. They include *Globicatella sulfidofaciens*, *Corynebacterium ammoniagenes*, *Corynebacterium urealyticum*, *Clostridium aminovalericum*, *Arthrobacter sp.*, and *Denitrobacter permanens* (Lu et al., 2003).

Farm workers and visitors are also possible vectors of microorganisms in livestock production systems (Nishiguchi et al., 2007). Nowadays, hygiene measures (such as changing clothes or UV disinfection) are increasingly being taken to prevent humans introducing microorganisms into livestock houses (Newell and Fearnley, 2003).

Microorganisms in ambient air can enter livestock production systems through ventilation ducts (Martin et al., 1996). These microorganisms contribute only a limited extent to the microorganism load in livestock systems: their concentrations are far lower than those inside of livestock systems. However, the ambient air pathway is still important, especially for the highly infectious viruses (Donaldson et al., 1987; Gibson and Donaldson, 1986; Gloster et al., 2003), because it needs only a few of these microorganisms to trigger an outbreak in a livestock farm.

Sources of dust in animal houses

Sources of airborne dust include feed, animal skin and feather debris, feces, litter, microorganisms, pollen, and insect parts (Aarnink et al., 1999; Donham et al., 1986). The contribution of these sources to airborne dust varies, depending on the animal species and the housing system. Heber et al (1988a) reported that the main source of airborne dust in pig houses was feed, which is consistent with the findings of Donham et al. (1986) and Aarnink et al. (1999). Muller and Wieser (1987) found that 55-68% of the airborne dust in floor layer systems with litter originated from the bedding materials in litter, while 80-90% of the airborne dust in layer systems with battery system originated from feedstuff (Table 1). In floor systems with wood shavings as litter for three-week old broilers, Aarnink et al. (1999) found that airborne dust mainly (> 10 %) originated from down feathers and urine components. The contribution of feed to the airborne dust largely depends on its composition and how it has been processed (Pearson and Sharples, 1995), e.g. meal or crumbles or pellets. The contribution of feces is probably related to the housing system, e.g. with or without litter (straw bedding versus liquid manure). Table 1 lists the main sources of dust and gives an estimation of their contributions.

Table 1. Sources of airborne dust in animal houses.

Animal	Housing type	Source (%)	Contribution	Reference
Layers	Floor housing with litter	Bedding material in litter	55-68%	(Muller and Wieser, 1987)
		Feathers	2-12%	
		Excrement	2-8%	
Layers	Battery housing	Feed	80-90%	(Muller and Wieser, 1987)
		Feathers	4-12%	
		Excrement	2-8%	
Broilers	Floor housing with litter	Feathers	>10%	(Aarnink et al., 1999)
		Crystalline dust	>10%	
		Feed, microorganisms	<1%	
Rearing pigs	Partially slatted floors	Feed	>10%	(Aarnink et al., 1999)
		Skin particles	>10%	
		Feces, crystalline dust	1-3%	

Species of airborne microorganisms

Most of the airborne microorganisms in livestock production systems are bacteria, of which the most dominant are gram-positive bacteria. Airborne gram-positive *Enterococci* were found to account

for up to 96% of the total bacteria recovered in poultry and pig houses (Clark et al., 1983). The most common species of these gram-positive bacteria are *Staphylococcus*, *Streptococcus* and *Enterococci* (Clark et al., 1983; Hartung, 1992; Matkovic et al., 2007). The gram-negative bacteria account for only a fraction of airborne bacteria (Zucker et al., 2000). Bakutis et al. (2004) reported that in terms of the total bacterial count, the proportion of gram-negative bacteria was approximately 10% in cattle houses, 4.9% in pig houses, and 2.6% in poultry houses. Zucker et al. (2000) found that the airborne gram-negative bacteria in pig and cattle houses are aerobic and include *Enterobacteriaceae*, *Pseudomonadacea*e and *Neisseriaceae*; no culturable obligate anaerobic gram-negative bacteria were isolated. In another study, although the genes of certain viable micro-aerophilic bacteria such as *Campylobacter* were detected in the air of poultry houses, none of them were still culturable (Olsen et al., 2009). A possible reason for the smaller proportion of airborne gram-negative bacteria in livestock production systems is that they are more vulnerable to environmental stress such as oxidation, radiation, and dehydration, probably because of their thinner cell walls (Pal et al., 2007; Theunissen et al., 1993). The proportion of fungi, molds and yeasts in the airborne microbial flora in animal houses is low (Hartung, 1992; Lee et al., 2006). The most frequently reported fungi in poultry, pig and dairy houses are *Aspergillus sp.*, *Alternaria sp.*, *Cladosporium sp.*, *Penicillium sp.*, *Fusarium sp.*, *Scopulariopsis sp.*, and yeast (Chang et al., 2001; Cormier et al., 1990; Martin et al., 1996; Matkovic et al., 2007; Vittal and Rasool, 1995; Wilson et al., 2002).

Size distribution of airborne microorganisms and dust

The size of an airborne particle determines its transportation, sedimentation and resuspension, as well as its deposition in the respiratory tracts of receptors. Investigations of the size distribution of microorganisms and dust in livestock production systems may provide a useful overview of their quantitative importance, indicate the health risk for human and animals, and facilitate the establishment and evaluation of control techniques. In this chapter, size of an airborne microorganisms refers to the size of the dust particle that contains this microorganism. Thus the size distribution of airborne microorganisms refers to the relative amounts of dust particles that contains microorganisms, sorted according to size class.

According to the definition related to occupational health, particle sizes are categorized into three categories: inhalable (< 100 μm), thoracic (< 10 μm) and respirable (particles which can go beyond the larynx and penetrate into the unciliated respiratory system, diameter < 4 μm) (Zhang, 2004). In scientific studies, the size of microorganisms is distinguished between non-respirable (> 4 μm) and respirable (Cambra-Lopez et al., 2010; Curtis et al., 1975; Madelin and Wathes, 1989). These occupational health fractions are also applied for sizing the dust, while recent research is increasingly classifying dust as PM_{10} (< 10 μm) or $PM_{2.5}$ (< 2.5 μm). The size distribution of airborne dust has been expressed either in mass or in counts.

Zhao et al. (2011a) found that in three pig houses about 73-95% of the airborne bacteria were in the non-respirable range (Figure 1). A similar result was reported by Curtis et al. (1975): non-respirable bacteria accounted for approximately 78-89% of the airborne bacteria in pig houses. The size distribution of microorganisms in poultry houses depends on the type of housing system. In broiler rooms with wood-shaving litter, most of the bacteria were non-respirable in the full life cycle of broilers (eight weeks); a similar distribution pattern was found in broiler rooms with raised netting floor only after the birds were older than six weeks. When the birds were two to five weeks old, the proportions of airborne respirable and non-respirable bacteria were similar (Madelin and Wathes, 1989).
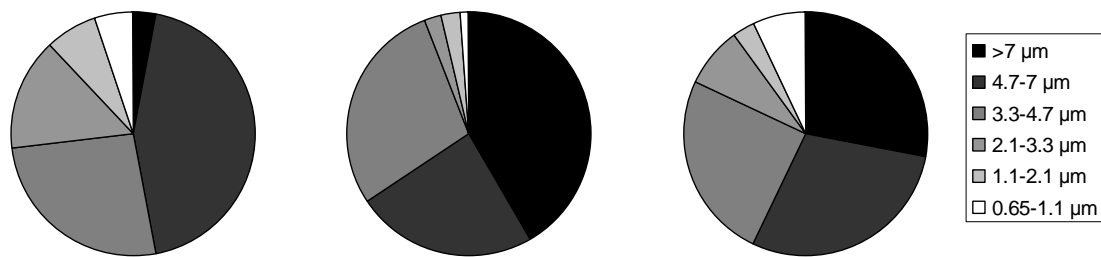
Figure 1. Size distribution of airborne bacteria in the exhaust air from three commercial fattening pig houses measured with an Andersen six stage viable bio-sampler (Zhao et al., 2011a).

Heber et al. (1988a) reported that non-respirable particles in pig houses accounted for more than 80% in mass, but less than 30% in terms of count. A very recent study (Lai et al., 2010) gives more detailed information. Expressed as percentage of total dust, the mass of PM_{10} was found to be 30-54% in pig houses, 41-69% in poultry houses, and 36% in cattle houses. In all three types of house, PM_{10} count was 99%. The equivalent figures for $PM_{2.5}$ mass were 1-3% in pig houses, 2-3% in poultry houses, and 5% in cattle houses. For PM_{25} counts the figures were 90-99% in pig houses, 88-92% in poultry houses and 99% in cattle houses. The difference in the mass and numeric size distribution is caused by the fact that small dust particles contribute little to mass.

That more microorganisms and less dust particles are found in the non-respirable range indicates that a non-respirable dust particle is more likely to be loaded with microorganisms than a respirable one. This is a reasonable hypothesis, because the larger a particle is, the greater the chance it may contain microorganisms. Nowadays, the size distribution of airborne microorganisms is normally determined with the Andresen stage impactor (Andersen, 1958). This sampler actually counts particles containing one or more microorganisms of different sizes; it does not count the microorganisms. In some cases, for instance a bio-security assessment for occupational health, it might be more reliable to assess the total count of microorganisms rather than the microbial-containing particles, because the former will give a better idea of the risk of infection. This requires corresponding research on size

distribution of microorganisms with sampling techniques that can detach the microorganisms from particles.

Concentrations of airborne microorganism and dust

Concentrations of airborne microorganisms and dust in livestock production systems have been investigated in previous studies (Kim et al., 2008; Radon et al., 2002; Zhao et al., 2011a). The results of these studies vary greatly as a consequence of multiple factors, and this makes it difficult to compare the data, even within the same animal species. So far, the studies by Seedorf et al. (1998) and Takai et al.(1998) still provide the most representative concentration data on microorganisms and dust in livestock production systems. These data are summarized in Table 2, together with the PM_{10} and $PM_{2.5}$ concentration measured by Lai et al (2010). For microorganisms, the highest concentrations of airborne bacteria and fungi were found in broiler houses. The concentrations found in layer, pig and cattle houses were lower, but still higher than those in ambient air (Wang et al., 2010). For dust, the highest dust concentrations were also found in poultry houses, and the lowest dust concentrations were always in cattle houses.

Table 2. Concentrations of airborne microorganisms and dust in livestock production systems.

Animal	Bacteria[a] $\log_{10}$ CFU m^{-3}	Fungi[a] $\log_{10}$ CFU m^{-3}	Inhalable[b] mg m^{-3}	Respirable[b] mg m^{-3}	PM_{10}[c] mg m^{-3}	$PM_{2.5}$[c] mg m^{-3}
Broiler	6.4	4-5	3.8-10.4	0.42-1.14	0.9-2.4	0.04-0.09
Layer	4-5	3-4	1.0-8.8	0.03-1.26	5.9-6.1	0.25-0.29
Pig	5.1	3.7	0.6-5.1	0.09-0.46	0.2-2.0	0.01-0.07
Cattle	4.3	3.8	0.1-1.2	0.03-0.17	0.1	0.01

[a] data from Seedorf et al. (1998).

[b] data from Taikai et al.(1998).

[c] data from Lai et al. (2010).

Factors affecting concentrations of airborne microorganisms and dust in animal houses

The concentrations of airborne microorganisms and dust in animal houses are affected by animal, housing system and management. In this chapter, these factors are discussed separately, but one should realize that these factors always collectively affect the concentration because they are inter-correlated. For instance, animal activity is associated with animal age, weight, and light schedule, and ventilation rate is affected by outdoor and set-point temperature, humidity and the animals themselves.

Animal

The animal factor can be further detailed into sub-factors such as age, weight, activity and stocking density. The concentrations of airborne microorganisms and dust generally increase concomitantly with animal age and weight (Hinz and Linke, 1998; Predicala et al., 2001; Yoder and Van Wicklen, 1988). However, an inverse relationship has also been found. Madelin and Wathes (1989) found a decrease of microorganisms and dust concentrations in the late fattening period of broilers. A similar

result was reported by Saleh et al. (2005). The decrease in concentration of microorganisms and dust is probably because the older broilers occupied all the floor space, which limited their activity.

In general, higher concentrations of bacteria, fungi and dust are measured when the animals are more active, as can be inferred from the finding that their concentrations were higher in day time than at night (Seedorf et al., 1998; Takai et al., 1998). Image and infrared technology allows animal activity to be automatically detected (Gloster et al., 2007; Pedersen and Pedersen, 1995). Using an infrared detector, Haeussermann et al. (2007) demonstrated that the indoor concentrations of PM_{10} were affected by pig activity. A similar study by Heber et al. (2006) showed that both total dust and PM_{10} were correlated with the pig activity. Surprisingly, Gloster et al. (2007) failed to establish the correlation between the concentration of airborne FMD virus and pig activity quantified by taking sequential pictures. The reason is not clear, but the authors explained that the virus production appears to be more closely associated with other factors, such as viraemia in the blood and physical symptoms (Gloster et al., 2007). Hardly any other information is available on the relation between quantified animal activity and concentrations of microorganisms.

Housing system

Compared to a cage system, an aviary system contained higher concentrations of microorganisms (De Reu et al., 2005) and dust (Appleby and Hughes, 1991). This is probably because in an aviary system, laying hens have more scope for moving horizontally and vertically and perform dust bathing behavior in the litter. Housing systems with bedded floors caused more air quality problems, although such housing systems are generally thought to be more beneficial for animal welfare (Kim et al., 2008; Madelin and Wathes, 1989; Quarles et al., 1970). The type of bedding material also affects the concentration of microorganisms in the air. For instance, in broiler houses, straw bedding released less bacteria in the air than wood shavings did (Banhazi et al., 2008a). The authors argued that this was probably because wood shavings provided a better micro-environment for bacteria viability and multiplication. Hence housing systems that provide more space and bedding material for animal activities are generally more contaminated with airborne microorganisms and dust than those with less space and without bedding material.

Comparisons of dust concentrations between natural and mechanical ventilation systems showed that with mechanical ventilation, less respirable (Phillips, 1986) and total dust was found in pig houses (Chiba et al., 1985) and there was less total dust in turkey houses (Janni and Redig, 1986). By contrast, concentrations of total bacteria and fungi were lower in naturally ventilated pig houses without bedding materials (deep-pit manure system with slats, and manure removal system by scraper) than in mechanically ventilated houses. The contradictory results found for the effect of type of ventilation (natural or mechanical) on microorganisms and dust are not fully understood, but it seems likely that the situations (including the management) of the ventilation systems vary between the different studies, making the data less comparable.

Management

Feed management may play an important role in dust concentration in livestock production systems. Previous studies have shown that dust concentrations were reduced by giving pelleted feed rather than powdered feed, wet feed rather than dry feed and coated feed rather than uncoated feed (Clark and McQuitty, 1988; Pearson and Sharples, 1995; Zeitler et al., 1987). The effect of feeding management on airborne microorganism concentration has not been extensively studied.

Maintaining good hygiene in livestock production systems may help to improve the air quality with respect to microorganisms and dust. For instance, cleaning (e.g. removing litter, scrubbing surfaces and disinfecting the house) between two production circles may reduce both airborne microorganisms and dust (Banhazi et al., 2008a). Investigations of the relation between hygiene and air quality have found they are not always positively correlated. Duchaine et al. (2000) reported that a housing system that appeared cleaner contained more airborne bacteria than one that appeared dirtier. The probable explanation is that houses with more settled dust on the surfaces are more readily ranked as dirtier, but dust accumulated on surfaces is not an appropriate indicator of the concentration of bacteria in the air.

Ventilation management (e.g. adjusting the rate at which indoor air is exchanged with outdoor air by mechanical ventilation systems) is to control the temperature and other aerial variables such as humidity and gas concentrations inside livestock houses. Previous studies have shown that lower concentrations of microorganisms and dust can be achieved by increasing the ventilation rate in livestock production systems (Duchaine et al., 2000; Hinz and Linke, 1998; Kim et al., 2007b). However, a non-significant correlation between these variables has also been reported in livestock production systems (Banhazi et al., 2008b; Seedorf et al., 1998). The probable reason for these contradictory findings is that ventilation affects the concentration of microorganisms and dust in two ways: by exhausting airborne microorganisms and dust to outdoors, thereby reducing their indoor concentrations, and by producing airflow turbulence above surfaces, agitating the particles and causing them to go into suspension in the air, thus compromising the removal effect. Smaller airborne particles might be more effectively removed from livestock houses by ventilation than bigger particles (Kuehn, 1988) because they are readily transported in the air streams.

A high relative humidity in the air reduced the dust concentration in livestock production systems (Guarino et al., 1999). In humid environments, the dust particles bind to the surface and are not easily suspended after animal activity, and those in the air aggregate and to settle faster (Heber et al., 1988b; Takai et al., 1998). Humidity seems not to affect the concentrations of total and gram-negative bacteria (Attwood et al., 1987; Banhazi et al., 2008b; Nicks et al., 1993). It is reasonable to suppose that when humidity is high, microorganisms are bound to surfaces and removed from the air, just as dust. However, a high humidity favors the multiplication of certain microbial species in the sources (De Rezende et al., 2001). The multiplication might result in there being more microorganisms on each particle going into suspension from the source.

Indoor temperature management has already been regulated for the poultry and pig industries, to optimize productivity. Al Homidan et al. (1997) reported that the total dust in broiler rooms increased when the temperature was set 2°C above the recommended level. The correlation between dust concentration and temperature reverses from positive to negative when the temperature is extremely high, apparently because animal activity decreases at high temperature and thus fewer particles from surfaces are disturbed and suspended (Donkoh, 1989; Guarino et al., 1999; Wylie et al., 2001). As well, a high temperature triggers a series of events that may affect the dust concentrations in livestock production systems, such as increasing the ventilation rate and activating wet cooling systems (Simmons and Lott. B. D., 1996). Although some researchers (Banhazi et al., 2008a) have suggested there is a relation between the concentration of microorganisms and temperature, little information is available so far.

DECAY OF MICROORGANISMS AND DUST IN THE AIR

The decay of microorganisms and dust is a parameter that cannot be ignored in empirical and theoretical models of airborne transmission (Lighthart and Frisch, 1976; Yu et al., 2004); it may help when assessing health impacts from exposure. The term "decay" has two meanings. Firstly, decay is the physical elimination of a particle from the air by means of a series of processes such as gravitational sedimentation, impaction and electrostatic precipitation. Secondly, decay is the loss of biological activity of an airborne microorganism owing to loss of enzyme activities or denaturing of membrane phospholipids, proteins or nucleic acids (Cox, 1989). Below, these definitions will be referred to as physical decay and biological decay, respectively. It is clear that physical decay applies to the elimination of dust particles, whereas both physical and biological decay apply to airborne microorganisms.

Physical decay

Physical decay can be quantified by the rate of deposition (particle rate loss coefficient or deposition velocity) that refers to the speed of particles eliminating from the airspace (Deshpande et al., 2009). A model of the deposition rate was proposed in the study by Lai (2002), in which various mechanisms are included as parameters (Table 3). In almost all practical cases, these mechanisms collectively affect the deposition of particles. The combined effect has been extensively investigated in different airspaces, e.g. experimental chambers with rough or smooth surfaces (Abadie et al., 2001; Chen et al., 1992), and furnished or unfurnished rooms (Fogh et al., 1997; He et al., 2005). It was found that, for particles ranging from 0.01 to 10 µm, the rate of deposition had a U-shaped pattern. The lowest deposition rate was found for particles ranging from 0.1 to 1.0 µm. Particles outside this range decayed faster. The probable explanation based on Lai (2006) is that the larger particles settled on surfaces by gravimetric sedimentation, and smaller particles are deposited by diffusion (Brownian

and turbulent) effect. These two physical decay mechanisms affect the deposition of 0.1-1 µm particles to a lesser extent.

Table 3. Mechanisms associating physical decay of microorganisms and dust.

Mechanism	Definition[a]	Reference
Advection	The mean transport of a particle by the mean motion of the atmosphere, and occurs when the spatial gradient is nonzero and the particle is transported along the mean wind	(Baldocchi et al., 1988)
Brownian diffusion	The process of mass transfer of particles brought about by a random molecular motion (Brownian motion) and associated with a concentration gradient	(Vaithiyalingam et al., 2002)
Thermophoresis	The motion of a particle under the influence of a temperature gradient	(Langer and Holcombe, 1999)
Gravitational sedimentation	The separation of dispersed particles from gaseous phase under action of gravity	(Wunsch, 1994)
Impaction	The deposition of particles due to their momentum causing them to deviate from airflow streamlines and impacting at bifurcations	(Katz et al., 2001)
Electrostatic precipitation	The use of an electrostatic field for precipitating or removing charged particles from a gas flow in which the particles are carried	(Shen and Pereira, 1979)

[a] Some definitions in this table that were originally for gases or molecules in non-aerial environments have been modified to make them suitable for describing the associating mechanisms of microorganisms and dust in the air.

Biological decay

The biological decay of airborne microorganisms has been expressed in different ways, e.g. decay rate (or death rate), survival or half-life. The decay rate is the decrease in concentration of microorganisms over time. A proportionality constant (k) indicates the extent of decay rate, and is shown in equation 1, where C_o is the initial concentration of airborne microorganisms, C_t is the concentration of microorganisms at t (time) after initial (Phillips et al., 1964). The survival represents the percentage of viable microorganisms left at a certain moment vis-à-vis the initial microbial count (Wu, 2009). The half-life, $t_{1/2}$, is the time taken for the concentration of viable microorganisms in the air to decrease by half, see equation 2 (Zhao et al., 2011c). Previous studies showed that the biological decay of airborne microorganisms was species-dependent and was determined by many external factors, such as humidity, oxygen concentration, temperature, ozone concentration, radiation (UV, γ-ray, X-ray), air ions and air pollutants (CO, SO_2 and NO_x) (Benbough, 1971; Lighthart, 1973).

$$k = \frac{\log(C_0/C_t)}{t} \tag{1}$$

$$t_{1/2} = \frac{(\log 2) \times t}{\log(C_0/C_t)} \tag{2}$$

Environmental factors affecting biological decay

The effect of humidity on the biological decay of airborne microorganisms has been investigated since the 1950s. In the early studies, the measure most used for humidity was relative humidity (RH, the ratio of the actual water vapor pressure of the air to the water vapor pressure of saturated air at a certain temperature). The results of these studies showed that the microorganisms were prone to decay at low RH (Lighthart, 1973), at median RH (Wright et al., 1968b) and at higher RH (Songer, 1967; Theunissen et al., 1993). More recently, a few studies have used absolute humidity (AH, the actual water content of the air) as another measure of humidity. For instance, Shaman and Kohn (2009) reported that the survival of airborne influenza virus was more significantly constrained by AH than by RH. They authors argued that RH is a meaningful physical quantity and for certain organisms may affect biological response; however, the AH can be of greater biological significance for many organisms. Some studies investigated other measures of humidity, such as evaporation potential (EP, the difference between actual water vapor content in the air and the water vapor content in saturated air at the same temperature). Zhao et al. (unpublished data) found that compared to RH and AH, EP was a more significant factor on the decay of airborne Gumboro vaccine virus. Although a bunch of studies have been carried out, it is not yet fully understood exactly how humidity influences microbial decay.

Temperature profoundly affects the biological decay of airborne microorganisms. The microbial cell temperature easily follows fluctuations in the ambient temperature because the microorganisms are usually unicellular (Prescott et al., 2005). In general, the higher the ambient temperature is, the faster the microorganisms decay. For instance, the decay rate of *Flavobacterium sp.* is 0.007 log min^{-1} at -2 to 24 °C, but increases to 0.017 log min^{-1} at 29 to 49 °C (Ehrlich et al., 1970a). A faster decay at higher temperature has also been reported for other microbial species, such as *E. coli*, *S. marcescens* (Ehrlich et al., 1970b) and Newcastle disease virus (Kournikakis et al., 1988). Prescott et al. (2005) have stated that high temperature may damage microorganisms either by denaturing the enzymes, transport carriers and other proteins, or by melting and disintegrating the lipid bilayer, or both.

The ambient environment is full of various types of radiation, including UV (10-400 µm) and visible light (400-750 µm), which may inactivate the microorganisms. The UV wavelength of 260 µm can be effectively absorbed by microbial genetic material, and is the UV wavelength most lethal to microorganisms. The 260 µm UV light inactivates microorganisms by inducing thymine dimmers that inhibit the replication and function of genetic material (Prescott et al., 2005). The bacteria decay more readily under UV radiation than RNA viruses (Harris et al., 1987; Hijnen et al., 2006). The probable reason for this is that the thymine of bacterial DNA is more vulnerable to dimerization induced by UV than the uracil of viral RNA. The mechanism whereby near-UV inactivates microorganisms is not fully understood, but is suspected to be the breaks in strands of genetic materials that are induced by the near-UV itself and toxic tryptophan photoproducts (Prescott et al., 2005). Visible light may be sufficient to damage microorganisms. Microbial pigments become excited when they absorb light

energy, which is transferred to O_2, generating singlet oxygen. Singlet oxygen ($^1O_2$) is a highly reactive oxidant, and it can inactivate microorganisms (Valduga et al., 1993). Some microorganisms may possess carotenoids which can absorb the excitation energy and reduce the formation of singlet oxygen, thus preventing cells from being damaged by light radiation (McCambridge and McMeekin, 1981)

Oxygen may accept electrons forming other toxic derivatives such as superoxide radical, hydrogen peroxide, and hydroxyl radical, which may easily destroy the cellular constituents. Many aerobic microorganisms contain enzymes such as superoxide dismutase and catalase, which protect the cell against oxidation by the derivatives; all the strictly anaerobic microorganisms lack these enzymes (Prescott et al., 2005). The effect of oxygen level on decay of airborne *Serratia marcescens* 8UK and *E. coli B* was studied by Cox (1989), who reported that oxygen was toxic for these microorganisms only when RH was lower than 70%. The toxicity increased with oxygen concentrations up to 30%; higher concentrations produced no additional toxicity. This finding was generally in agreement with other similar studies (Benbough, 1967; Hess, 1965). Viruses seem to be less sensitive to oxygen than bacteria. The decay of airborne viruses that included Semliki Forest virus, Langat virus, T7 coliphage, Poliovirus, and Encephalomyocarditis virus was no different whether they were aerosolized in the air or in nitrogen (Benbough, 1971; de Jong et al., 1975).

Bacteria exposed to ozone (O_3) may be inactivated due to damage to the cell surface (Giese and Christensen, 1954; Scott and Lesher, 1963) and destruction of the intracellular enzymes, protein and genetic material (Barron, 1954; Ingram and Haines, 1949; Kim et al., 1999). Ozone may damage the viral nucleic acids of viruses and alter the polypeptide chains of the viral protein coat (De Mik and De Groot, 1977; Kim et al., 1999; Roy et al., 1981). Investigations of the ozone effect on decay of airborne microorganisms showed that fungi seemed more resistant to ozone than bacteria; gram-negative bacteria were more resistant than gram-negative ones (Heindel et al., 1993; Kowalski et al., 1998). Ozone alone can be toxic to airborne microorganisms; however, its toxicity is enhanced when ozone reacts with compounds in the ambient air, known as open air factors (OAF). Studies have shown that mixture of ozone with olefins (de Mik et al., 1977; Druett and Packman, 1972), and ozone with negative air ions (Fan et al., 2002) are more toxic to airborne microorganisms than ozone alone.

The dust particles to which microorganisms adhere may protect them from microbial decay. When microorganisms are carried by the dust, they suffer less radiation and exposure to toxic gas, and less fluctuation in micro-climate (Milling et al., 2005). It has been found that single bacteria are effectively inactivated by ozone; however, when these bacteria were covered with a coating of organic matter, as in aerosols naturally emitted during a cough or sneeze, ozone in permissible concentration had no effect (Elford and van den Ende, 1942). As well as providing physical protection, the composition of dust particles might give bio-chemical support to microorganisms. In order to metabolize, microorganisms require carbon, oxygen, nitrogen, phosphorus, sulfur and other elements (Maus et al., 2001). Compounds containing these elements are abundant in airborne dust from livestock production systems (Aarnink et al., 1999; Muller and Wieser, 1987). An interesting hypothesis is that microbial

decay in particles differs, depending on the composition of the particles. There is some support for this hypothesis in the differences found in the decay of microorganisms aerosolized from liquid suspensions with different chemical compositions (Benbough, 1971; Hess, 1965).

Studies on biological decay

There have been extensive studies on the biological decay of microorganisms, using aerosol experiments. In these experiments, microorganisms were aerosolized in airspace which was sampled at certain intervals. The biological decay was indicated by the amounts of collected microorganisms at different sampling moments. In order to avoid the confounding effect of physical decay, inert tracers or labeled microorganisms were used (Ijaz et al., 1987; Ijaz et al., 1985b; Verreault et al., 2008). The biological decay of airborne microorganisms was investigated at different temperatures and humidities. The studies on biological decay with respect to these two factors have been summarized in Tables 4 and 5; studies in which the physical loss was not distinguished have been excluded. The tables show the highest biological decay at the least favorable temperature/RH, and the lowest biological decay at the most favorable temperature/RH (both the least and most climate conditions refer to "Extreme RH" and "Extreme temp" in the tables). The values of biological decay have been presented in three ways: decay rate, survival and half-life. These cover the results reported in most of the relevant studies. Some of the microorganisms show a bi-phase biological decay, with a fast initial decay rate in the first few seconds or minutes after aerosolization, followed by a slow secondary decay (Cox, 1971; Songer, 1967; Webb, 1959). Because long transporting distance and duration are probably more influential in airborne transmission (Brankston et al., 2007), only the secondary decay is presented in the tables.

Most of the previous studies have used wet aerosolization in which microbial suspensions were aerosolized. Wet aerosolization does indeed closely mimic the fate of microorganisms expelled from animal respiratory tracts in wet aerosols that dehydrate in the air. However, after they have been generated, the large wet aerosols containing microorganisms settle on surfaces and the small ones shrink into dry nuclei; both processes are very fast, taking only seconds (Kincaid and Longley, 1989; Sun and Ji, 2007; Wells, 1934). The microorganisms in wet aerosols can only be transported over short distances and induce infections in limited areas (Brankston et al., 2007). In reality, airborne microorganisms are also generated from dry sources (dry feces, bedding material, feed etc.) in livestock production systems, and are likely to be combined with dry dust particles in airborne transmission. Dry aerosolization is then recommended, but this may give a picture of the biological decay of microorganisms that differs from the decay in wet aerosolization because the microorganisms may suffer dehydration stress at low ambient RH or rehydration at high ambient RH (Cox, 1971).

Table 4. Biological decay of airborne bacteria under different RH and temperature.

Bacteria	RH tested (%)	Temp tested (°C)		Extreme RH for decay (%)	Extreme Temp for decay (°C)	Decay	Survival (mins after aerosolization)	Half-life	Reference
Chlamydia pneumoniae	5, 50, 95	8.5, 15, 25, 35	H[a]	50	35	-	0% (1.5)	-	(Theunissen et al., 1993)
			L[b]	95	15, 25	-	>10%* (5.5)	-	
	32-87	15, 30	H	<50%	30	-	-	3 min	(Wathes et al., 1986)
			L	High RH	15	-	-	83 min	
E. coli (lyophilized)	20*-100*	26.5	H	50*, 90*-100*	26.5	-	0.9* - 2*% (30)	-	(Cox, 1970)
			L	20*	26.5	-	20* - 30 % (30)	-	
Flavobacterium	25, 45, 65, 85, 99	24	H	99	24	2.6% min⁻¹	-	-	(Ehrlich et al., 1970a)
			L	85	24	1.3% min⁻¹	-	-	(Ehrlich et al., 1970a)
	85, 100	-40, -18, -2, 24, 29, 38, 49	H	85	49	< 0.01% min⁻¹	-	-	(Ehrlich et al., 1970a)
			L	100	-40, -18	4.7% min⁻¹	-	-	
Legionella pneumophila strain	30, 60, 90	20	H	60	20	-	< 1% (120)	-	(Dennis and Lee, 1988)
			L	90	20	-	> 10*% (120)	-	
Mycoplasma laidlawii	10, 25, 40, 50, 60, 75, 90	27	H	40	27	-	< 1% (300)	-	(Wright et al., 1968b)
			L	10, 90	27	-	10* - 100*% (60)	-	
Mycoplasma gallisepticum	10, 25, 40, 50, 60, 75, 90	27	H	50, 60	27	-	0% (300)	-	(Wright et al., 1968b)
			L	10	27	-	100%* (60)	-	
Mycoplasma pneumoniae	10, 25, 30, 40, 50, 60, 75, 80, 90	27	H	60, 80	27	-	0% (240)	-	(Wright et al., 1968a)
			L	10	27	-	50% (240)	-	
Pasteurella multocida	28, 40, 59, 79	22.6	H	79	22.6	-	2.1% (45)	-	(Thomson et al., 1992)
			L	40	22.6	-	8.9% (45)	-	
Pasteurella tularensis	0*-90*	26.8	H	50*	-	-	0.2* - 0.3*% (15)	-	(Cox and Goldberg, 1972)
			L	90*	-	-	100%* (15)	-	
	20*-95*	-	H	55	-	-	0.2* - 1*% (15)	-	(Cox, 1971)
			L	95*	-	-	> 50%* (15)	-	
Pasteurella tularensis (lyophilized)	0*-90*	26.8	H	81	-	-	0.4* - 0.6*% (15)	-	(Cox and Goldberg, 1972)
			L	0*	-	-	> 10*% (15)	-	
	20*-95*	-	H	75	-	-	0.01* - 0.02*% (15)	-	(Cox, 1971)
			L	20*	-	-	10* - 20*% (15)	-	
Pseudomonas fluorescens	23, 39, 60, 79	-	H	79	-	-	0.01* - 0.1*% (60)	-	(Handley and Webster, 1993)
			L	39, 60	-	-	1* - 3.2*% (60)	-	
Rhizobium meliloti	30, 50, 70, 95	20	H	30	20	-	0.1* - 1*% (300)	-	(Won and Ross, 1969)
			L	50, 70, 95	20	-	> 10*% (300)	-	

Table 4. Biological decay of bacteria under different RH and temperature. (Continued)

Bacteria	RH tested (%)	Temp tested (°C)		Extreme RH for decay (%)	Extreme Temp for decay (°C)	Decay	Survival (mins after aerosolization)	Half-life	Reference
Sarcina lutea	1/3, 23/27, 45/52, 73/76, 88/96	15	H	3-Jan	15	0.346 log % h^{-1}	-	-	(Lighthart, 1973)
			L	45/52	15	0.001 log % h^{-1}	-	-	
Serratia marcescens	40, 97	25	H	40	25	-	2% (32)	-	(Hess, 1965)
			L	97	-	-	100% (32)	-	
	45/52, 73/76, 88/96	15	H	45/52	15	0.368 log % h^{-1}	-	-	(Lighthart, 1973)
			L	73.0/75.5	15	0.057 log % h^{-1}	-	-	
Staphylococcus no. 1600	39, 75	-	H	75	-	0.0189 log min^{-1}	-	-	(Strasters and Winkler, 1966)
			L	39	-	0.0037 log min^{-1}	-	-	
Streptococcal L-Forms	20, 40, 60, 80	27	H	40	27	-	0% (30)	-	(Stewart and Wright, 1970)
			L	20	27	-	$>10^6$ % (240)	-	

* Estimated data from figures.

[a] Highest biological decay of airborne microorganisms (i.e. worst survival and shortest half-life time).

[b] Lowest biological decay of airborne microorganisms (i.e. optimal survival and longest half-life time).

Table 5. Biological decay of viruses under different RH and temperature.

Virus	RH tested (%)	Temp tested (°C)		Extreme RH for decay (%)	Extreme Temp for decay (°C)	Decay	Survival (mins after aerosolization)	Half-life	Reference
Bacteriaphage S-13	20, 50, 80	21	H[a]	50	21	-	0.1* - 1 % (120)	-	(Dubovi and Akers, 1970)
			L[b]	80	21	-	$>10^6$ % (120)	-	
Bacteriaphage MS-2	20, 50, 80	21	H	50	21	-	0.01* - 0.1 % (120)	-	(Dubovi and Akers, 1970)
			L	20	21	-	1* - 10 % (120)	-	
Bovine parainfluenza type 3	30, 90	6, 32	H	90	32	-	0% (180)	-	(Elazhary and Derbyshire, 1979)
			L	90	6	-	1.6 – 4.0% (180)	-	
Newcastle disease virus	10, 35, 90	23	H	35	23	-	0.1* – 10⁶% (90)	-	(Songer, 1967)
			L	10	23	-	$>10^6$% (90)	-	
Infectious bovine rhinotracheitis virus	10, 35, 90	23	H	35	23	-	0.1* – 10⁶% (90)	-	(Songer, 1967)
			L	90	23	-	$>10^6$% (90)	-	
Vesicular stomatitis virus	10, 35, 90	23	H	35	23	-	1* – 10⁶% (90)	-	(Songer, 1967)
			L	10	23	-	>10% (30)	-	

Table 5. Biological decay of viruses under different RH and temperature. (Continued)

Virus	RH tested (%)	Temp tested (°C)		Extreme RH for decay (%)	Extreme Temp for decay (°C)	Decay	Survival (mins after aerosolization)	Half-life	Reference
E. coli B T3 bacteriophage	10, 35, 90	23	H	35	23	-	0% (90)	-	(Songer, 1967)
			L	90	23	-	>10*% (90)	-	
Bovine rotavirus UK	30, 50, 80	20	H	80	20	-	-	3 h	(Ijaz et al., 1994)
			L	50	20	-	-	18 h	
Mouse rotavirus	30, 50, 80	20	H	80	20	-	-	2 h	(Ijaz et al., 1994)
			L	50	20	-	-	24 h	
Poliovirus type 1 Sarbin	30, 50, 80	20	H	30, 50	20	-	-	n.r.	(Ijaz et al., 1985b)
			L	80	20	-	-	9 hs	
Human corona virus 229E	30, 50, 80	6, 20	H	80	20	-	-	3.3 h	(Ijaz et al., 1985a)
			L	50	6	-	-	102.5 h	
Human rotavirus	30, 50, 80	6, 20	H	80	6	-	-	1.7 h	(Ijaz et al., 1985c)
			L	50	6	-	-	57.4 h	
Bovine rotavirus	20, 50, 80	10, 20, 30	H	50	30	$2.39\ \text{log h}^{-1}$	-		(Moe and Harper, 1983)
			L	90	10	$0.03\ \text{log h}^{-1}$	-		
E. coli B T3 coliphage	8, 30, 50, 80, 95	21	H	8	21	-	0% (240)		(Hatch and Warren, 1969)
			L	95	21	-	>10*% (240)		
Pasteurella pestis Bacteriophage	20, 40, 50, 60, 72, 95	21	H	40, 50, 60	21	-	0.1* − 10*% (240)	-	(Hatch and Warren, 1969)
			L	20, 72, 95	21	-	>10*% (240)	-	
Encephalomyocarditis Virus	5* − 90*	10, 20, 30, 37	H	10* − 20*	37	-	0.001* - 0.01 % (30 - 35)	-	(de Jong et al., 1975)
			L	80* − 90*	20	-	100* % (30 - 35)	-	
Foot and Mouth disease virus O₁ BFS 1860	20, 30, 40, 50, 60, 70	19 - 22	H	20	19 - 22	-	0.01* - 0.1*% (5)	-	(Barlow and Donaldson, 1973)
			L	50, 60, 70	19 - 22	-	0.01* − 11*% (5)	-	
	55, 70	18 - 23	H	55	18 - 23	n.c.	-	-	(Donaldson, 1972)
			L	70	18 - 23	$3.15\ \text{log h}^{-1}$	-	-	
Foot and Mouth disease virus O₂ Brescia	55, 70	18 - 23	H	55	18 - 23	n.c.	-	-	(Donaldson, 1972)
			L	70	18 - 23	$2.60\ \text{log h}^{-1}$	-	-	
Foot and Mouth disease virus O₁ Lombardy	55, 70	18 - 23	H	55	18 - 23	n.c.	-	-	(Donaldson, 1972)
			L	70	18 - 23	$2.38\ \text{log h}^{-1}$	-	-	

Table 5. Biological decay of viruses under different RH and temperature. (Continued)

Virus	RH tested (%)	Temp tested (°C)		Extreme RH for decay (%)	Extreme Temp for decay (°C)	Decay	Survival (mins after aerosolization)	Half-life	Reference
Foot and Mouth disease virus C Noville	55, 70	18 - 23	H	55	18 - 23	2.90 log h^{-1}	-	-	(Donaldson, 1972)
			L	70	18 - 23	1.88 log h^{-1}	-	-	
Foot and Mouth disease virus A5 Eystrup	55, 70	18 - 23	H	55	18 - 23	2.60 log h^{-1}	-	-	(Donaldson, 1972)
			L	70	18 - 23	1.78 log h^{-1}	-	-	
Foot and Mouth disease virus C Lebanon	55, 70	18 - 23	H	55	18 - 23	2.40 log h^{-1}	-	-	(Donaldson, 1972)
			L	70	18 - 23	1.43 log h^{-1}	-	-	
Foot and Mouth disease virus A22 Iraq	55, 70	18 - 23	H	55	18 - 23	3.28 log h^{-1}	-	-	(Donaldson, 1972)
			L	70	18 - 23	1.25 log h^{-1}	-	-	
Foot and Mouth disease virus O1 Pacheco	55, 70	18 - 23	H	55	18 - 23	2.05 log h^{-1}	-	-	(Donaldson, 1972)
			L	70	18 - 23	1.06 log h^{-1}	-	-	
Influenza virus	20/25, 34/36, 49/51, 64/65, 81/82	7.0/8.0, 20.5/24.0, 32.0	H	81	32	-	0% (240)	-	(Harper, 1961)
			L	23/25	7.0/8.0	-	61% (1380)	-	
Vaccinia virus	17/20, 48/51, 80/84	10.5/11.5, 21.0/23.0, 31.5/33.5	H	80/83	31.5/33.5	-	0% (1380)	-	(Harper, 1961)
			L	20	10.5/11.5	-	66% (1380)	-	
Venezuelan equine encephalomyelitis virus	19/23, 48/50, 81/86	9.0/9.5, 21.0/23.0, 32.0/33.0	H	81/85	32.0/33.0	-	0% (360)	-	(Harper, 1961)
			L	19	9.0/9.5	-	26% (1380)	-	
Poliomyelitis virus	18/23, 35/36, 49/51, 64/65, 80/81	20.5/23.5	H	49/51	20.5/23.5	-	0% (360)	-	(Harper, 1961)
			L	80/81	20.5/23.5	-	85% (1380)	-	
	20, 80	-	H	20	-	-	2.5% (60)	-	(Benbough, 1971)
			L	80	-	-	53% (60)	-	
Japanese B Encephalitis Virus	30, 55, 80	24	H	80	24	-	-	28 min	(Larson et al., 1980)
			L	30	24	-	-	62 min	
Langat virus	20, 80	-	H	80	-	-	10% (60)	-	(Benbough, 1971)
			L	20	-	-	52% (60)	-	
Semliki Forest virus	20, 80	-	H	80	-	-	51% (60)	-	(Benbough, 1971)
			L	20	-	-	67% (60)	-	

Table 5. Biological decay of viruses under different RH and temperature. (Continued)

Virus	RH tested (%)	Temp tested (°C)		Extreme RH for decay (%)	Extreme Temp for decay (°C)	Decay	Survival (mins after aerosolization)	Half-life	Reference
E. coli B T7 coliphage	20, 80	-	H	20	-	-	0.05% (60)	-	(Benbough, 1971)
			L	80	-	-	57% (60)	-	
Lassa virus Josiah	30, 55, 80	24, 32, 38	H	80	32	6.9% min^{-1}	0.3% (60)	10.1 min	(Stephenson et al., 1984)
			L	30	24	1.3% min^{-1}	16.9% (60)	54.6 min	
Pseudorabies virus	55, 85	4, 22	H	85	22	-	-	17.4 min	(Schoenbaum et al., 1990)
			L	55	4	-	-	43.6 min	
Newcastle disease virus	20/30, 50, 80	10, 15, 20, 25, 30	H	80	25, 30	-	8% (360)	-	(Kournikakis et al., 1988)
			L	20/30	10	-	56% (360)	-	
PRRSV	5-90	5-41	H	63.8	30	-	-	3.3 min	(Hermann et al., 2007)
			L	17.1	5	-	-	192.7 min	
Psittacosis agent	30, 50, 80	26.7	H	80	26.7	6.73% min^{-1}	-	-	(Mayhew and Hahon, 1970)
			L	30	26.7	0.64% min^{-1}	-	-	
Reovirus type 1 Lang	25/35, 45/55, 65/75, 85/95	21/24	H	65/75, 25/35	21/24	3.2 - 3.3% min^{-1}	-	-	(Adams et al., 1982)
			L	85/95	21/24	1.5 - 2.5% min^{-1}	-	-	
Yellow fever virus	30, 50, 80	26.7	H	50	26.7	7.04% min^{-1}	-	-	(Mayhew and Hahon, 1970)
			L	30	26.7	3.26% min^{-1}	-	-	
Variola virus	30, 50, 80	26.7	H	30	26.7	0.86% min^{-1}	-	-	(Mayhew and Hahon, 1970)
			L	80	26.7	0.56% min^{-1}	-	-	
Respiratory Syncytial Virus	20, 30, 40, 50, 60, 70, 80, 90	20.5	H	80	20.5	1.49 log h^{-1}	-	-	(Rechsteiner and Winkler, 1969)
			L	20	20.5	0.47 log h^{-1}	-	-	
Rift Valley fever virus ZH-501	30, 55, 80	24	H	80	24	10.1% min^{-1}	-	6.9 min	(Brown et al., 1982)
			L	30	24	0.9% min^{-1}	-	77.0 min	
Rift Valley fever virus SA-51	30, 55, 80	24	H	80	24	6.1% min^{-1}	-	11.4 min	(Brown et al., 1982)
			L	30	24	1.3% min^{-1}	-	53.3 min	
Rotavirus SA11	25, 50, 80	20	H	80	20	-	-	< 2 h	(Sattar et al., 1984)
			L	50	20	-	-	40 h	

Table 5. Biological decay of viruses under different RH and temperature. (Continued)

Virus	RH tested (%)	Temp tested (°C)		Extreme RH for decay (%)	Extreme Temp for decay (°C)	Decay	Survival (mins after aerosolization)	Half-life	Reference
St. Louis encephalitis (SLE) virus	29, 46, 60, 80	21	H	80	21	-	14[b]% (360)	-	(Rabey et al., 1969)
			L	29	21	-	79[a]% (360)	-	
Venezuelan equine encephalomyelitis virus	30, 60	22	H	60	22	-	0.006 – 77.3% (60)	-	(Berendt and Dorsey, 1971)
			L	30	22	-	0.02 – 88.7% (60)	-	
Rhinovirus-14	30, 50, 80	20	H	30, 50	20	-	<0.25 % (15)	-	(Karim et al., 1985)
			L	80	20	-	30 % (1440)	13.7 hs	

* Estimated data from figures.

[a] Highest biological decay of airborne microorganisms (i.e. worst survival and shortest half-life time).

[b] Lowest biological decay of airborne microorganisms (i.e. optimal survival and longest half-life time).

DEPOSITION AND INFECTIVE DOSE

Particle deposition in respiratory tract of humans and animals

According to Heyder et al. (1986), the probability of deposition will be different for each particle even if all the particles in the air inhaled in one breath are identical, because the inhaled air with particles penetrates the respiratory tract to different depths where it remains for different periods of time, and because of the stochastic nature of particle transport. Therefore, "particle deposition" (in the respiratory tract) refers to the mean probability of an inspired particle being collected on airway surfaces. However, the deposition may be defined differently in different studies (Corbanie et al., 2006). Particle deposition in the respiratory tract depends on particle characteristics (e.g size, shape, density) and breathing pattern (e.g. nasal/oral breath, respiratory flow rate and cycle period), and is commonly expressed as a function of particle size.
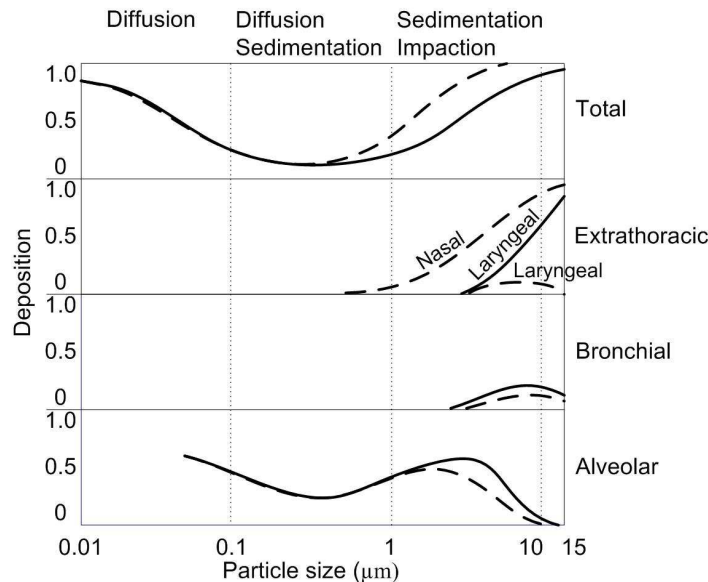
Figure 2. Deposition of unit density spherical particles in human respiratory tract at a mean flow rate of 250 cm^3 s$^-$^{1} and a breathing cycle period of 8 s. Solid curves show the deposition curve for steady oral breathing, and dashed curves for steady nasal breathing. Adapted from Heyder et al. (1986).

Particle deposition in the human respiratory tract has been well documented (Brown et al., 2002; James et al., 1991; Lippmann et al., 1980). In principle, the deposition of particles is governed by the mechanisms of diffusion (particles < 0.1 μm), or by diffusion and sedimentation (0.1-1 μm particles), or by sedimentation and impaction (particles > 1 μm) (Heyder, 2004). On the basis of previous experimental studies, Heyder et al. (1986) developed a semi-empirical deposition model for particles ranging from 0.005 μm to 15 μm. Figure 2 shows a deposition pattern for slow inspiration over a long period for both oral and nasal breathing. The deposition has been shown for three regions of the respiratory tract - extrathoracic, bronchial and alveolar - based on how far down the tract particles may

be deposited. In addition, total deposition is given (the sum of the deposition in the three regions). It can be seen that the total deposition is least for particles of 0.1-1 μm, and that the deposition increases as the size of small particles (< 0.1 μm) decreases, and the size of the larger particles (> 1 μm) increases. Particles larger than 10 μm are mainly deposited in the extrathoracic region, and cannot penetrate the alveolar region during slow and fast oral and nasal inspiration.

The deposition in the human respiratory tract cannot be extrapolated to livestock because of the unique morphology of the human respiratory systems (Corbanie et al., 2006). Particle deposition in guinea pig head, trachea and lungs was investigated by Harper and Morton (1953). They found an increasing regional deposition in guinea pig head for larger particles: 35.7% of the 1 μm particles were deposited in the guinea pig head, compared with 98.1% of the 10 μm particles. The reverse was true for lung deposition: 55.2% of 1 μm particles and 0.6% of 10 μm were deposited in the guinea pig lung The particle size most deposited in the trachea was 2.5 μm. A model of particle deposition in the guinea pig respiratory tract was established by Schreider and Hutchens (1979), who found that 99% of unit density particles of 10 μm or more could be deposited in the nasopharyngeal-tracheobronchial region (Figure 3). The lowest deposition was 10% for a particle size of 0.8 μm. About 17% of particles ranging from 0.08 to 4 μm could be deposited in the pulmonary region. However, this model was compromised by several assumptions that were made by the authors, e.g. laminar airflow in the respiratory tract, equal expansion of all lobes and alveoli, and complete mixing of the particles in the alveoli.

Hayter and Besch (1974) investigated the regional deposition of five particle sizes (0.091, 0.176, 0.312, 1.1, and 3.7 to 7 μm) in chicken. The particles deposited in the head and anterior trachea were in the 3.7 to 7 μm size range, those deposited in the lung and posterior air sacs were 1.1 μm size, and those deposited in the caudal regions of the birds were in the size classes 0.091 and 0.176 μm. Those of size 0.312 μm were deposited mainly in upper airways. These early data of particle deposition in chicken airways are suspected to be compromised by the use of anesthetized chickens, because anesthesia alters the animals' breathing pattern. Corbanie et al. (2006) investigated the deposition of particles in a wider range (1, 3, 5, 10 and 20 μm) in unanaesthetized chickens of three ages. Unlike the definition of "deposition" that was proposed by Heyder et al. (1986), Corbanie et al. (2006) defined deposition as the percentage of particles deposited in a particular region of the respiratory tract among those in the entire tract. They found that particles larger than 5 μm were too large to be deposited in the lungs and air sacs in 2- and 4-week-old chickens, as low percentages of particles were recovered in these regions (Figure 4). For 1-day-old chicks, however, the particle deposition in lungs and air sacs was independent of particle size and even particles of 20 μm were deposited in the lower airway, probably due to the chicks having a different breathing pattern than older chickens.

The deposition pattern of monodispersed particles (3.3 μm) in calf airways was studied by Jones et al (1987). They found those particles were preferentially deposited in the trachea and major bronchi.
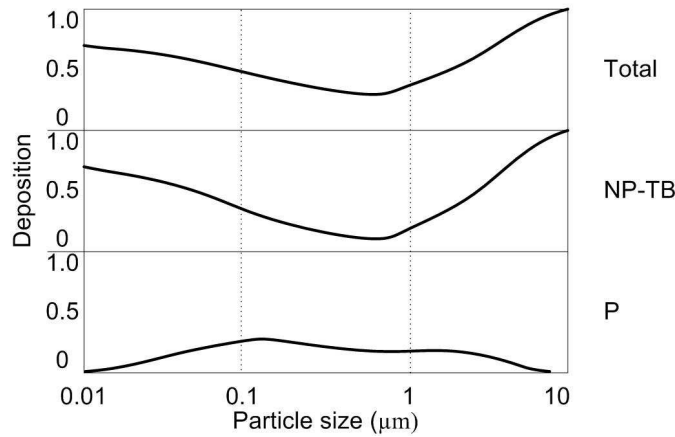
Figure 3. Deposition of unit density spherical particles in the respiratory tract of guinea pig at a tidal volume of 4.44 cm³ and a respiratory rate of 60 breaths min⁻¹. NP-TB: nasopharyngeal-tracheobronchial region. P: pulmonary region. Adapted from Schreider and Hutchens (1979).
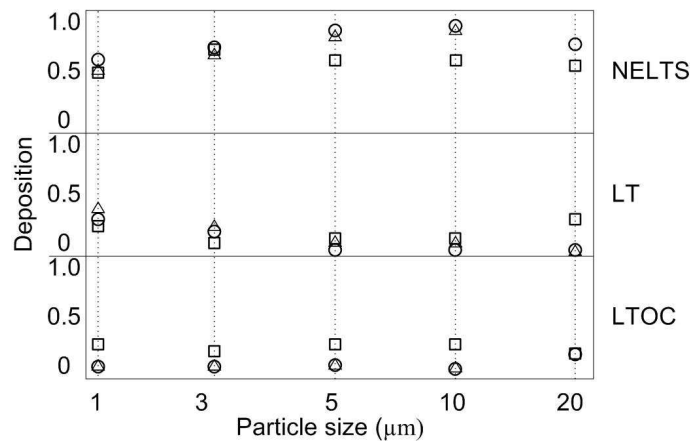

Figure 4. Deposition of fluorescent particles in the respiratory tract of 1-day-old (□), 2-week-old (○), and 4-week-old (Δ) broilers. NELTS: nose, eyes, larynx, trachea and syrinx. LT: lungs and thoracic air sacs. LTOC: lower beak, tongue, esophagus and crop. Adapted from Corbanie et al. (2006).

Infective dose

Infection is more likely to fit a single-hit model, which means that one pathogenic microorganism may trigger an infection in a recipient. From this point of view, the infective dose (ID, or occurrence of infection) is therefore related to the probability that a recipient becomes infected after taking in a certain dose of pathogenic microorganisms, following a Poisson distribution. The ID of pathogenic microorganisms to human and animals has generally been expressed in two ways. In one way, ID is expressed as the number of infected recipients out of a population after a dose of microorganisms has been administered. The other way is to determine the microbial concentrations required to infect 50% of a population (ID_{50}). Table 6 lists the ID of several pathogenic microorganisms. The ID of the same microorganism varies, depending on the recipient animal species. For instance, a lower ID of FMD virus is needed to infect sheep and cattle than to infect s pigs (Alexandersen and Donaldson, 2002;

Donaldson et al., 1987; Gibson and Donaldson, 1986). It can be also seen that a certain dose of a microorganism is not always capable of infecting all recipients, probably because of a difference in the resistance of individual recipients (due to e.g. age, breed etc.) (Roy, 1980). Furthermore, the route by which the microorganisms are administered may also be responsible for variation in ID (Cafruny and Hovinen, 1988; Zimmerman et al., 1993). In previous studies, the administration routes were either nasally or orally, or via aerosols, and these reflect different deposition situations and regions for microorganisms in the respiratory tract. Nasal administration simulates infection because larger microbial particles are deposited in the upper airways; the oral ministration may simulate oral breathing; and the aerosol administration simulates infection because smaller microbial particles are deposited in deeper airways. It was reported that the microorganisms had preferential sites for multiplication and infection because of the complex vulnerability of regions in respiratory tracts (Cafruny and Hovinen, 1988; Druett et al., 1953; Druett et al., 1956). This being so, infection occurs more readily when the microorganisms are administered to the more vulnerable region, thus resulting in a lower ID value. Baskerville (1981) summarized the preferred infection regions of some microorganisms to animals by categorizing nose, pharynx and tonsils as the upper respiratory tract and the trachea, bronchi and bronchioles, and alveoli as the lower respiratory tract (Table 7).

SAMPLING AIRBORNE MICROORGANISMS AND DUST

Sampling protocols for dust in ambient air have been legislated by both the US Environmental Protection Agency (US EPA, 2006) and the European Committee for Standardization (European Commission, 1998; European Commission, 2005). Nowadays, the official sampling protocols focus increasingly on small particles, e.g. PM_{10} and $PM_{2.5}$, because these have the potential to be suspended for longer time, transported for longer distance, and deposited in the lower respiratory tract; in addition, they are more hazardous to human health. To ensure unbiased sampling, these protocols specify many details, such as sampling duration, type of sampler, and sample handling.

The protocols for ambient air may not be directly applicable for dust sampling in livestock production systems where the dust concentrations are much higher than those in ambient air. In addition, to date there is no protocol for sampling airborne microorganisms. Given that the sampling of microorganisms and dust in livestock production systems is increasingly being performed for assessing the bio-security of air environments and for evaluating mitigation techniques, the sampling protocol must be well designed, in order to assure reliable data. Below, sampling strategies and samplers for airborne microorganisms and dust are highlighted taking into consideration their sampling in livestock production systems.

Table 6 Infective dose (ID) of pathogenic microorganisms.

Microorganism	Dose	Animal	Administration	Infected/Total (or % infected)	Reference
Campylobacter jejuni	90 CFU	3 day chickens	Orally	9/10	(Ruiz-Palacios et al., 1981)
E. coli (O157:H7)	6000 CFU	3 month pigs	-	6/8	(Cornick and Helgerson, 2004)
E. coli (O157:H7)	< 300 CFU	10 week calves	Orally	2/17	(Besser et al., 2001)
E. coli (O157:H7)	10^7 CFU	3 year steer	Stomach tube	2/5	(Cray and Moon, 1995)
Salmonella typhimurium	1000 CFU	10 – 14 day pigs	Intranasal	1/5	(Loynachan and Harris, 2005)
Salmonella enteritides	< 10 CFU	> 52 week molted layers	Orally	50%	(Holt, 1993)
Salmonella enteritides	6500 – 56000 CFU	> 52 week unmolted layers	Orally	50%	(Holt, 1993)
FMD virus (strain O1 Lausanne)	1700 $TCID_{50}$	20 – 30 kg pigs	Aerosol	5/8	(Alexandersen and Donaldson, 2002)
FMD virus (strain O_1 BFS 1860)	13 – 398 $TCID_{50}$	43 – 166 kg calves	Aerosol	10/12	(Donaldson et al., 1987)
FMD virus (strain O_1 BFS 1860)	10 – 50 $TCID_{50}$	26 – 82 kg sheep	Aerosol	7/12	(Gibson and Donaldson, 1986)
FMD virus (strain SAT 2 SAR 3/79)	25 – 251 $TCID_{50}$	118 – 150 kg calves	Aerosol	11/15	(Donaldson et al., 1987)
PRRSV	10 $TCID_{50}$	4 – 5 week pigs	Intranasal	2/3	(Yoon et al., 1999)
Porcine rotavirus	1 PFU	2 hour piglets	Pharynx	2/2	(Graham et al., 1987)
Encephalomyocarditis virus	$10^{8.8}$ $TCID_{50}$	4 – 6 week pigs	Intranasal	2/5	(Zimmerman et al., 1993)
Influenza A/Texas/91 (H1N1) virus	100000 $TCID_{50}$	18-33 year old humans	Intronasal	24/33	(Hayden et al., 1996)
Influenza A2/Bethesda/10/63	1-5 $TCID_{50}$	21-40 year old man	Aerosol	4/14	(Alford et al., 1966)
Rotavirus	0.9 FFU	18-45 year old man	Orally	1/7	(Ward et al., 1986)
Salmonella newport	150000 CFU	Human	Orally	1/6	(McCullough and Eisele, 1951)
Salmonella derby	1.5×10^7 CFU	Human	Orally	3/6	(McCullough and Eisele, 1951)
Salmonella bareilly	13000 CFU	Human	Orally	1/6	(McCullough and Eisele, 1951)

CFU: colony forming unit; $TCID_{50}$: 50% tissue culture infective dose; PFU: plaque forming unit; FFU: focus forming unit.

Table 7. Initial infection region of microorganisms (Baskerville, 1981).

Microorganism	Animal	Infection region
Bordetella bronchiseptica	Pig	URT[a]
Haemophilus spp.	Pig	URT
Pasteurella spp.	Pig	URT
	Cattle	URT
	Sheep	URT
Mycoplasma	Poultry	URT
	Pig	URT
	Cattle	URT
Bovine herpesvirus-1	Cattle	URT
Parainfluenza-3	Cattle	URT
	Sheep	URT
Infectious laryngo-tracheitis virus	Poultry	URT
Infectious bronchitis virus	Poultry	URT
Aujeszky's disease virus	Pig	URT
Aspergillus fumigatus and other fungi	Poultry	LRT[b]
Respiratory syncytial virus	Cattle	LRT
Adenoviruses	Cattle	LRT

[a] URT: Upper respiratory tract, including nose, pharynx and tonsil.

[b] LRT: Lower respiratory tract, including trachea, bronchi and bronchioles, and alveoli.

Sampling strategy

Isokinetic sampling is the ideal sampling method, because it has been devised to sample the true numbers of particles in the air. Such sampling can be achieved if the sampler inlet is in alignment with and facing the direction of air flow and if the air velocity within the sampler is the same as the ambient air velocity (Zhang, 2004). However, true isokinetic sampling is impossible in practice due to the variation in the surrounding air flow pattern (air direction and velocity) and/or the limited possibilities of some samplers (Liu and Pui, 1981). As a concession, the current legislation aims to reduce the sampling bias in non-isokinetic samplings by stipulating the range of conditions under which the samplings may be performed.

The sampling location should be chosen bearing in mind the research purpose. When human health is of concern, sampling should be carried out near the human breathing zone. One option is to fit a portable sampler on a worker's body at a height of 150-170 cm above floor level and within a radius of 30 cm around the mouth (Ouellette et al., 1999). There are difficulties in doing the same with animals, so therefore stationary samplers in their breathing zones are recommended. The level of the breathing zone is 30-40 cm above floor level for pigs, 10-25 cm for poultry and shoulder height for cattle (Kim et al., 2007a; Topisirovic, 2003). For growing animals these figures should be adjusted to the animal height at a certain age. When emissions of microorganisms and dust are of interest, the best sampling location is in or near the air outlet. Care should be taken not to place the samplers at a

location where the air speed is high, because the efficiency of the sampler (the ratio of the concentration calculated from the air samples to the true concentration in the air) may drift far from 100% (Grinshpun et al., 1994; Hofschreuder et al., 2007).

To protect against the adverse health effects associated with short-term and long-term exposure, the daily and annual thresholds for PM_{10} in ambient air have been set at 50 and 40 μg m^{-3} respectively (European Commission, 1999); the annual threshold for $PM_{2.5}$ has been set at 25 μg m^{-3} (European Comission, 2008). To assess the concentration of fine dust, the sampling period is generally 24 hours. This sampling duration may also be applied when sampling dust in animal houses to obtain daily mean concentrations. For studies collecting information on dust fluctuations during the day, successive samplings for short periods should be performed. This kind of sampling can be achieved by interval sampling or sampling with online optical samplers.

Due to the lack of a standard, the sampling duration for airborne microorganisms in different studies varies, but is normally less than 1 hour. The duration is determined by taking account of the estimated concentrations of microorganisms and the characteristics of the sampler in use. The sampling duration can be set at a short period (a few minutes) when the total microorganisms normally abundant in livestock houses is of interest. When the microorganism of interest is sporadically present (this is especially true for viruses indoors), the period should be set long enough to collect enough microorganisms for further analysis. Some samplers have not been designed to be used for long sampling duration. For instance, severe evaporation of liquid medium in All Glass Impinger (AGI-30) occurs when the sampling period is long and this may affect its sampling efficiency. The recommended maximum duration of the sampling period for the AGI-30 is 30 minutes. The Andersen impactor may easily become overloaded when samples are taken in livestock houses (Thorne et al., 1992), therefore, the sampling duration is limited to minutes or even to seconds.

Other aspects in a sampling strategy include practical and economic considerations. Active sampling instruments require a power supply to operate the pump. These instruments are unlikely to be used in many outdoor measurements unless the manufacturers supply rechargeable batteries. The portability of the instrument and its ancillaries, e.g. weight and dimensions etc., are factors that should be considered. Instruments and sample analysis add to the costs. For instance, a high quality pump is required for dust filtration sampling, and it must be intelligent enough to provide constant airflow when ambient temperature changes and pressure increases because dust accumulates on the filter. The post-sampling culturing and counting of microorganisms, especially viruses, is time consuming and particularly expensive.

Samplers for microorganisms

Current samplers for airborne microorganisms are generally based on one of three main principles, i.e. impaction, impingement, or filtration (Table 8). These different sampling principles have their advantages and disadvantages. Samplers using the impaction principle, e.g. Andersen six stage

impactor, can be used to distinguish the microorganisms according to their sizes (Andersen, 1958). In addition, the bacteria impacted on agar plates may be directly incubated for viable counts. However, its susceptibility to overloading limits its sampling in livestock houses to short periods, which may result in non-representative samples. The problem of overloading can be overcome by using samplers with the impingement principle, because then the liquid samples may be decimally diluted and analyzed. A disadvantage of this sampler, e.g. AGI-30, is that it may not be able to sample for a long time due to evaporation of the collection liquid (Lin et al., 1997). Filtration is a user-friendly method in a practical situation, but not suitable for sampling microorganisms that are vulnerable to dehydration stress.

Table 8. Common samplers for airborne microorganisms.

Sampling principle	Collection medium	Example samplers
Impaction	Agar plate	Andersen One/Two/Six stage Impactor (Andersen Instruments Incorporated, Atlanta, USA)
		Casella Slit Sampler (Casella Ltd, Bedford, UK)
		Surface Air Systems (Cherwell Laboratories, Bicester, UK)
Impingement	Liquid medium	AGI-30 (Ace Glass, Vineland, USA)
		Multistage May Liquid Impinger (AW Dixon, Beckenham, Kent)
Filtration	Filter	Dissolvable gelatin filter (Sartorius, Göttingen, Germany)
		Polytetrafluoroethylene (Sartorius, Göttingen, Germany)

A sampler with known efficiency is a prerequisite for a reliable evaluation of the microbial concentration. The efficiency of a bio-sampler includes physical and biological efficiency. The physical efficiency describes how well non-viable airborne particles are aspirated by the device's inlet and transported to the collection medium, referred to as inlet sampling efficiency, and how well the bio-sampler retains these particles in its medium, referred to as collection efficiency (Griffiths and Stewart, 1999; Nevalainen et al., 1992). For particles in the range from 1 to 10 μm and an airflow rate between 0 and 500 cm s^{-1}, the inlet sampling efficiency of the Andersen Six Stage Impactor is 90 – 150% when the opening faces in direction of the air flow and 8 – 100% when it is oriented perpendicularly to the horizontal aerosol flow (Grinshpun et al., 1994). The inlet efficiency of the AGI-30 for particles of 1 μm is close to 100%, but it is reduced to 70-90% for 5 μm particles and to 20-30% for 10 μm particles (Grinshpun et al., 1994). When the 50% collection efficiency of the Andersen Six Stage Impactor and AGI-30 was investigated it was found that the Andersen Six Stage Impactor has 50% collection efficiency for 6.6-7.0 μm particles at the first stage and 0.57-0.65 μm particles for the last stage (Andersen, 1958; Nevalainen et al., 1992). The 50% collection efficiency of AGI-30 was for particles of 0.31 μm (Nevalainen et al., 1992).

Filters vary in their physical efficiency. Some are highly effective. By measuring the particle concentration upstream and downstream of filters with an optical particle counter,

polytetrafluoroethylene (PTFE) filters and gelatin filters were confirmed to collect more than 93% of particles, even down to 80 nm (Burton et al., 2007).

If the particles are living organisms, they may be inactivated during sampling due to impaction stress (Stewart et al., 1995), impingement stress (Shipe et al., 1959; Tyler and Shipe, 1959; Tyler et al., 1959) and/or dehydration stress (Li et al., 1999). Therefore, in order to indicate how well a bio-sampler maintains the microbial viability and prevents cell damage during sampling, the concept of biological efficiency has been introduced (Griffiths and Stewart, 1999). Different samplers may differ in their biological efficiency. Moreover, due to the vulnerability of microorganisms to the sampling stress, a sampler's biological efficiency may vary.

In some studies, the efficiency has been evaluated by comparing samplers side-by-side in an environment with unknown microbial concentration (Engelhart et al., 2007; Henningson et al., 1982; Thorne et al., 1992). This method easily ranks the performance of different samplers; however, it does not reveal whether the amount of microorganisms collected in the air samples accurately represents the microorganism content of the air, nor does it distinguish between the physical and biological efficiency. Other studies separately investigated the efficiency of bio-samplers in aerosol experiments, in which a known amount of microorganisms together with an indicator (either labeled microorganisms, or inert tracer compound) were nebulized in an isolator. The physical efficiency can be determined by comparing the amount of tracer collected by a bio-sampler with that collected by the reference sampler (a sampler that has a high physical efficiency); the biological efficiency is subsequently indicated by the change in the ratio of microorganisms/indicator. Using this method, Zhao et al. (2011b; 2011c; 2011d) reported the efficiency of the Andersen Six Stage Impactor, AGI-30, OMNI-3000 and gelatin filter in sampling *Enterococcus faecalis*, *E. coli*, *Campylobacter jejuni*, *Mycoplasma synoviae* and Gumboro vaccine virus.

Samplers for dust

Filters are most commonly used for sampling airborne dust. Both US and European reference samplers use the filtration principle to collect dust from the air (European Commission, 1998; European Commission, 2005; US EPA, 1997). For sampling dust in certain size fractions (e.g. PM_{10} and $PM_{2.5}$), a pre-separator for separating the coarse dust from the target dust particle sizes has to be installed in front of the filter. Sampling systems with pre-separators using the impaction principle have been legislated as reference methods for measuring dust in ambient air in US (US EPA, 1997) and European countries (European Commission, 1998; European Commission, 2005). These systems show steep collection curves for sampling fine dust in ambient air where dust load is low (Kenny et al., 2000). However, the pre-separator with impaction principle may become overloaded when sampling in dusty livestock production systems, thereby resulting in overestimated concentrations of fine dust (Zhao et al., 2009). In contrast, a pre-separator with cyclone principle has been found to be less

vulnerable to overloading in dusty environments than pre-separators based on impaction (Zhao et al., 2009).

Optical dust samplers are now commercially available. These dust samplers can monitor the real-time dust concentrations, and no further process is needed after sampling (unlike the gravimetric method, in which filters must be weighed). Moreover, some optical samplers may separately record the concentrations of dust in different size ranges. However, the optical samplers have limitations in humid environments, because water droplets are also counted as dust particles.

MITIGATION TECHNIQUES FOR AIRBORNE MICROORGANISMS AND DUST

Many techniques have been applied in practice to reduce the concentrations and emissions of airborne microorganisms and dust in the livestock industry. They vary in their utility, but can be grouped into two main principles. The first principle, to control particles at source, includes techniques such as feed coating and oil spraying, which has been stated to be "the most effective means" of controlling airborne particles in the space (Pearson and Sharples, 1995). The second principle is air purification. Ionization (electrostatic) and air scrubbers are examples.

Control at source

The fatty substances often added to feed to increase metabolizable energy content may reduce the airborne microorganisms and dust in animal houses (Pearson and Sharples, 1995). Gore et al. (1986) reported that concentrations of airborne bacteria were reduced by 27%, and settled dust by 45 to 47%, when 5% soybean oil was added to the pig diet. This result is consistent with the study by Welford et al. (1992), who found a 31% reduction of inhalable dust with 2% oil addition to the feed. Other substances, such as tallow, lecithin and lignin have also been used as effective feed additives for the purpose of particle control in animal houses (Dawson; Pearson and Sharples, 1995).

Spraying techniques reduce the particle concentrations mainly by coating the surfaces, thereby, preventing particles from being suspended or resuspended from their sources (Takai, 2007). Kim et al. (2006) reported that spraying 60 ml m^{-2} of tap water, salt water, treated manure, microbial additive, soybean oil, artificial spice, and essential oil may all reduce particles in pig houses. These authors found an average reduction of 53% for airborne bacteria, 51% for fungi and 30% for total dust. The substance for spraying found to be the most effective additive for reducing dust was soybean oil. In broiler rooms, Aarnink et al. (2009) reported that PM_{10} reduction increased linearly from 55 to 85% when daily rapeseed oil application rates increased from 6 to 24 ml m^{-2}. The $PM_{2.5}$ reduction was not related to application rate and was about 80%. In aviary systems for layers, a 34% reduction for PM_{10} and 50% reduction for $PM_{2.5}$ were achieved by daily spraying with 20 ml m^{-2} (Aarnink et al., 2009). Although a high oil application rate achieves high PM_{10} reduction in broiler rooms, it may adversely affect animal health. When spraying 24 ml m^{-2} daily, there was a tendency for statistically higher footpad lesion in broilers. Aarnink et al. (2009) recommended limiting oil application to 16 ml m^{-2} .

Air purification

The ionization (also referred to as electrostatic) technique produces negative ions in the air, which causes airborne particles to become negatively charged. The negatively charged particles are attracted to earthed or positively charged surfaces. Previous studies have shown promising reduction effects on dust in animal houses Using ionization techniques, total dust was reduced by 13 to 61% in poultry houses (Lyngtveit and Eduard, 1997; Mitchell et al., 2000; Mitchell et al., 2004; Richardson et al., 2003), and by 45 to 58% in pig houses (Rosentrater, 2003; Tanaka and Zhang, 1996). Cambra-Lopez et al. (2009) reported that the technique produced average reductions of 36% for PM_{10} and 10% for $PM_{2.5}$. The disparity is probably caused by the different charging mechanisms to particles. The small particles (< 0.1 μm) are charged by the thermal charging mechanism, which is proportional to the diameter; large particles (> 0.5 μm) are charged by field charging mechanism, which is proportional to the square of the diameter (Bundy, 1984). Ionization has the potential to prevent airborne transmission of microorganisms (Gast et al., 1999; Holt et al., 1999), however, no reduction of airborne bacteria, fungi and mold was found in broiler houses by this technique (Cambra-Lopez et al., 2009). Knowledge of ionization on reduction of airborne microorganisms in animal houses is still limited and needs to be augmented.

End-of-pipe techniques, dedusters and air scrubbers have been installed at air outlets of animal houses to minimize emissions of air pollutants. Zhang et al. (2001) found a deduster removed 90% total dust from the air exhausted from a pig house. Because dedusters are based on the centrifuge principle, they are more effective at removing larger particles than smaller ones. The deduster studied by Zhang et al. (2001) had a removal efficiency of 90% for particles larger than 10 μm, 77% for 7 μm particles, and 50% for 4 μm particles. The low removal efficiency for small particles was assumed to be particle re-entrainment due to high air turbulence. Acid and biological air scrubbers were originally developed to reduce ammonia and odor emissions, and they also appear to be effective in reducing particle emissions. An acid scrubber which uses sulfuric acid may achieve a 70% reduction of total bacteria (Aarnink et al., 2005). In a lab-scale experiment, Aarnink et al. (2005) found that *E. faecalis* and Gumboro virus could be reduced by 100% when per-acetic acid was used as the circulation solution in a scrubber. The biological scrubbers are not consistent in reducing microorganisms (Seedorf and Hartung, 1999), probably because the microorganisms for digesting odorous compounds can also be emitted to the ambient air. The removal of total dust by biological scrubbers was found to be 22 to 96% (Seedorf and Hartung, 1999).

Combined techniques have been applied in practice: for instance, oil spraying combined with feed coating (Takai and Pedersen, 2000) and multi-stage air scrubbers (Zhao et al., 2011a). These techniques are more consistent and effective in reducing emissions of airborne microorganisms and dust, as well as other gaseous pollutants (Ogink and Bosma, 2007; Zhao et al., 2008). A disadvantage of combined scrubbing techniques is the relatively high energy use and the complexity for use on

practical farms. Therefore, further research is needed to develop energy-saving and simple-to-operate combined scrubbing techniques.

CONCLUSIONS

- The most important source of airborne microorganisms is the animals themselves by means of respiration, coughing, sneezing and excretion. The sources of dust include excrement, litter, feed, skin and feathers.

- Airborne microorganisms in animal houses are mostly bacteria, with gram-positive bacteria predominating; fungi account for only a small proportion of microorganisms. Concentrations of both microorganisms and dust are high in animal houses. They are affected by animal type, housing system, management and environmental factors. Because these factors play an interrelated role on the concentrations of microorganisms and dust, integrated research on the effects is required.

- The microorganisms transmitted in the air suffer physical and biological decay. The physical decay largely depends on their size, and the biological decay is mainly determined by environmental factors, such as humidity, temperature, radiation, toxic gases. In airborne transmission, microorganisms may be carried by dust particles which may protect microorganisms from decay. Knowledge of the role of dust in the transportation of microorganisms is still lacking, and needs to be expanded.

- Microorganisms are deposited on different regions in the respiratory tract, mainly depending on their size. They have different preferred infection regions in the respiratory tract. The amount of microorganisms needed for an infection varies with microorganism species and animal species.

- Reference methods for microorganism and dust sampling in animal houses need to be legislated. These methods should be suitable for highly microbial and dusty environments, and the efficiency of the sampling devices needs to be investigated.

- Different techniques have been applied to reduce airborne microorganisms and dust in and from animal houses. Combining several abatement techniques may achieve higher and more consistent reduction. Energy-saving and simple-to-operate combined techniques are of interest for further development.

EFFECTIVENESS OF MULTI-STAGE SCRUBBERS IN REDUCING EMISSIONS OF AIR POLLUTANTS FROM PIG HOUSES

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ABSTRACT. Emissions of air pollutants from livestock houses may raise environmental problems and pose hazards to public health. They can be reduced by scrubbers installed at the air outlets of livestock houses. In this study, three multi-stage scrubbers were evaluated in terms of their effectiveness in reducing emissions of airborne dust, total bacteria, ammonia, and CO_2 from pig houses in winter. The three multi-stage scrubbers were one double-stage scrubber (acid stage+ bio-filter), one double-stage scrubber (acid stage + bio-scrubber), and one triple-stage scrubber (water stage + acid stage + bio-filter). Results showed that these scrubbers reduced concentrations of PM_{10} by 61% to 93%, concentrations of $PM_{2.5}$ by 47% to 90%, concentrations of airborne total bacteria by 46% to 85%, and concentrations of ammonia by 70% to 100%. Concentrations of CO_2 were not affected. Most of the airborne bacteria emitted from the pig houses were larger than 3.3 μm (73% to 95%). The multi-stage scrubbers removed 53% to 92% of them, compared with -42% to 20% removal effectiveness of the bacteria in the size range of 0.65 to 3.3 μm. The triple-stage scrubber was the most efficient in removing dust and ammonia. Compared to single-stage scrubbers, all the three multi-stage scrubbers performed more consistently in reduction of PM_{10}, $PM_{2.5}$, total bacteria, and ammonia emissions from livestock houses and removed these pollutants more efficiently. It should be noted that all measurements were performed in winter at low ventilation rates, thus at low loadings of the multi-stage scrubbers.

Keywords. Aerosol, Bioaerosol, Livestock, Particulate matter, Pathogen, Microorganism.

INTRODUCTION

The air emitted from livestock houses is abundant in pollutants (e.g., dust, ammonia, and odors) that may cause public health and environmental problems (Larsson et al. 1994; Wing and Wolf 2000). It has been estimated that in The Netherlands approximately 20% of PM_{10} (particulate matter smaller than 10 μm) emissions originated from livestock houses in 2000 (Chardon and Van der Hoek 2002). The ambient ammonia is mainly from livestock production, which accounts for over 80% of the total national ammonia emissions in the U.S. and Europe (van der Hoek 1998; Liang et al. 2005; USEPA 2005). Although the contribution of livestock to airborne microorganisms in the ambient air is not well documented, it is well known that air from livestock houses contains large amounts of pathogenic microorganisms that, transmitted aerially, may infect animals and humans in the vicinity (Seedorf et al. 1998; Stark 1999; Gloster and Alexandersen 2004; Power 2005). To safeguard human health and protect the environment, there must be effective technologies to abate the emissions of aerial pollutants from livestock houses.

One straightforward way to reduce emissions of aerial pollutants is to install air scrubbers at the ventilation outlets of livestock houses. The scrubbers act like screens to filter the exhaust air by physically trapping pollutant compounds and/or by converting them biologically or chemically. The simplest scrubber uses only water in its recirculation system. Aarnink et al. (2005) reported that such a scrubber reduced emissions of total dust by 88%, ammonia by 25%, *Enterococcus faecalis* by 33%, and odor by 55%. The reduction of ammonia can be improved by adding acid to the recirculated water, resulting in a so-called acid scrubber. This kind of scrubber is usually packed with acid-resistant porous material through which acid water is recirculated continuously or intermittently. When polluted air passes through the wet packed material, ammonia is bound in the acid water and converted into NH_4^+. The pH of the acid water is normally kept at less than 4. A well designed acid scrubber can reduce the ammonia concentration in the air by over 90% (Melse and Ogink 2005). Acid scrubbers can also be effective in reducing airborne microorganisms. A sulfuric acid scrubber achieved approximately 70% reduction of total bacteria (Aarnink et al. 2005). In a lab-scale experiment Aarnink et al. (2005) showed that *E. faecalis* and *Gumboro* virus could be reduced by 100% when per-acetic acid was used. However, acid scrubbers are less effective at removing odor. Ogink and Groot Koerkamp (2001) reported an odor reduction of 29% when using an acid scrubber in a pig house. The reason for the lower efficiency for odor removal is thought to lie in the various odorous compounds; some of them cannot be captured by the acid water (Ogink and Aarnink 2003).

Another type of scrubber, the biological scrubber, uses microbial activity to degrade and convert pollutant compounds into less harmful substrates. There are two types of biological scrubbers: bio-filters with organic packing materials, and bio-scrubbers (or bio-trickling filters) with inert packing materials (Melse 2009). Compared to acid scrubbers, biological scrubbers are more efficient in odor removal but less efficient in ammonia removal. Melse and Ogink (2005) reported that, on average,

bio-scrubbers removed 44% of odor and 70% of ammonia. The reduction of total dust was reported to be 79% to 96% with a bio-filter (Seedorf and Hartung 1999) and 22% to 45% with bio-scrubbers (Seedorf and Hartung 1999; Kosch et al. 2005). The drawback of biological scrubbers is that they may emit more microorganisms. Seedorf and Hartung (1999) found that the exhaust air of a bio-scrubber contained 2.7 times more mesophilic fungi than the incoming air. Similar results were reported by Aarnink et al. (2005).

In general, acid scrubbers are more effective at removing ammonia and microorganisms, whereas biological scrubbers are more effective at removing odor. Therefore, using a single-stage acid or biological air scrubber to purify the exhaust air from animal houses has limited effect, as each scrubber targets different specific pollutants. It has been suggested that combining these two types of scrubbers into one multi-stage air scrubber would reduce most aerial pollutants (Seedorf et al. 2005). Some preliminary studies confirmed that such multi-stage scrubbers effectively abate ammonia, odor, and dust (Snell and Schwarz 2003; Schlegelmilch et al. 2005; Ogink and Bosma 2007). However, the previous studies were not performed in a comparative way for different scrubbers, and no information on reduction of microorganisms has been reported so far. Furthermore, the improvement of multi-stage scrubbers is still proceeding. The effect of newly developed scrubbers on reducing airborne pollutants needs re-assessment. In our study, we therefore set out to evaluate three new types of multi-stage air scrubbers under practical conditions in terms of their reduction of PM_{10}, $PM_{2.5}$, airborne bacteria, ammonia, and CO_2 from pig houses.

MATERIALS AND METHODS

Pig houses and scrubbers

Three multi-stage scrubbers were evaluated: a double-stage scrubber (acid stage + bio-filter, henceforth referred to as ABF); a double-stage scrubber (acid stage + bio-scrubber, henceforth ABS); and a triple-stage scrubber (water stage + acid stage + bio-filter, henceforth WABF). They were installed in three pig houses.

The ABF (Figure 1) was installed in a pig house containing 3,200 growing-finishing pigs with an average weight of 67.5 kg. Exhaust air from 15 compartments was conveyed to a central ventilation room and from there to the ABF. The maximum ventilation capacity of the pig house was 100,000 m^3 h^{-1}. This low maximum ventilation capacity was possible because a cooling system was implemented for the incoming air. The actual ventilation rate of the scrubbers on each measuring day was determined by the CO_2 balance method (Pedersen et al. 2008). For the ABF, the ventilation system was running on average at 29,000 m^3 h^{-1} (ranging from 24,800 to 39,810 m^3 h^{-1}). The total volume of the ABF was 85.5 m^3 (2.5 m deep, 9.5 m wide, and 3.6 m high). It consisted of an acid stage and a bio-filter. The acid stage was packed with acid-resistant packing material (2Hnet, specific surface of 150 m^2 m^{-3}) with a volume of 10.26 m^3 (0.4 m deep, 9.5 m wide, and 2.7 m high). Underneath was a 3.46

m^3 reservoir containing a diluted solution of sulfuric acid. The acidic solution was recirculated over the packing material of the acid stage at a rate of 54 m^3 h^{-1}. When the pH reached 4.0, sulfuric acid was automatically added until the pH was again 2.0. The interval for adding acid depended on the ammonia concentration in the exhaust air, which varied with factors such as air temperature, humidity, rearing period, season, and ventilation rate. The recirculated acidic solution was replaced every three months. Expressed as an hourly discharging rate, this was 0.018 m^3 h^{-1} on average. The bio-filter was packed with shredded tree roots with a volume of 13.68 m^3 (0.4 m deep, 9.5 m wide, and 3.6 m high). Underneath was a water reservoir. The acid stage and the bio-filter were 1.0 m apart. During the measurement period, the theoretical residence time, i.e., the time that polluted air was in contact with the packing materials (Equation 1), was 3.0 s on average. The ABF was in use for one month prior to starting this study:

$$RT = Vol / (Vent / 3600) \tag{1}$$

RT = residence time (s);

$Vent$ = ventilation rate (m^3 h^{-1});

Vol = total volume of packing materials (m^3).
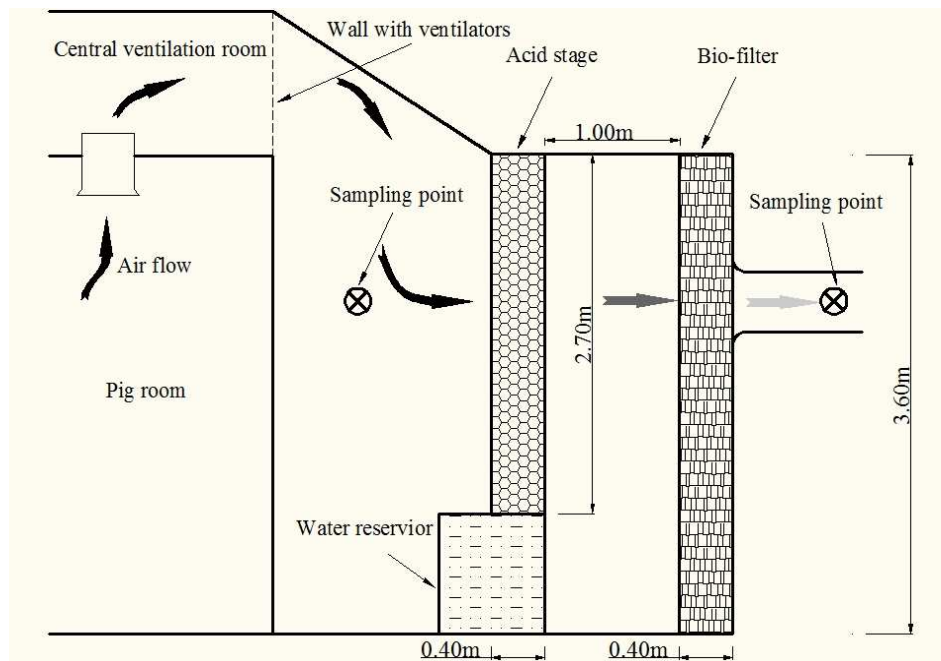
Figure 1. Schematic diagram of the double-stage scrubber (ABF).

The ABS (Figure 2) was more compact than the other two multi-stage scrubbers. It was installed in a pig house containing 1,200 growing-finishing pigs with an average weight of 80 kg. The air from ten compartments was first conveyed to a central ventilation room, and then three extractor fans (maximum capacity per fan 20,000 m^3 h^{-1}) at the end of the ventilation room directed it to the ABS. The ventilation system ran on average at 12,600 m^3 h^{-1} (ranging from 9,975 to 16,323 m^3 h^{-1}) during the measuring period. The total volume of the ABS was 23.81 m^3 (3.15 m deep, 2.7 m wide, and 2.8 m high). It consisted of an acid stage and a bio-scrubber. The acid stage was packed with acid-resistant

material (Fyban, specific surface of 140 m^2 m^{-3}) with a volume of 1.5 m^3 (0.5 m deep, 1.5 m wide, and 2.0 m high). Underneath was a 1 m^3 reservoir containing a diluted solution of sulfuric acid. The acidic solution was pumped to the packing material of the acid stage at a rate of 2.25 m^3 h^{-1}. When the pH reached 4.0, sulfuric acid was automatically added to adjust it back to 1.5. After five pH adjustments, the recirculation acidic solution was replaced with fresh solution at a pH of 1.5. The solution was discharged at 0.055 m^3 h^{-1} on average. The volume of the bio-scrubber was 0.45 m^3 (0.15 m deep, 1.5 m in width, and 2 m high). The packing material was made of polypropylene with a specific surface of 300 m^2 m^{-3} (2H plastic FKP 156). Downwind from the bio-scrubber was a droplet catcher (0.15 m deep, 1.5 m wide) and under it was a 0.8 m^3 reservoir filled with water at a pH of 6.0 to 7.0. All but the bottom 2 cm of water in the reservoir was replaced once a week; expressed per hour, this equaled 0.045 m^3 h^{-1}. The acid stage and bio-scrubber were 0.55 m apart. The residence time was about 0.6 s. The ABS was in use for approximately one year prior to starting this study.
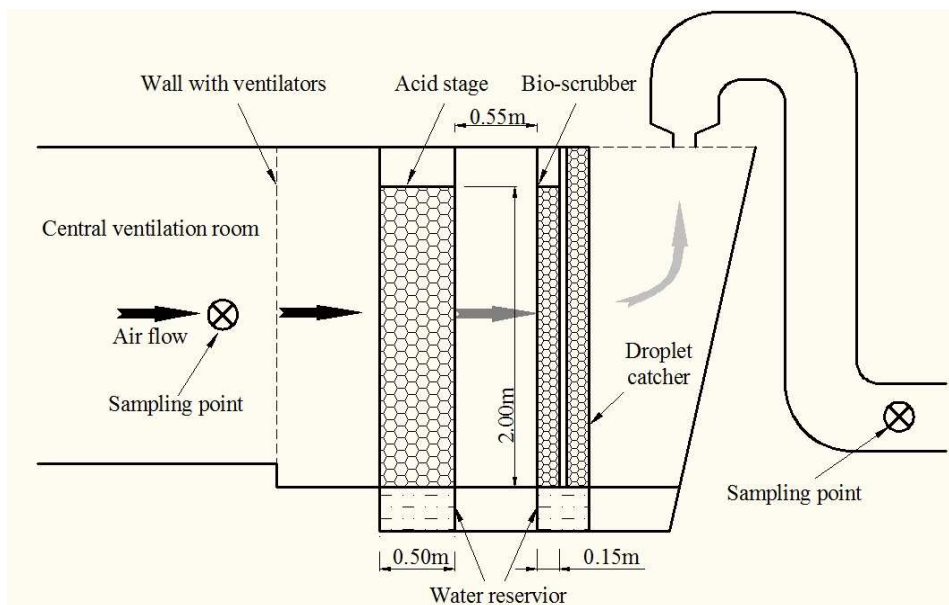
Figure 2. Schematic diagram of the double-stage scrubber (ABS).

The WABF (Figure 3) was installed in a growing-finishing pig house containing five compartments for 520 pigs in total. The average live weight of the pigs during the measurements was approximately 70 kg. Air from all the rooms was collected in a central ventilation room and then exhausted to the WABF by three fans with a capacity of 19,500 m^3 h^{-1} each. During the measurements, the ventilation system ran on average at 8,775 m^3 h^{-1} (ranging from 7,711 to 9,788 m^3 h^{-1}). The volume of the WABF was 110.4 m^3 (4.6 m deep, 9.6 m wide, and 2.5 m high). It consisted of a scrubbing stage with only water, an acid stage, and a bio-filter. The same polypropylene packing material (2H plastic FKP 158, specific surface of 320 m^2 m^{-3}) was used in the water and acid scrubbing stages. The water-scrubbing stage removed large dust particles in particular, to prevent the following scrubbing stages from becoming clogged, which would have caused an undesirable drop in pressure. Its volume was 2.73 m^3 (0.15 m deep, 9.6 m wide, and 2 m high); under it was a reservoir for the recirculated water. The water

was recirculated at 1.2 m^3 h^{-1}. The acid stage had the same dimensions as the water stage. The capacity of its reservoir was 4.8 m^3. The acidic solution was recirculated at a rate of 2.25 m^3 h^{-1}. The water in both scrubbing layers was replaced completely with fresh water every three months. The discharging rates of the water and acid stages were 0.1 to 2 m^3 h^{-1} and 0.01 to 0.2 m^3 h^{-1}, respectively. The volume of the bio-filter was 12.6 m^3 (0.6 m deep, 8.4 m wide, and 2.5 m high). It was filled with shredded tree roots that were kept wet by sprayers mounted above. The water percolating through the bio-filter was caught in a small reservoir below. The acid scrubber and bio-filter were 1.3 m apart to ensure that no acid droplets were transmitted to the bio-filter. The residence time was 7.5 s. The WABF was in use for more than one year prior to starting this study.
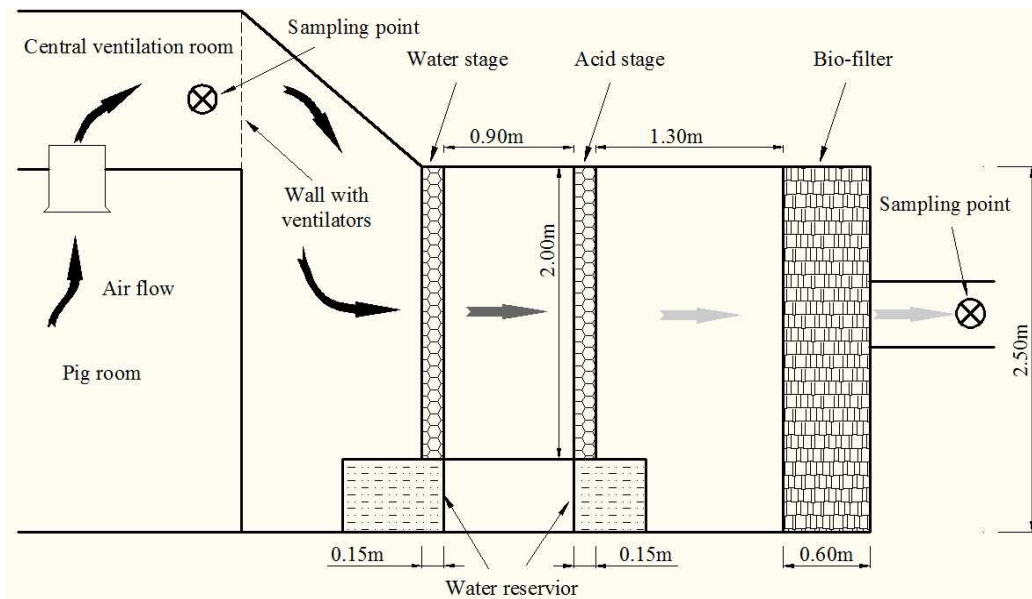
Figure 3. Schematic diagram of the triple-stage scrubber (WABF).

Measurements of air pollutants

All measurements were taken in winter (Table 1). The concentrations of the air pollutants in the incoming and outgoing air (indicated as "sampling point" in Figures 1, 2, and 3) of the multi-stage scrubbers were determined. For the ABS and WABF, the air pollutants in the incoming air were measured in the central ventilation chamber near the fans; for the ABF, they were measured just before the acid stage.

Table 1. Sampling dates for dust, total bacteria, ammonia, and CO_2.

Scrubber	Dates for dust, ammonia, and CO_2	Dates for total bacteria
ABF	4, 5, 6, 27, 28, 29 Dec., and 4 Jan.	28 Dec. and 3 Jan.
ABS	11, 12, 15, 18, 20, 21 Dec.	21 Dec. and 9 Jan.
WABF	15, 17, 19, 29, 31 Jan., and 1 Feb.	17 Jan. and 6 Feb.

To prevent the pollutant concentrations in the outgoing air from being underestimated because of dilution by the ambient airflow, plastic tubes of 45 cm diameter were installed in the outer surfaces of the last stages of the scrubbers so that the pollutant concentrations could be measured at that point.

The velocity of the air leaving the ABS was above 2 m s^{-1}, too high to obtain a representative dust concentration (Hofschreuder et al. 2008). To overcome this problem, the beginning of the tube was made into funnel shape (Figure 2). The diameter of the air inlet of the funnel-shaped tube was 15 cm.

PM$_{10}$ and PM$_{2.5}$

Concentrations of PM$_{10}$ and PM$_{2.5}$ were measured six times for the ABS and WABF, and seven times for the ABF. Each measurement lasted 24 h. The PM$_{10}$ concentrations in incoming and outgoing air of all three multi-stage scrubbers were sampled by EU reference samplers (European Commission 1998; European Commission 2005) with impaction pre-separators (IPS). Particles sucked into the IPS hit its greased plate, and those larger than 10 μm were retained on the plate by inertia. The PM$_{10}$ particles were conveyed in the air stream to the glass fiber filter inside the filter holder, where they were collected. The PM$_{2.5}$ concentrations in the outgoing air of the three scrubbers were also measured with the EU reference sampler by means of the same impaction principle (European Commission 1998; European Commission 2005). However, Zhao et al. (2009) reported that the EU reference PM$_{2.5}$ sampler was not suitable for use in dusty environments like livestock houses because of problems with overload. The overload, which was attributed to the greased plate's low capacity to retain larger particles (>2.5 μm), resulted in PM$_{2.5}$ concentrations being overestimated. Therefore, cyclone pre-separators (CPS), which were less vulnerable to overload in dusty environments, were used to collect PM$_{2.5}$ in the incoming air of the ABF and ABS. The PM$_{2.5}$ concentrations of the incoming air of WABF was measured with IPS, but was calibrated with Equation 2 (Aarnink et al. 2007):

$$PM_{2.5CPS} = 20.7 + 0.156 \times PM_{2.5IPS} \ (R^2 = 0.55) \tag{2}$$

Charlie HV pumps (TCR Tecora SRL, Milan, Italy) were used to provide a constant airflow of 2.3 m^3 h^{-1} through the IPS and 1 m^3 h^{-1} through the CPS. The airflow was adjusted for temperature and pressure variations in the samplers. In this way, a stable airflow within 2% of the nominal value was maintained.

A glass fiber filter was weighed before and after dust sampling in the same way (Zhao et al. 2009): it was weight four times in two days after two days of stabilization at 20°C ±1°C and 50% ±5% RH. The filter weight was determined as the mean value of the results of four times of weighing. The dust weight collected on a filter was the difference in weight of this filter before and after sampling. Combining the total air volume passing through a filter, dust concentration was expressed in mg per cubic meter of air (mg m^{-3}).

Airborne bacteria

Total bacteria in the incoming and outgoing air of the scrubbers were measured by a six-stage ambient viable sampler (Andersen sampler, Pacwill Environmental, Ltd., Beamsville, Ontario, Canada) and an Airport MD8 (Sartorius AG, Göttingen, Germany) for each scrubber on two sampling days (Table 1).

The Andersen sampler has six stages, each of which consists of a plate with agar placed under a screen with 400 holes. The number of holes remains the same for each stage, but the diameter of the holes becomes progressively smaller in successive stages. In this way, air speed increases from the first to the sixth stages. The sampling airflow rate in the Andersen sampler was 28.3 L min^{-1}. Airborne microorganisms are retained on the agar plates in different stages, depending on their size (the largest in the first stage, and the smallest in the last stage) in the following sequence: >7.1 µm in stage 1, 4.7 to 7.1 µm in stage 2, 3.3 to 4.7 µm in stage 3, 2.1 to 3.3 µm in stage 4, 1.1 to 2.1 µm in stage 5, and 0.65 to 1.1 µm in stage 6. Plates containing plate count agar (PCA) were used in the Andersen sampler. The sampling duration was 10 s.

The Airport MD8 uses a gelatin filter to collect airborne microorganisms. It has been reported that the gelatin filter is highly effective for sampling particles at low relative humidity (Parks 1996). However, under high relative humidity, it may be destroyed during sampling. In our experiment, the humidity of the exhaust air from the scrubber sometimes reached 100%. In order to prevent the gelatin filter from being destroyed, the sampling time was limited to less than 5 min. The airflow rate of the MD8 was set to 50 L min^{-1}.

On each sampling day, the Andersen sampler took one sample in the incoming and one in the outgoing air, while the MD8 took four samples. The bacteria-loaded agar plates from the Andersen sampler were directly incubated at 30°C for 72 h. The bacterial colony forming units (CFU) on the plate in each stage were counted after incubation and corrected with the positive hole conversion table (Andersen 1958). The bacteria-loaded gelatin filters from the Airport MD8 were dissolved in buffered peptone water (BPW, Oxiod, Ltd., Cambridge, U.K.). Thereafter, decimal dilutions were made, and each dilution was plated on PCA. The incubation and enumeration methods were performed according to the international standard for total bacteria (ISO 4833 2003).

Ammonia and CO$_2$

Ammonia and CO$_2$ concentrations were measured with Kitagawa gas detection tubes (Komyo Rikagaku Kogyo K.K., Tokyo, Japan) during daytime. For high ammonia concentrations, Kitagawa tubes No. 105SC were used (measuring range: 5 to 260 ppm; uncertainty: 5% to 10%); for low ammonia concentrations, Kitagawa tubes No. 105SD were used (measuring range: 0.2 to 20 ppm; uncertainty: 5% to 10%). Tubes No. 126SG were used for measuring CO$_2$ (measuring range: 0.02% to 1.4%; uncertainty: 10%). On seven sampling days, measurements were performed for the ABF. For the ABS and the WABF, sampling was performed on six days.

Temperature, relative humidity, and air velocity

The temperature, RH, and air velocity of the incoming and outgoing air were measured near the dust and bacteria samplers. Temperature and relative humidity (RH) were recorded with sensors (Hygroclip, Ettlingen, Germany) and data loggers (CR10, Campbell Scientific, Shepshed, U.K.) every

5 min for 24 h. Air velocity was measured with a hot-wire anemometer (model 642, Wilh. Lambrecht GmbH, Göttingen, Germany). All these measuring instruments were calibrated with reference equipments before the experiment started.

Table 2. Measurements of dust, total bacteria, ammonia, CO_2, temperature and humidity, and air velocity (frequency, number of sampling days, and duration).

Parameter	Frequency (times per day)	No. of Sampling Days	Duration (per measurement)
Dust	1	6 or 7[a]	24 h
Total	1[b]	2	10 s
bacteria	4[c]	2	<5 min
Ammonia	1	6 or 7[a]	1 to 2 min
CO_2	1	6 or 7[a]	1 min
T and RH	1	6 or 7[a]	24 h (5 min interval)
Air velocity	Incoming air = 1;	6 or 7[a]	Instantaneous
	Outgoing air = 3 or 5[d]		

[a] Six for ABS and WABF; seven for ABF.

[b] Measured with Andersen sampler.

[c] Measured with Airport MD8.

[d] Three for ABF; five for ABS and WABF.

Data analysis

When there was more than one measurement moment per sampling day, as was the case for total bacteria concentrations measured with the MD8 and velocity of the outgoing air, the results of those measurements on that day were averaged. The reduction of air pollutants by the scrubbers was calculated as the concentration difference between the incoming and outgoing air divided by the concentration of the incoming air (Equation 3):

$$R = (C_i - C_o) / C_i \times 100\% \tag{3}$$

R = reduction (%);

C_i = concentration of air pollutant in incoming air of the scrubber;

C_o = concentration of air pollutant in outgoing air of the scrubber.

A linear model (GLM procedure in SAS) was set up for the analysis of dust reduction (Y) as the response, with multi-stage scrubber and dust type as two fixed factors (Equation 4). The differences in the reductions achieved by the multi-stage scrubbers for total bacteria, ammonia, and CO_2 were analyzed with the ANOVA procedure. All analyses were done with SAS software (SAS 9.1.3 Service Pack 4, SAS Institute, Inc., Cary, N.C.):

$$Y_{ijk} = \mu + S_i + PM_j + S_i \times PM_j + \varepsilon_{ijk} \tag{4}$$

Y_{ijk} = dust reduction (%);

μ = overall mean (%);

S_i = effect of multi-stage scrubber (i = ABF, ABS, WABF);

PM_j = effect of dust (j = PM$_{10}$, PM$_{2.5}$);

ε_{ijk} = residual error.

RESULTS AND DISCUSSION

Temperature, RH, and air velocity

Table 3 shows the temperature, RH, and air velocity during the sampling period. All the RH values of the outgoing air reached 100% due to water evaporation in the scrubbing stages. The mean air velocities were all lower than 2 m s^{-1}.

Table 3. Temperature (day mean), RH (day mean), and air velocity of the incoming and outgoing air of the multi-stage scrubbers.

Scrubber	Temperature (±SE, °C)		RH (±SE, %)		Air Velocity (±SE, m s^{-1})	
	Incoming	Outgoing	Incoming	Outgoing	Incoming	Outgoing
ABF	23.6 (±0.3)	18.0 (±0.5)	71 (±2)	100 (±0.0)	1.18 (±0.20)	0.54 (±0.08)
ABS	21.8[a]	17.7[a]	75[a]	100[a]	1.65 (±0.44)	1.80 (±0.71)
WABF	18.2 (±0.2)	12.7[a]	66 (±2)	100[a]	0.75 (±0.07)	0.45 (±0.02)

[a] Only one day's data were collected due to malfunctioning of the sensors.

Reduction of PM$_{10}$ and PM$_{2.5}$

Table 4 shows the airborne PM$_{10}$ and PM$_{2.5}$ concentrations in the incoming and outgoing air of the multi-stage scrubbers and the reductions they achieved. GLM analysis showed that the reduction depends on the type of scrubber and dust. The R^2 value was 0.77. Both the type of scrubber ($p < 0.01$) and the type of dust ($p < 0.01$) were significant factors.

Table 4. Concentrations of PM$_{10}$ and PM$_{2.5}$ in the incoming and outgoing air and the reduction achieved by the multi-stage scrubbers (n = 6 for ABS and WABF; n = 7 for ABF; p = 0.05).

Scrubber	PM$_{10}$			PM$_{2.5}$			p
	Concentration (±SE, µg m^{-3})		Reduction[a] (±SE, %)	Concentration (±SE, µg m^{-3})		Reduction[a] (±SE, %)	Value[b]
	Incoming	Outgoing		Incoming	Outgoing		
ABF	341 (± 19)	63 (± 8)	81[a] (± 3)	32[c] (± 5)	12 (± 4)	62[a] (± 9)	0.02
ABS	679 (± 18)	267 (± 25)	61[b] (± 3)	46[c] (± 3)	24 (± 1)	47[a] (± 2)	0.30
WABF	711 (± 35)	51 (± 7)	93[a] (± 1)	85[d] (± 4)	8 (± 1)	90[b] (± 2)	1.00

[a] Means in the same column with the same superscript letter are not significantly different ($p > 0.05$).

[b] Probability that the reduction of PM$_{10}$ and the reduction of PM$_{2.5}$ by the same scrubber are not significantly different.

[c] PM$_{2.5}$ concentrations measured by the CPS.

[d] PM$_{2.5}$ concentrations calibrated with Equation 2 after being measured by the IPS.

The PM$_{10}$ concentrations in the incoming air varied between the scrubbers and ranged from 341 to 711 µg m^{-3}. The reductions in PM$_{10}$ concentrations were 81% for the ABF, 61% for the ABS, and 93%

for the WABF, respectively. Multiple comparisons revealed that the ABF and the WABF were significantly more effective in reducing PM_{10} than the ABS ($p < 0.05$).

The $PM_{2.5}$ concentrations in the incoming air varied between the scrubbers and ranged from 32 to 85 µg m^{-3}. The reductions in $PM_{2.5}$ concentrations were 62% for the ABF, 47% for the ABS, and 90% for the WABF. Statistical analysis showed that the WABF was significantly more effective in reducing $PM_{2.5}$ than the ABF and the ABS. The difference between the ABF and the ABS was not significant.

During this winter-time experiment, none of the three multi-stage scrubbers ran at its maximum capacity. The airflow loadings of the multi-stage scrubbers were 29,000 m^3 h^{-1} for the ABF (29% of maximal capacity), 12,600 m^3 h^{-1} for the ABS (21% of maximal capacity), and 8,775 m^3 h^{-1} for the WABF (15% of maximal capacity). The residence time was calculated as 3.0 s for the ABF, 0.6 s for the ABS, and 7.5 s for the WABF. The long residence time in the WABF could be the main reason why it achieved the highest reduction of PM_{10} and $PM_{2.5}$.

All three multi-stage scrubbers reduced PM_{10} more than $PM_{2.5}$. Statistical analysis revealed that the ABF reduced significantly more PM_{10} than $PM_{2.5}$ ($p = 0.02$). This finding is consistent with another study, which reported that the removal efficiency of the ABF was superior for larger particles (Ogink and Hahne 2007). There was no significant difference between PM_{10} and $PM_{2.5}$ reduction by both the ABS and the WABF ($p = 0.30$ vs. $p = 1.00$). In this experiment, $PM_{2.5}$ accounted for 7% to 12% of the PM_{10} by weight in the incoming air and 8% to 19% of the PM_{10} by weight in the outgoing air.

Reduction of total bacteria

Table 5 shows the concentrations and reduction of total airborne bacteria. Because the pig houses with scrubbers differed in their construction, management, and the numbers and average weight of pigs, the bacteria concentrations in the incoming air of the scrubbers varied. The total bacteria concentrations in the incoming air ranged between 3.7×10^4 and 88.2×10^4 CFU m^{-3} when measured with the Andersen sampler and between 1.4×10^4 and 8.6×10^4 CFU m^{-3} when measured with the MD8. The concentrations in the outgoing air ranged between 1.5×10^4 and 11.5×10^4 CFU m^{-3} when measured with the Andersen sampler and between 0.1×10^4 and 1.2×10^4 CFU m^{-3} when measured with the MD8. The reduction in total bacteria emissions achieved by the three scrubbers ranged from 46% to 85% according to the Andersen sampler data and from 69% to 96% according to the MD8 data. There was no significant difference in bacterial reduction between the scrubbers. It has been reported that a single-stage bio-filter could reduce airborne microorganism emissions, whereas bio-scrubbers increased the emissions (Seedorf and Hartung 1999; Aarnink et al. 2005), but in our study the multi-stage scrubbers with either a bio-filter or with a bio-scrubber reduced total bacteria emissions. It is questionable whether an increase of microorganisms in the air resulting from a biological scrubber poses a threat to the environment. The microorganisms that convert the aerial pollutants in biological scrubbers are generally harmless. However, it cannot be excluded that pathogenic microorganisms also

grow in the environment of a biological scrubber (Aarnink et al. 2005). It should be noted that reduction of total bacteria measured by the Andersen sampler was the mean of two measurements. In future investigations, more samples are needed for more reliable analysis and conclusions.

Table 5. Concentrations of total bacteria in the incoming and outgoing air as measured by Andersen sampler and MD8, and the reduction achieved by the multi-stage scrubbers ($n = 2$, $p = 0.05$).

Scrubber	Andersen ($\pm$SE, $\times 10^4$ CFU m^{-3})		Reduction[a] ($\pm$SE, %)	MD8 ($\pm$SE, $\times 10^4$ CFU m^{-3})		Reduction[a] ($\pm$SE, %)
	Incoming	Outgoing		Incoming	Outgoing	
ABF	3.7 ($\pm$3.1)	1.5 ($\pm$1.1)	46[a] ($\pm$16)	3.0 ($\pm$0.9)	1.2 ($\pm$1.1)	69[a] ($\pm$26)
ABS	88.2 ($\pm$44.1)	11.5 ($\pm$2.8)	85[a] ($\pm$5)	8.6 ($\pm$0.8)	1.0 ($\pm$0.1)	88[a] ($\pm$0)
WABF	8.6 ($\pm$1.0)	2.0 ($\pm$0.5)	77[a] ($\pm$3)	1.4 ($\pm$0.7)	0.1 ($\pm$0.0)	96[a] ($\pm$1)

[a] Means in the same column with the same superscript letter are not significantly different in reduction of total bacteria between the three multi-stage scrubbers ($p > 0.05$).

When sampling in the same environment, the MD8 collected less bacteria than the Andersen sampler in all cases. This finding is consistent with that of a former study by Lundholm (1982). The explanation put forward by Lundholm (1982) was the dehydration of bacteria on the gelatin filter of the MD8 during sampling. Certain bacteria are particularly susceptible to dehydration (Macher and First 1984). The reduction in the viable bacteria count might also be caused by sample transportation: viruses start to lose some of their infectivity after only 30 min in contact with gelatin filters (Weesendorp et al. 2008). In our experiments, the filter samples were kept in plastic bags and transported to the lab directly after sampling. However, it still took approximately 4 h before the bacteria were cultured. Another contributing factor is that the gelatin filters dissolved readily when sampling under high RH. In our experiment, the RH of the outgoing air leaving the scrubbers was 100%. Small pores were noticed on the gelatin filters when sampling the outgoing air. These pores would have allowed bacteria particles to pass through without being filtered, leading to underestimation of the bacteria concentration of the outgoing air. The dehydration stress on bacteria is much less when sampling with an Andersen sampler (Zhao et al. 2011; Zhao et al. 2011). An Andersen sampler can also differentiate between airborne bacteria on the basis of their size. However, the drawback of the Andersen sampler is that when sampling in highly bacterial-contaminated environments, such as livestock houses, it easily becomes overloaded (over 300 colonies on one agar plate). This limits the sampling duration to seconds in such environments. This was why the sampling duration applied in our study was 10 s.

The bacteria concentrations in the incoming and outgoing air were further classed by size ranges (Figure 4). In the incoming air, bacteria were predominately recovered from the first three stages of the Andersen sampler, which collected particles >3.3 µm. Bacteria in these three stages accounted on average for 73% of the total number of bacteria in the incoming air before the scrubber for the ABF,

95% for the ABS, and 82% for the WABF. Only small amounts of bacteria were present in the last three stages of the Andersen sampler, which collected particles ranging from 0.65 to 3.3 μm. This result was consistent with the study done by Crook et al. (1991), who found more airborne bacteria in larger particles than in smaller ones. The concentrations of bacterial particles >3.3 μm dropped sharply in the outgoing air compared to the concentrations in the incoming air.
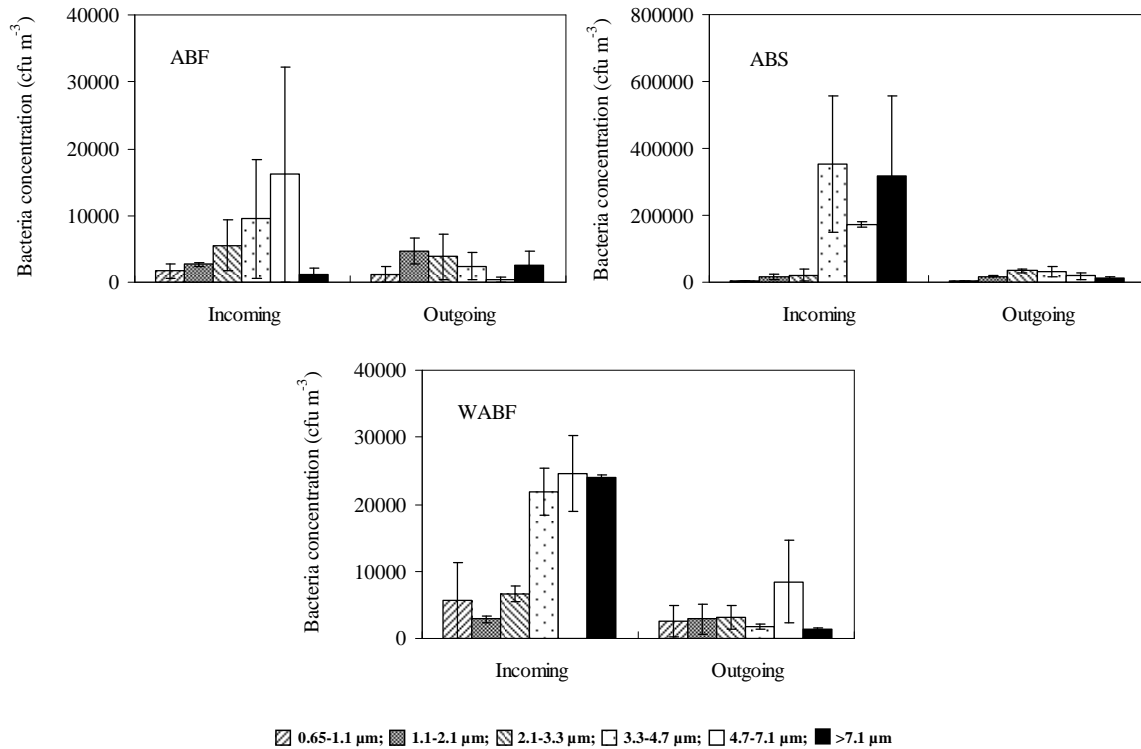

0.65-1.1 μm; 1.1-2.1 μm; 2.1-3.3 μm; 3.3-4.7 μm; 4.7-7.1 μm; >7.1 μm

Figure 4. Bacteria concentrations in incoming and outgoing air measured by Andersen sampler in different size ranges.

To summarize the data on bacteria collected in the first three stages of the Andersen sampler, the overall removal efficiency of bacterial particles >3.3 μm by the scrubbers was 53% for the ABF, 92% for the ABS, and 84% for the WABF. The scrubbers were less effective in removing of bacterial particles <3.3 μm: 12% for the ABF, -42% for the ABS, and 20% for the WABF. However, the statistical analysis showed that the difference in reduction of >3.3 μm bacteria particles compared to that of smaller particles was not statistically significant, probably due to the low power of analysis ($n =$ 2).

Reduction of ammonia and CO_2

The reductions in ammonia and CO_2 achieved by the different types of scrubbers are given in Table 6. The standard error for ammonia concentrations in the outgoing air of the ABF was high. This was caused by the problem of the pH-adjusting system on three sampling days, which led to an increase in pH of the acid solution and higher ammonia emissions. The WABF achieved the highest removal efficiency of 100% for ammonia, compared with the ABF (70%) and ABS (83%). Manuzon et al.

(2007) reported that air scrubbers were more effective when treating air with low ammonia concentrations. In our experiment, the ammonia levels were the lowest in the air entering the WABF. This was possibly one of the reasons that the WABF was more efficient at removing ammonia. Furthermore, the WABF had the longest residence time, which gave more chances for mass transfer. Concentrations of CO_2 did not differ between the incoming and outgoing air of the scrubbers.

Table 6. Average concentrations and reduction of ammonia and CO_2 in the incoming and outgoing air, and the reduction by the multi-stage scrubbers (p = 0.05, n = 6 for ABS and WABF; n = 7 for ABF).

Scrubber	Ammonia (±SE, ppm)		Reduction[a] (±SE, %)	CO_2 (±SE, vol %)		Reduction[a] (±SE, %)
	Incoming	Outgoing		Incoming	Outgoing	
ABF	44.7 (±1.6)	10.0 (±5.8)	70[a] (±13)	0.29 (±0.02)	0.30 (±0.02)	1[a] (±6)
ABS	39.3 (±1.3)	7.0 (±0.6)	83[ab] (±2)	0.32 (±0.02)	0.32 (±0.02)	-3[a] (±6)
WABF	28.3 (±2.5)	0.1 (±0.1)	100[b] (±1)	0.18 (±0.01)	0.18 (±0.01)	4[a] (±6)

[a] Means in the same column with the same superscript letter are not significantly different (p > 0.05).

Multi-stage vs. single-stage scrubbers

The reduction of air pollutants by single-stage scrubbers varies hugely depending on their structure or design. A review of former studies revealed that single-stage scrubbers (acid or biological) reduced total dust by 22% to 88% (Seedorf and Hartung 1999; Marsh et al. 2003; Aarnink et al. 2005) and ammonia by 35% to 99% (Melse and Ogink 2005).

Assuming that the multi-stage scrubbers truly reduced larger particles more than the smaller ones (Ogink and Hahne 2007), the reduction of total dust by these scrubbers would be at least 61% to 93% (reduction for PM_{10} in our study). With respect to total bacteria, the three multi-stage scrubbers constantly decreased the CFU in the outgoing air, whereas some single-stage scrubbers have been found to increase the CFU (Aarnink et al. 2005). These multi-stage scrubbers also proved to be very effective in removing ammonia (70% to 100%). In general, they performed more consistently in reducing emissions of dust, total bacteria, and ammonia from livestock houses and achieved higher average removal efficiency than single-stage scrubbers. However, more stages may create a higher pressure drop over the scrubber, which may increase the energy consumption. Further research is required to develop energy-saving multi-stage scrubbers.

In this article, the removal efficiency of airborne dust, bacteria, ammonia, and CO_2 by three multi-stage air scrubbers of different designs was evaluated. The scrubbers were effective in reducing all the air pollutants except CO_2. It should be noted that all measurements were done within a short period during the winter, which means that the scrubbers were not tested at high ventilation rates. Measurements during a longer time frame that includes periods with maximum ventilation rates will give a complete insight into the overall efficiency of the multi-stage scrubbers and the consistency of their removal efficiency.

CONCLUSIONS

The three scrubbers reduced concentrations of PM_{10} by 61% to 93% and concentrations of $PM_{2.5}$ by 47% to 90%. The double-stage scrubbers (ABF) were more effective in reducing PM_{10} than $PM_{2.5}$. The triple-stage scrubber (WABF) reduced dust effectively from the pig house (93% reduction for PM_{10} and 90% for $PM_{2.5}$), but the difference in the reductions of PM_{10} and $PM_{2.5}$ were not statistically different.

The multi-stage scrubbers reduced concentrations of airborne total bacteria by 46% to 85%. The bacteria were predominantly in particles >3.3 µm in the air flowing into the scrubbers from the pig houses. These bacteria accounted for 73% to 95% of the total bacteria count. The removal efficiency was 53% to 92% for bacterial particles >3.3 µm and -42% to 20% for bacterial particles in the range of 0.65 to 3.3 µm.

The reduction in ammonia achieved by the multi-stage scrubbers ranged between 70% to 100%. No difference in CO_2 concentration could be found between the air entering and leaving the scrubbers.

All measurements were performed during winter period at low ventilation rates. A year-round sampling period would give a full picture of the scrubbers' performance.

ACKNOWLEDGEMENTS

EVALUATION OF AN IMPACTION AND A CYCLONE PRE-SEPARATOR FOR SAMPLING HIGH PM$_{10}$ AND PM$_{2.5}$ CONCENTRATIONS IN LIVESTOCK HOUSES

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ABSTRACT. When used in dusty environments, such as livestock houses, the current EU reference sampler for ambient dust (PM_{10} and $PM_{2.5}$) with an impaction pre-separator (IPS) is expected to become overloaded, because of the low dust-retaining capacity of the greased impaction plate in the IPS. The two objectives of this study were 1) to verify this by regularly replacing the loaded greased plates by clean ones, and 2) to evaluate a sampler with a cyclone pre-separator (CPS) as an alternative dust sampler in ambient environments and in livestock houses (fattening pig, broiler and dairy) following the EU standard procedure. Results showed that PM_{10}-IPS did not become overloaded in 24 h measurements in layer houses, whereas $PM_{2.5}$-IPS became overloaded within 1 h. $PM_{2.5}$-CPS did not become overloaded during 48 h sampling in a layer house. Both PM_{10}-CPS and $PM_{2.5}$-CPS showed good precision and comparability with IPS in less dusty environments. In pig and broiler houses with high dust loads, PM_{10}-CPS could give comparable results with IPS by introducing a correction factor. It is concluded that $PM_{2.5}$-IPS is not suitable for use in livestock houses, and that $PM_{2.5}$-CPS is very resistant to high dust load. For PM_{10} sampling, both IPS and CPS can be used.

Keywords. *Overloading, Evaluation, Impaction, Cyclone, Pre-separator, Animal houses.*

INTRODUCTION

PM_{10} and $PM_{2.5}$ are well-known to have negative effects on public health (Brunekreef and Holgate 2002). These dust fractions originate from fuel exhausts, paved roads, cooking, wood combustion, and sea salt (Schauer et al. 1996; Buzcu et al. 2003; Fraser et al. 2003). In recent years, dust from livestock houses has aroused much concern, not only because of its potential physiological hazards to humans, but also because it combines with other air pollutants and is suspected to act as a carrier of pathogens in airborne disease transmission between farms (Stark 1999; Iversen et al. 2000; Von Essen and Romberger 2003). The emissions of dust from livestock houses account for a significant proportion of total dust emission, especially in countries with intensive livestock production (Erisman et al. 2008). In the Netherlands, for example, total primary PM_{10} emission in 2000 was estimated to be 50 ktons, of which approximately 9.3 ktons, or 18.6%, originated from livestock production (Chardon and Van der Hoek 2002).

Appropriate samplers are needed for unbiased and precise measurement of dust emissions from livestock houses. These samplers (which are based on gravimetric principles) should be capable of effectively pre-separating large particles (larger than PM_{10} or $PM_{2.5}$) and trapping PM_{10} or $PM_{2.5}$ on filters. Pre-separation by the impaction principle is a common method for less dusty environments, such as ambient air. The WINS impactor, a secondary single-stage greased impactor, has been described in US federal reference method (FRM) for $PM_{2.5}$ in ambient atmosphere (Kenny et al. 2000). A PM_{10} impactor inlet for a high airflow rate has also been developed (Misra et al. 2003). The currently used reference and equivalent PM samplers have recently been listed and described by the US Environmental and Protection Agency (US EPA 2008).

The EU standard samplers for PM_{10} and $PM_{2.5}$ in ambient air are also based on the impaction pre-separation principle. The advantage of impaction pre-separators (IPS) is that they have a sharp cut-off curve, which does not alter significantly during long sampling periods (96 h) in ambient environments for $PM_{2.5}$ (Kenny et al. 2000). On the other hand, one of the drawbacks of an IPS is suggested to be overloading caused by large particles from heavily loaded impaction plates bouncing off onto the downstream filter, which results in overestimated dust concentrations (Kenny 1998).

One solution, or compromise, to avoid overloading is only to operate an IPS below certain dust load limits. Under EU legislation, for a 24 h measurement a $PM_{2.5}$ IPS can be operated in the environment with a dust load of < 200 µg m^{-3} (European Commission 1998; European Commission 2005). However, this threshold was set in order to avoid variations in airflow rate caused by filter clogging, rather than to prevent overloading. Nowadays, special pumps can be used to ensure a constant flow of air. So, the current usefulness of the 200 µg m^{-3} threshold in relation to overloading is unclear. Furthermore, no such threshold has been set for PM_{10}-IPS.

It would be useful to know whether an IPS system could be used outside its registered environments (> 200 µg m^{-3}), e.g. to sample dust in livestock houses. It might be possible to extend the

IPS's capacity to retain large particles, by regularly cleaning the impaction plate, but this is difficult, given that we do not yet know how the ambient dust load and the particle size distribution affect IPS performance (Hofschreuder et al. 2007). Another option would be to use samplers based on a different principle to take samples in very dusty environments.

A promising alternative to the impaction pre-separator might be a cyclone pre-separator (CPS). This technique for sampling dust was devised decades ago (Higgins and Dewell 1967; Wedding et al. 1982). Early studies showed that CPSs did not have particle size selection curves as sharp as IPS. Attempts to evaluate two CPSs as reference pre-separators of dust samplers failed, either because the cut-off curve was too shallow, or because of the shift in cut-off diameter (Peters et al. 1996). These disadvantages seemed to have been overcome in more recent CPSs. A CPS with a sharp cut-off curve similar to that of the WINS impactor for collecting $PM_{2.5}$ has been described (Kenny et al. 2000; Gussman et al. 2002). It has been designated as a reference equivalent sampler (US EPA 2002). A dry CPS is more user-friendly than an IPS with grease on the impaction plate. Furthermore, a CPS can collect large quantities of dust and is less vulnerable than an IPS to the problem of particle bouncing (Saltzman 1984; Pui and Liu 1988). So, using a CPS might be a good option for sampling in dusty environments.

Our study had two objectives: 1) to investigate the occurrence and extent of overloading of the current EU reference PM_{10} and $PM_{2.5}$ samplers with IPS in the dusty environment of layer houses, and 2) to evaluate CPS as an alternative pre-separator for use in the dusty environments of livestock houses following the EU standard procedure.

MATERIAL AND METHODS

Samplers

Note that in this paper the abbreviations of "IPS" and "CPS" refer either to two types of pre-separators or to two types of samplers (a combination of an air inlet, a pre-separator and a downstream filter holder).

IPS

An IPS is described as the reference dust sampler in CEN-EN 12341 (1998) for PM_{10} and in CEN-EN 14907 (2005) for $PM_{2.5}$. Figure 1 shows the samplers for both dust fractions.

An IPS consists of an air inlet head, an impaction pre-separator and a filter holder. For this study, a flat impaction plate was rubbed with grease and placed under eight impactor nozzles (Figure 1). Larger particles strike the plate at speed and are retained because of their inertia, but the smaller target particles are carried along in the air stream and are collected on the downstream filter. The airflow rate through the inlet head of an IPS is 2.3 $m^3 h^{-1}$.

(A)   (B)

Figure 1. The complete IPS system (A) and the differently sized openings of the impactor nozzles (B) that cause the air above the impaction plate to accelerate (larger openings for PM$_{10}$; smaller openings for PM$_{2.5}$).

CPS

A CPS consists of an air inlet head, a cyclone pre-separator (URG corp., US) and a filter holder (Figure 2). The design of the air inlet head of a CPS is based on that of an IPS. Instead of eight impactor nozzles there are eight short tubular screws. The body of the air inlet head is a cylinder with a hollow cone frustum inside. All surfaces are smooth, to avoid dust loss in the air inlet head. In the CPS used in our study, a metal pipe connected the end of the air inlet head to the pre-separator. The pre-separator uses the centrifugal principle to separate large particles trapped in a dust collector. PM$_{10}$ or PM$_{2.5}$ are conveyed in the air stream and collected by a glass fibre filter in the filter holder. The airflow used for a CPS is set at 1 m^3 h^{-1}.

(A)   (B)

Figure 2. The air inlet head, the PM$_{10}$ cyclone pre-separator, the PM$_{2.5}$ cyclone pre-separator and the filter holder (A, from left to right), and the construction of the air inlet head (B).

Pumps

Pumps of type Charlie HV (Ravebo Supply b.v., Brielle) were used to suck air through the samplers. These pumps automatically adjust the airflow on the basis of the temperature measured at

the sampling head, and the temperature, pressure and airflow of the gas meter installed inside the pumps. The airflow of the pumps remains constant when the pressure difference over the filter increases. This control system ensured that during sampling the airflow remained constant, varying by no more than 2% from the nominal value. The clocks in the pumps were programmed to start and stop sucking air automatically.

Filters

PM$_{10}$ and PM$_{2.5}$ were collected on glass fibre filters with a diameter of 47 mm. The dust-free filters were stabilized for 48 h under standard conditions: 20 ± 1°C and 50 ± 5% relative humidity. Using a precise balance with a resolution of 10 µg, each filter was then weighed once in the morning and afternoon of the first and second days after the stabilization (4 times in total). The mean value per filter was calculated as the filter weight. The dust-loaded filters were weighed following the same procedure. Subtracting the weight of the dust-free filter from the dust-loaded filter yielded the amount of dust collected.

Measurements

Verifying overloading

The tests were performed in a poultry house (aviary system, some litter on the floor and loose housing) with about 100 laying hens, in Wageningen, the Netherlands. We first tested whether the IPSs could be overloaded within 8 h sampling. Four IPSs and two CPSs for both PM$_{10}$ and PM$_{2.5}$ were used. During the 8-hour sampling period, four rates of replacing the greased IPS plates were tested: 0 times (no replacement), 3 times (every 2 h), 7 times (every 1 h) or 15 times (every 0.5 h). Secondly, PM$_{10}$-IPSs were further challenged with a longer sampling period of 24 h and with fewer plate replacements in a dustier layer room containing about 300 hens. The replacement rates were 0 times (no replacement), 1 time (every 12 h), 2 times (every 8 h) or 3 times (every 6 h). Thus the numbers of plates used per treatment were 1, 2, 3 and 4. In both tests, measurements were repeated twice. The CPSs were not cleaned during the sampling period.

To ascertain whether the PM$_{2.5}$-CPSs also suffered from overloading, we performed a test in duplicate. Six of the CPSs were run for 24 h with three cleaning frequencies 0 times (no cleaning of the dust collector), 1 time (cleaning every 12 h) and 2 times (cleaning every 8 h). In the mean time, two more CPSs ran for 48 h without cleaning.

Test comparing CPS with IPS

96 pairs of 24 h measurements, 48 for PM$_{10}$ and 48 for PM$_{2.5}$, were conducted in various environments: livestock houses (three fattening pig houses, one broiler house and one dairy barn); an industrial workplace; and in the ambient air. For each pair of measurements we used one IPS (as the reference sampler) and two CPSs (as the candidate sampler).

In the three fattening pig houses the there were respectively 1200 pigs (80 kg each), 520 pigs (312 were 85 kg, 208 were 45 kg) and 3200 pigs (67.5 kg each). Before exhausted, the air from each of the houses was sucked through a ventilation room, where the dust was sampled. The broiler house contained eight similar compartments, in each of which approximately 2675 broilers were raised on the ground with bedding of wood shavings. In two of these compartments samples were taken on the same day in the first, second, third and fifth weeks of bird age. The dairy barn with approximately 100 dairy cows was naturally ventilated. The industrial workplace was a machinery room in Wageningen University in which farm robots were stored and where several people worked daily. IPSs were installed in duplicate in this workplace (so, two IPSs and two CPSs for one pair of measurements). Measurement of the ambient air was performed outdoors, about 20 m from the machinery room.

In all the locations, the samplers were installed at 1.2 m height, with one exception: the ventilation room of the pig house with 3200 pigs. Here the samplers were suspended 2.0 to 3.0 m above the floor. The three samplers (one IPS and two CPSs) were placed about 30 cm apart, to avoid mutual interference. The air velocity near the samplers was measured with a hot-wire anemometer and varied from 0.1 to 1.8 m s^{-1}.

Nomenclature	
N	total number of data pairs
n	number of valid data pairs
X_i	ith measured value of the reference sampler (IPS) concentration
Y_{i1}	ith measured value of the candidate sampler (CPS$_1$) concentration 1
Y_{i2}	ith measured value of the candidate sampler (CPS$_2$) concentration 2
Y_i	mean concentration value of the ith parallel measurement of the candidate samplers 1 and 2
D_i	difference between the ith measured value of the candidate samplers 1 and 2
mean$_D$	mean of all D in a dataset
sd	standard deviation of all D in a dataset
s_a	absolute standard deviation for low concentration group
s_r	relative standard deviation for high concentration group
f	degree of freedom
t	test statistic according to the Student t distribution
$t^2_{(\alpha/(2N),N-2)}$	the critical value of the t distribution with (N-2) degrees of freedom and a significance level of $\alpha/(2N)$, in which α was set at 5% in this analysis
CI$_{95}$	two-sided 95 % confidence interval
Z	Grubb's test statistic
CV	critical value in Grubb's test

Data analysis for comparing CPS with IPS

Data were analysed according to the EU standard procedure for PM$_{10}$ (European Commission 1998). See sections "*Analyses of comparability of candidate samplers (CPS-CPS test)*" test and "*Test*

of comparability of the candidate sampler with the reference sampler (CPS-IPS test)" below. Since no specified standard procedure was available for $PM_{2.5}$, the procedure for the PM_{10} sampler was also used for the $PM_{2.5}$ sampler. The only difference was that the $PM_{2.5}$ data were classed according to the sampling location (group one: livestock houses; group two: workplace/ambient air), instead of by dust concentration (threshold of 100 µg m^{-3}).

To qualify as a reference equivalent dust sampler, two requirements have to be met: 1) the two candidate samplers are comparable to each other in a CPS-CPS test; 2) the candidate samplers are comparable with the reference sampler in a CPS-IPS test. Before the two tests, outliers from the datasets of PM_{10} and $PM_{2.5}$ were excluded (see "Detecting outliers").

Detecting outliers

The outliers were detected first for the CPS-CPS test, then for the CPS-IPS test, using Grubb's test. For the outliers in the CPS-CPS test, the Z value of each data pair (data from two CPSs) could be calculated with Equation 1,

$$Z_i = \left|mean_D - D_i\right|/sd \tag{1}$$

Values for "D" were calculated as absolute differences when mean PM_{10} concentration from the CPSs was $\leq$ 100 µg m^{-3}, and as relative differences when PM_{10} concentration was > 100 µg m^{-3}. Only absolute differences were calculated for $PM_{2.5}$. When Z_i exceeded a critical value (Equation 2), "D_i" was considered to be an outlier and the corresponding data pair was subsequently excluded. If a data pair was excluded as an outlier in the CPS-CPS analysis, it did not appear in the subsequent CPS-IPS analysis.

$$CV = (N-1)\Big/N \cdot \sqrt{t^2_{(\alpha/(2N),N-2)}\Big/(N-2+t^2_{(\alpha/(2N),N-2)})} \tag{2}$$

Outliers in CPS-IPS test were determined according to the same procedure. "D" here indicated the difference between the mean concentration of two CPSs and the concentration of the IPS in one data pair. Outliers were checked for the whole dataset in the CPS-CPS test for both PM_{10} and $PM_{2.5}$. The CPS-IPS outlier of $PM_{2.5}$ was only tested for data from the work place/ambient air environment. The standard procedure prescribes that the number of outliers should be within 5% of the total number of data.

Analyses of comparability of candidate samplers (CPS-CPS test)

The test focused on whether the differences between the concentration values measured by the two CPSs were within the acceptable boundary defined by the EU standard (European Commission 1998). Ideally, the candidate samplers would be identical, i.e. they sampled the same dust fraction, implying that $D_i = 0$.

The PM_{10} data were classified into two groups: low concentration ($\leq$ 100 µg m^{-3}) and high concentration (> 100 µg m^{-3}). An absolute standard deviation, s_a, was calculated for the low

concentration group (Equation 3). CI_{95} was obtained by multiplying s_a by the corresponding student factor t (Equation 4), which was defined as the 0.975 quantile of the two-sided 95% confidence interval of the Student t-distribution with $f_\leq = n_\leq - 2$ degrees of freedom. If $CI_{95} \leq 5$ µg m^{-3}, the candidate samplers met the requirement for comparability in the low concentration group. A relative standard deviation was used for the high concentration group (Equations 5 and 6). To meet the comparability requirement, CI_{95} should be $\leq 5\%$.

$$s_a = \sqrt{\sum D_i^2 / 2n_\leq} \tag{3}$$

$$CI_{95} = s_a \cdot t_{f\leq;0.975} \tag{4}$$

$$s_r = \sqrt{\sum (D_i / Y_i)^2 / 2n_>} \tag{5}$$

$$CI_{95} = s_r \cdot t_{f>;0.975} ; \tag{6}$$

Test of comparability of the candidate sampler with the reference sampler (CPS-IPS test)

The test focused on the differences between the concentration values measured by CPSs (y) and IPSs (x). Ideally, the candidate sampler was sampling the same dust fraction as the reference one, implying y = x.

The relationship y = f(x) between the candidate and the reference concentration values was computed with linear regression analysis. It was compared with the two-sided acceptance envelope, i.e. y = (x ± 10) µg m^{-3} when x ≤ 100 µg m^{-3}, and y = (1 ± 0.1)x µg m^{-3} when x > 100 µg m^{-3}. If the variance coefficient R^2 of the calculated reference equivalence function was ≥ 0.95 over the relevant concentration range, and the reference equivalence function calculated was within the limits of the acceptance envelope, the candidate sampler met the requirement for reference equivalence.

RESULTS

The overloading verification test

Table 1 shows the results of the overloading verification test and the linear regression analysis of dust concentrations from IPSs against the number of plate replacements. The 24 h overloading test of CPS is also included. A p-value < 0.05 indicates that as the number of plate replacements increased, the measured dust concentrations were significantly reduced, i.e. in the given environment, the samplers were overloaded.

The PM_{10} IPSs did not become overloaded (p = 0.40) during an 8 h sampling period. When PM_{10} was sampled with IPS for 24 h with 0 to 3 plate replacements the number of plate replacements did not affect the measured PM_{10} concentration (p = 0.80). The PM_{10} concentrations measured with CPSs were 10%-16% higher than those measured with IPSs: 666 vs 607 µg m^{-3} in the 8 h test and 2403 vs 2075 µg m^{-3} in the 24 h test.

Table 1. PM$_{10}$ and PM$_{2.5}$ concentrations (each value is the mean of duplicate measurements) and linear regression analysis in 8 h and 24 h overloading tests. In the 8 h test for IPS, the greased plates in the IPS were replaced 0 (no replacement), 3 times (every 2 h), 7 times (every 1 h) or 15 times (every 0.5 h); in the 24 h test for IPS, the greased plates in the IPS were replaced 0 (no replacement), 1 time (every 12 h), 2 times (every 8 h) or 3 times (every 6 h). During the test CPS was operated without cleaning. In the 24 h test for CPS, the coarse dust collectors were cleaned 0 times, 1 time (every 12 h) or 2 times (every 8 h).

Sampling period	Dust	Concentration (μg m^{-3}) CPS (number of collector cleanings)			Mean	IPS (number of plate replacements)				Mean	Linear regression line Slope (se)	Constant (se)	P[a] Slope
8 h	PM$_{10}$	666 (0)			666	624 (0)	609 (3)	588 (7)	608 (15)	607	-0.94 (1.03)	613.4 (8.7)	0.40
8 h	PM$_{2.5}$	49 (0)			49	171 (0)	158 (3)	142 (7)	123 (15)	149	-3.20 (0.54)	168.5 (4.5)	0.00
24 h	PM$_{10}$	2403 (0)			2403	2110 (0)	2115 (1)	1941 (2)	2135 (3)	2075	-9.63 (35.82)	2089.8 (67.0)	0.80
24 h	PM$_{2.5}$	103 (0)	101 (1)	99 (2)	101	-	-	-	-	-	-1.78 (1.21)	102.9 (1.6)	0.24

[a] Probability that the slope of the linear regression line is not different from zero (null hypothesis). A p-value > 0.05 indicates the null hypothesis should be accepted, while a value ≤ 0.05 indicates the null hypothesis should be rejected.

Table 2. Number of data pairs obtained, concentration ranges, and two-sided 95% confidence interval, CI$_{95}$, for comparability test of candidate sampler (CPS) and reference sampler (IPS) in workplace environment.

Dust	Group	Number	Concentration range (μg m^{-3}) Min	Max	Mean	Absolute CI$_{95}$ (μg m^{-3})	Relative CI$_{95}$ (%)
PM$_{10}$	≤ 100 μg m^{-3} (CPS)	28	4.0	40.5	20.0	2.20	15.3
	> 100 μg m^{-3} (CPS)	20	313.7	4392.0	1126.0	66.50	6.0
	Workplace (IPS)	6	5.0	43.1	13.7	1.95	29.7
PM$_{2.5}$	Workplace/ambient (CPS)	19	2.8	40.6	10.5	2.30	31.5
	Animal house (CPS)	27	6.1	168.2	39.1	9.54	20.2
	Workplace (IPS)	9	4.2	43.4	11.9	1.66	24.7

Increasing the number of plate replacements during an 8 h sampling period significantly affected (p = 0.00) the measured $PM_{2.5}$ concentration: with no replacement the mean concentration was 171 µg m^{-3}, with 15 replacements it was 123 µg m^{-3}. The mean $PM_{2.5}$ concentration measured with CPSs (49 µg m^{-3}) was much lower than the concentrations measured with IPSs (149 µg m^{-3}).

The regression coefficient of the dust concentration against number of cleanings of the coarse dust collectors of CPS was not significant (p = 0.24). The two CPSs that continuously collected dust for 48 h without their dust collectors being cleaned gave an average $PM_{2.5}$ concentration of 106 µg m^{-3}. ANOVA showed there was no significant difference between the measured $PM_{2.5}$ measurements. This indicated the CPS was not overloaded.

Comparison of CPS with IPS

Detecting outliers

No outlier was detected in the PM_{10} dataset. Two pairs of $PM_{2.5}$ data were eliminated as outliers in the CPS-CPS test. No outlier was found in the CPS-IPS test for PM_{10} and $PM_{2.5}$. The number of outliers accounted for 4.2% of the total number of $PM_{2.5}$ data pairs, which was within 5%. The valid data pairs of PM_{10} and $PM_{2.5}$ were 48 and 46. Both were more than 40 pairs, the minimum required in the EU standard.

(A): ≤ 100 µg m^{-3}   **(B): > 100 µg m^{-3}**
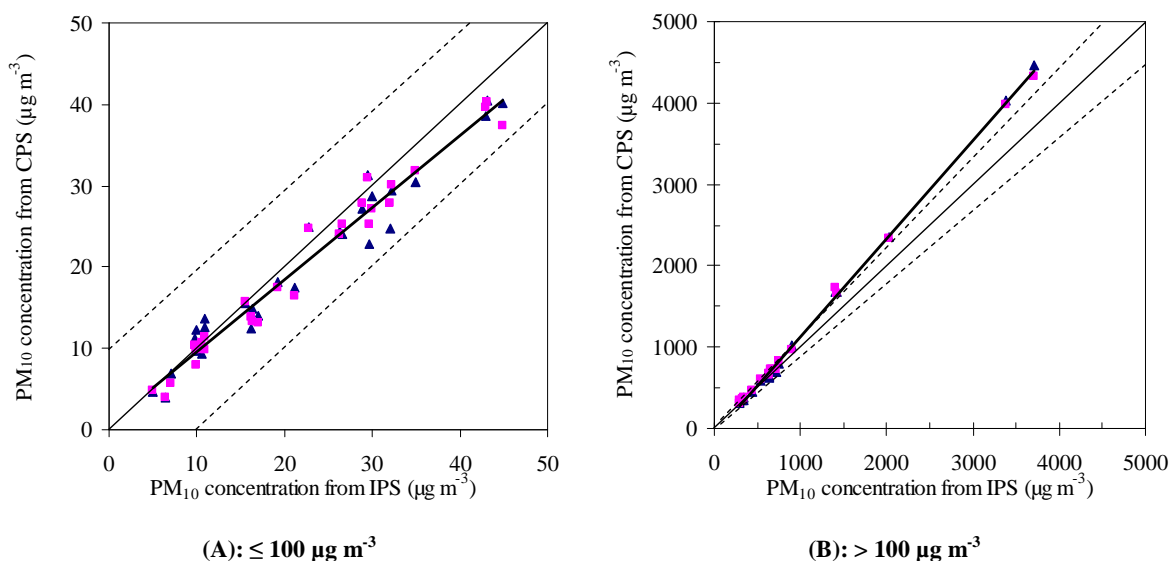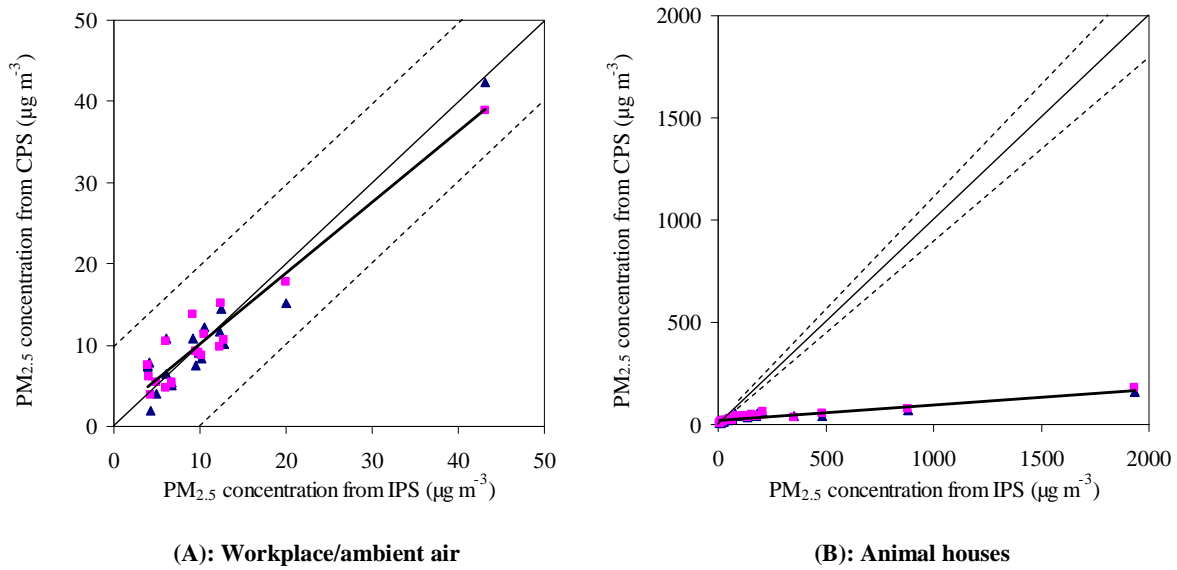
Figure 3. PM_{10} concentrations in CPS-IPS comparability test (- - - : acceptable boundary according to CEN 1998; —: y = x; ▬, regression line; ▲: concentrations from CPS$_1$; ▪: concentrations from CPS$_2$).

Comparability of CPSs

The number, range and absolute and relative CI_{95} of the data pairs are listed in Table 2. The CI_{95} for the PM_{10} low concentration group (2.20 µg m^{-3}) was within the 5 µg m^{-3} boundary. The relative CI_{95} value for PM_{10} high concentration group was 6.0%, slightly exceeding the 5% boundary. The CI_{95} values for $PM_{2.5}$ were 2.30 µg m^{-3} for the workplace/ambient air and 9.54 µg m^{-3} for the livestock

houses. The IPSs showed good absolute CI_{95} values in the workplace (1.66 µg m^{-3}). All the relative CI_{95} values for $PM_{2.5}$ were > 5%.

(A): Workplace/ambient air   **(B): Animal houses**

Figure 4. $PM_{2.5}$ concentrations in CPS-IPS comparability test (- - - : acceptable boundary according to CEN 1998; —: y = x; ▬, regression line; ▲ : concentrations from CPS_1; ■: concentrations from CPS_2).

Table 3. Regression analysis of CPS-IPS test (concentrations from CPSs against concentrations from IPSs).

Dust	Data group	Pairs of data	Slope[a] (se)	Constant[b] (se)	R[b]
PM_{10}	≤ 100 µg m^{-3}	28	0.89 (0.03)[***]	0.6 (0.8)	0.97
	> 100 µg m^{-3}	20	1.20 (0.01)[***]	-68.0 (12.3)[***]	0.99
$PM_{2.5}$	Workplace/ambient air	19	0.87 (0.06)[***]	1.5 (0.8)	0.93
	Animal houses	27	0.07 (0.01)[***]	22.3 (2.6)[***]	0.86

[a] Tested against the null hypothesis that the slope equals 1 (data from the CPS and the IPS were the same). *** signifies the slope deviated significantly from 1 (p < 0.001).

[b] Tested against the null hypothesis that the constant equals 0. *** signifies the constant deviated significantly from 0 (p < 0.001).

Comparability of CPSs with IPS

Figure 3 and Figure 4 show the relationship between dust concentrations measured with CPSs and IPSs. Table 3 gives the results of the regression analysis. It can be seen that the values of R^2 were higher than 0.95 for both PM_{10} groups. The slopes showed different trends in the low (0.89 ± 0.02) and high (1.20 ± 0.01) concentration groups. The regression line fell within the acceptable boundary for PM_{10} concentrations ≤ 100 µg m^{-3} (Figure 3 A). All data were spread evenly in the range from 0 to 50 µg m^{-3}, but we had no data in the range from 50 to 100 µg m^{-3}. Combined with the results of the CPS-CPS comparability test, the PM_{10}-CPS totally fulfilled the standard requirements in EU legislation as a reference equivalent dust sampler when sampling in less dusty environments. In dusty environments, higher concentrations were obtained with the CPSs than with the IPSs (Figure 3 B). Most concentrations were below 1000 µg m^{-3}. PM_{10} data points above 1000 µg m^{-3} fell outside the boundary.

Most of the $PM_{2.5}$ concentrations obtained in the workplace/ambient air were between 0 and 20 µg m^{-3}. The regression line fitted well within the boundary (Figure 4 A). R^2 was 0.93, slightly below the required 0.95. The slope was 0.87 ± 0.04 (se). Due to the severe overloading of the IPS, the regression line of data from the livestock houses drifted far from the $PM_{2.5}$ boundary (Figure 4 B). The slope of regression line was 0.07 ± 0.01 (se).

DISCUSSION

Overloading verification

Overloading of the IPS was verified by ascertaining the effect of replacing the greased plate on the $PM_{2.5}$ concentration. In an environment with low dust concentration – for example, in ambient air – plate replacement is not expected to have any effect, because one plate has sufficient capacity to retain large particles during a 24 h sampling period (Kenny et al. 2000). In a dusty environment –for example, an livestock house – a greased plate rapidly loses its retaining ability. If the plate is not replaced by a clean one, the large particles already collected on it bounce off, re-enter the airstream and are collected on the filter together with the target fraction. Therefore, if the dust concentration decreases when the greased plates are exposed to dust collection for a shorter period, i.e. if there is a negative correlation between dust concentration and the number of plate replacements in the sampling period, there is overloading.

Our results from measuring PM_{10} concentrations with IPSs for 8 h in dusty layer houses and then for 24 h in a dustier layer house demonstrate that PM_{10}-IPS could not be overloaded. The slightly higher PM_{10} concentrations measured by CPSs compared with IPSs might be due to a "shallow penetration curve" as was found for $PM_{2.5}$-CPS by (Kenny et al. 2000).

The measured $PM_{2.5}$ concentrations (8 h test) were on average about 3.5 times higher when using IPSs without plate replacement compared with using CPSs (171 vs 49 µg m^{-3}). The significant inverse correlation we found between $PM_{2.5}$ concentration and number of plate replacements shows that the $PM_{2.5}$-IPS was overloaded. It can be expected that $PM_{2.5}$-IPS will be more vulnerable to overloading than PM_{10}-IPS, because its greased impaction plate is challenged with more particles (including particles between 2.5 and 10 µm). Also, the air impacts the plate in $PM_{2.5}$-IPS at a velocity six times higher than that of PM_{10}-IPS (15 vs 2.4 m s^{-1}). At such a high air speed, bouncing and re-entrainment of the large particles from the impaction plate is more likely. The EU standard stipulates that $PM_{2.5}$-IPS should be operated in environments having a total dust concentration ≤ 200 µg m^{-3}. The reason for imposing this limit is to avoid filter clogging and to minimize fluctuations in flow rates from the pumps. However, this standard takes no account of overloading of greased plates. We have demonstrated that $PM_{2.5}$-CPS is very resistant to high dust loading: even 48 h of continuous sampling without cleaning the dust collector did not give rise to an overloading problem.

Comparison of CPS with IPS

The EU legislation set IPS as the standard dust sampler. To qualify as a "reference-equivalent sampler", all other samplers have to be subjected to a comparability test. Within this test, comparability should be proven between the candidate samplers (precision test) and between the candidate and the reference samplers. Ideally, two similar candidate samplers provide identical results when taking samples in the same environment. But it is impossible to obtain identical results because system and measuring errors are present, which could be indicated by CI_{95}. The lower the CI_{95} value, the better the precision of the candidate samplers. The upper limits of CI_{95} for PM_{10} samplers are set at 5 µg m^{-3} for dust concentrations ≤ 100 µg m^{-3} and at 5% for higher concentrations.

In our study, PM_{10}-CPS showed quite good precision when sampling in less dusty environments (CI_{95} = 2.20 µg m^{-3}), i.e. the workplace/ambient air and a dairy farm. It was comparable with the results of the reference sampler (CI_{95} = 1.95 µg m^{-3}). The relative CI_{95} of PM_{10}-CPS was even lower than that of the reference sampler (15.3% vs 29.7%). The CI_{95} of 6 % for dusty environments, with data from the pig and broiler houses, was slightly higher than the standard. However, CEN-EN 12341 does not specify a CI_{95} for livestock houses, where other factors might affect sampler characteristics, such as dust concentration and particle size distribution. For comparability tests in livestock houses, the boundary CI_{95} of 5% needs to be reconsidered.

In our $PM_{2.5}$-CPS data, 45 out of the 46 pairs were ≤ 100 µg m^{-3} (the upper range of 168.2 µg m^{-3} was determined by the one extreme high value in livestock houses). Therefore, grouping the data into above and below 100 ug/m3 was not useful. Instead, we split the $PM_{2.5}$-CPS data into the workplace/ambient air group and the livestock house group, on the justification that the IPS was more likely to become overloaded in the livestock houses than in the workplace/ambient air. There is no standard CI_{95} for $PM_{2.5}$-CPS. The absolute CI_{95} values showed a big difference between the two groups (2.30 vs 9.54 µg m^{-3}). Compared to the reference sampler, the calculated value of 2.30 µg m^{-3} for CPS in workplace/ambient air seemed to be acceptable. The data from livestock houses showed a very high absolute CI_{95} value of approximately 5.7 times the reference (9.54 vs 1.66 µg m^{-3}).

The linear regression of CPS-IPS test showed good R^2 values for PM_{10}-CPS. The regression line of PM_{10} concentrations measured with CPS against those with IPS fell between the two-sided boundary in the range of ≤ 100 µg m^{-3} (Figure 3 A). The results of all the CPS versus IPS tests revealed that the PM_{10}-CPS fulfilled all the requirements to qualify as an equivalent sampler in this range. In the range of > 100 µg m^{-3}, the regression line lay outside the boundary (Figure 3 B), although the R^2 was high (0.99). The different patterns of the regression lines in these two ranges are probably caused by the interaction between the pre-separation principles (impaction vs cyclone) and the dust load.

The EU procedure (European Commission 1998) recommends that the concentrations measured should cover the widest possible range. Moreover, comparability would be more convincing if the concentration data were uniformly distributed over the whole range. In our study, PM_{10} data were spread over a wide range; however, there were no data in the range from 50 to 300 µg m^{-3}. Although

we expect that the relationship between the CPS and the IPS will not be different within this range, additional collection of data within this range is recommended.

Assuming that the reference sampler gave the true PM_{10} concentration, the slope of the regression line for an ideal candidate sampler against the reference sampler should not be significantly different from 1. For a candidate sampler with a slope significantly different from 1, a correction factor should be introduced to calibrate PM_{10} concentration to the reference. In our study, the linear regression analysis showed different lines for the two PM_{10} concentration groups. PM_{10} concentrations measured with CPSs were systematically lower than values measured with IPSs in the concentration range ≤ 100 μg m^{-3}, but higher in the concentration range > 100 μg m^{-3}. Therefore, the calibration should be treated separately: see Equations 7 and 8. The two regression lines intersected at 223 μg m^{-3} (Figure 5). The equation regressed from data in the range of ≤ 100 μg m^{-3} should be used for the calibration when PM_{10} concentrations measured with CPS are lower than 223 μg m^{-3} (Equation 7). For the higher concentrations, the equation regressed from data in the range > 100 μg m^{-3} should be used (Equation 8).

$$y = 1.09x \ (\text{x} \leq 223 \ \mu g \ m^{-3}) \tag{7}$$

$$y = 0.83x + 57.5 \ (\text{x} > 223 \ \mu g \ m^{-3}) \tag{8}$$

x: concentration measured with CPS (μg m^{-3});

y: calibrated concentration (μg m^{-3}).

A high $PM_{2.5}$ concentration of 40.5 μg m^{-3} was noticed (Figure 4 A) in the workplace/ambient air, but the other concentrations were all lower than (or equal to) 20 μg m^{-3}. Without this value, the regression line would be y = 0.81x + 2.0 (CPS on y axis against IPS on x axis). The slope and constant both changed (but not significantly) compared to the former regression line (y = 0.87x + 1.5). With the data available in this experiment, the regression line of $PM_{2.5}$ concentrations from the workplace/ambient air falls within the boundary.
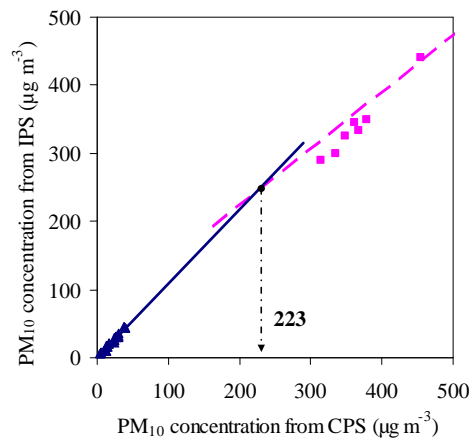

Figure 5. Relationships between PM_{10} concentrations measured with CPS and IPS samplers for values ≤ 100 μg m^{-3} and for values > 100 μg m^{-3} (━: regression line for the range of ≤ 100 μg m^{-3}; - - - : regression line for the range of > 100 μg m^{-3}; ▲ data ≤ 100 μg m^{-3}; ▪ data > 100 μg m^{-3}; note that not all data points > 100 μg m^{-3} are shown).

The $PM_{2.5}$ data from livestock houses drifted from the boundary because of the overloading problem of the IPSs. The regression line was rather flat, with a slope of 0.07 (CPS on y axis against IPS on x axis). It was highly affected by the data collected in the pig and the broiler houses with high $PM_{2.5}$ concentrations (44.9 to 1933.3 µg m^{-3}).
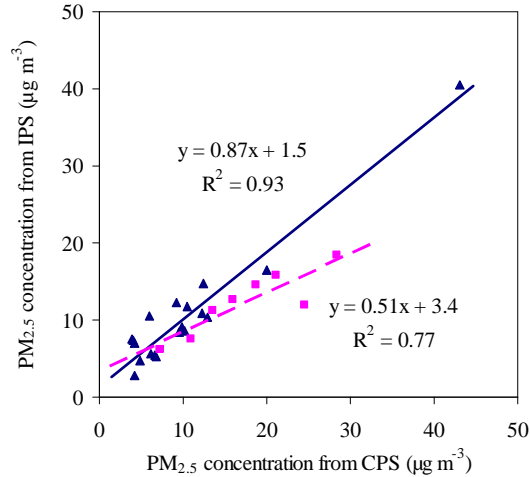

Figure 6. Relationships between $PM_{2.5}$ concentrations measured with CPS and IPS samplers in workplace/ambient air and in a dairy house (▬: regression line, workplace/ambient air; - - - : regression line, dairy house; ▲ data from workplace/ambient air; ■ data from the dairy house).

The $PM_{2.5}$ concentrations measured by the CPSs were quite similar in the workplace/ambient air and on the dairy farm: on average 10.5 µg m^{-3} and 12.3 µg m^{-3}, respectively. It is of interest to examine the data collected in these two environments in more detail (Figure 6). The regression slope with only the data of the dairy farm is 0.51, which is higher than the slope for pig and poultry farms. However, it is still quite different from 1. This indicates that overloading of IPS is a problem on dairy farms, although the $PM_{2.5}$ concentration is similar to the workplace/ambient air. In this case, the particle size distribution probably plays a role, with much of the dust being composed of large particles. The R^2 remained low (0.77). It can be concluded that $PM_{2.5}$-IPS cannot be used on pig, poultry and dairy farms.

CONCLUSIONS

Our study has verified that the current impaction pre-separator sampler (IPS) is vulnerable to overloading in the dusty environment of layer houses and has investigated whether the cyclone pre-separator sampler (CPS) is an alternative for sampling dust in livestock houses. Our key results and conclusions can be summarized as follows:

- PM_{10}-IPS was not overloaded during a 24 h sampling period in a layer house with high dust concentrations (in this study the average PM_{10} concentration was 2 075 µg m^{-3}). $PM_{2.5}$-IPS was overloaded within a 1 h sampling period. It cannot be used as a pre-separator for $PM_{2.5}$ in pig,

poultry and dairy houses. PM_{10}- and $PM_{2.5}$-CPS were not overloaded in the dusty environment of a layer house during a 24 h sampling period.

- PM_{10}-CPS proved to be an equivalent sampler in the PM_{10} concentration range of ≤ 100 µg m^{-3}. The CI_{95} is slightly out of the acceptable boundary and not all plotted concentration points are within the two-sided boundary range for PM_{10} concentrations > 100 µg m^{-3} (all data from livestock houses). Data from the CPS should be corrected with calibration lines in the whole PM_{10} concentration range.

- For workplace/ambient air environments the sampling performance of $PM_{2.5}$-CPS was proved to be comparable to $PM_{2.5}$-IPS.

- For livestock houses, $PM_{2.5}$-CPS is not comparable with $PM_{2.5}$-IPS, because of the overloading of IPS. $PM_{2.5}$-CPS has a clearly superior ability to store the larger particles for a long period of sampling.

Investigation of the Efficiencies of Bioaerosol Samplers for Collecting Aerosolized Bacteria Using a Fluorescent Tracer. I: Effects of Non-sampling Processes on Bacterial Culturability

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ABSTRACT. By sampling aerosolized microorganisms, the efficiency of a bioaerosol sampler can be calculated depending on its ability both to collect microorganisms and to preserve their culturability during a sampling process. However, those culturability losses in the non-sampling processes should not be counted toward the sampling efficiency. Prior to the efficiency assessment, this study was designed to investigate the culturability losses in three non-sampling processes: 1) the tracer uranine induced loss; 2) the loss during aerosolization (pre-sampling process); and 3) the bacteria and uranine recovery in air sample handling procedures for the samples of the Andersen 6-stage impactor and the Airport MD8 (post-sampling process).

The results indicated that uranine had no significant effect on the culturability of *Enterococcus faecalis*, *Escherichia coli*, and *Mycoplasma synoviae* in suspensions (P > 0.05), but negatively affected the culturability of *Campylobacter jejuni* (P = 0.01). The culturability of *E. faecalis*, *E. coli*, and *M. synoviae* was not affected by stresses caused by aerosolization (P > 0.05). Only 29% of *C. jejuni* were still culturable during aerosolization (P = 0.02) . In the air sample handling procedures, the four species of bacteria were recovered without significant losses from the samples of the Andersen impactor, but only 33%-60% uranine was recovered. *E. faecalis*, *E. coli* and *M. synoviae* were recovered without significant losses from the samples of the Airport MD8. More *C. jejuni* was recovered (172%), probably due to multiplication or counting variation. It is suggested that tracer and bacteria should be aerosolized separately when the tracer negatively affects the bacterial culturability. In both pre- and post-sampling processes, losses of bacterial culturability (or multiplication) may occur, which should be taken into account when assessing the efficiencies of bioaerosol samplers.

Keywords. *Bacteria, Sampling, Tracing, Nebulizing, Stress, Bioaerosol.*

INTRODUCTION

Bioaerosol samplers are increasingly being used to measure airborne microorganisms in occupational and home environments to assess bioaerosol exposure. For an accurate measurement of the concentration of microorganisms in the air, the sampling efficiencies of the bioaerosol samplers should be known.

The efficiencies of bioaerosol samplers have been investigated using different methods based on their abilities to retrieve culturable microorganisms from the air (Henningson et al. 1982; Thorne et al. 1992; Lin and Li 1999; Yao and Mainelis 2006; Engelhart et al. 2007). One of these different methods entails the samplers in an isolator sampling the microorganisms that have been aerosolized in suspensions by using a tracer to determine the physical efficiencies and the deposition of bioaerosols. Detailed steps in such an efficiency assessment study include: 1) a pre-sampling process, i.e. aerosolization of microbial suspensions; 2) a sampling process, i.e. collection of bioaerosols with samplers; and 3) a post-sampling process, i.e. air sample handling procedure. Microorganisms may lose culturability in all these steps and due to the addition of tracer to the microbial suspensions, yet only the loss in the sampling process is ascribed to the sampler efficiency. There is a risk that culturability losses caused by these non-sampling processes (tracer-induced loss, loss in the pre-sampling process, and loss in the post-sampling process) may be wrongly attributed to underperforming bioaerosol samplers (Reponen et al. 1997; Li and Lin 2001), resulting in their efficiencies being underestimated. Therefore, the culturability losses in the non-sampling processes should be investigated prior to the efficiency assessment study, and should be excluded when calculating the sampling efficiencies of bioaerosol samplers.

Different microbiological and physical tracers have been used in efficiency assessment and microbial survival studies (Ijaz et al. 1985; Ijaz et al. 1994; Verreault et al. 2008). The main advantages of microbiological tracers, including *Bacillus subtilis* spores and genetic materials of microorganisms (PCR technology), are that they are harmless to microorganisms and their aerodynamic characteristics are similar as those of microorganisms. However, the microbiological tracers might also decay in bioaerosols, rendering themselves as "unsuitable" and "premature" tracers (Wang et al. 2001; Verreault et al. 2008). For this reason, some non-decay physical tracers remain popular. A prerequisite for the application of a physical tracer is that it does not destroy the culturability of microorganisms in bioaerosol suspensions and in aerosol state. Uranine, a physical fluorescent tracer, has been reported to be virus-friendly and is detectable in tiny doses (Ijaz et al. 1985; Ijaz et al. 1985; Auckenthaler et al. 2002). The effect of uranine on bacterial culturability has not been studied extensively, however, and must be assessed for species for which no data are available.

In pre-sampling process, microorganisms may lose culturability due to the shear force produced by the spraying device (Reponen et al. 1997), and the dehydration stress when water evaporates from the

bioaerosols (Marthi et al. 1990). The loss in this process can be assessed by gently collecting the microorganisms during the aerosolization.

During the post-sampling process, microorganisms may be lost depending on the way how the air samples are handled (Tseng and Li 2005). The generally used bioaerosol samplers collect airborne microorganisms either on agar plates, in liquid media, or with gelatin filters. The liquid air samples can be diluted directly and incubated for further identification and quantification without further processing. In some cases, non-liquid air samples need to be transferred into liquid media, but microorganisms may lose culturability during this process. The Andersen impactor, for example, collects bacteria on its agar plates that are usually incubated immediately after air sampling. However, direct incubation is not feasible if the agar plates are overloaded with bacteria (more than 400 colonies per plate), which often occurs when sampling highly contaminated air (Zhao et al. 2011). Furthermore, direct incubation is not applicable when an Andersen impactor is used to collect non-culturable aerosols, e.g. a physical tracer. To overcome these problems, the collected microorganisms and tracer can be transferred from the agar plates into liquid media, for instance by homogenizing microbially-loaded agar with liquid medium (Lundholm 1982) or by rinsing the microorganisms from the agar surface with liquid medium. Similarly, the gelatin filter of the Airport MD8, which is used for filtering the airborne microorganisms, needs to be dissolved in liquid medium after sampling. In order to accurately assess the efficiencies of bioaerosol samplers, the effects of the post-sampling processes on the culturability of microorganisms should be quantified.

Two studies (Part I and Part II) were designed to investigate the sampling efficiencies of four bioaerosol samplers, when collecting four species of bacteria using uranine as a fluorescent tracer, by correcting with the culturability losses in the non-sampling processes. These four samplers were an Andersen 6-stage impactor, an all glass impinger (AGI-30), a high flow rate sampler (OMNI-3000), and an Airport MD8 with a gelatin filter. The four bacterial species were *Enterococcus faecalis*, *Escherichia coli*, *Campylobacter jejuni*, and *Mycoplasma synoviae*. In Part II (Zhao et al. 2011), the focus was on the efficiency assessment. In Part I (this paper), the effects of the non-sampling processes on the culturability of these four species of bacteria were evaluated. The outcomes of Part I are believed to provide valuable correction information for Part II (Zhao et al. 2011). Specifically, this study investigated:

1) the effect of uranine on the culturability of four species of bacteria in broth suspensions, and on the culturability of *E. faecalis* in aerosol state;

2) the effect of aerosolization on the culturability of bacteria nebulized by a spray-head;

3) the effects of air sample handling procedures on the recovery of culturable bacteria and uranine from samples of the Andersen impactor and the Airport MD8.

MATERIALS AND METHODS

Overview of the study design

Figure 1 is an overview of the design of the two studies. Details on the methods are given in section "Uranine test" for the uranine test, in section "Effect of pre-sampling process on bacterial culturability" for pre-sampling effect on bacterial culturability, and in section "Effects of post-sampling processes on recovery of culturable bacteria and uranine" for post-sampling effects on bacterial culturability. The sampling efficiency assessment will be presented in Part II (Zhao et al. 2011).
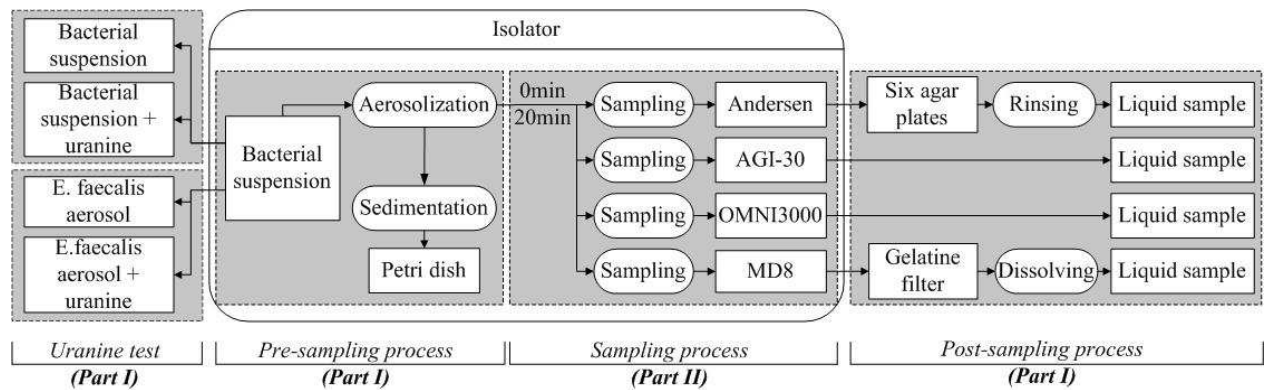
Figure 1. Overview of the study design.

Preparation of bacterial suspensions and bacterial counting

Frozen beads containing *E. faecalis* were rolled on a sheep blood agar plate which was then incubated at 37°C overnight. The colonies of *E. faecalis* were scraped off and transferred into buffered peptone water (BPW, bioTRADING Benelux B.V., Mijdrecht, the Netherlands). To determine the *E. faecalis* concentration, the suspension was diluted in 10-fold steps from 10^{-1} to 10^{-8} in physiological salt solution (bioTRADING Benelux B.V., Mijdrecht, the Netherlands). One tenth ml of each dilution was plated on a sheep blood agar plate, which was incubated at 37°C for 48 h. On plates with 30 – 300 colonies the colonies were counted and the concentration of the bacteria in the original suspension was calculated, following international standard procedure (ISO 7402 1985). The final concentration of *E. faecalis* was approximately 9 $\log_{10}$ colony forming units (CFU) ml^{-1}. The concentrations of the other three bacterial species in suspensions and in the samples were determined similarly. However, the type of agar and the incubation conditions were bacteria-specific.

A suspension of *E. coli* was prepared in the same way as for *E. faecalis*, but with McConkey No. 3 agar (Oxoid, Cambridge, UK). The concentration of *E. coli* in the suspension was approximately 9 $\log_{10}$ CFU ml^{-1}.

C. jejuni was enriched in heart infusion (HI) broth (bioTRADING Benelux B.V., Mijdrecht, the Netherlands) at 41.5°C under micro-aerial conditions (6% oxygen, 10% CO_2 and 84% N_2) for 48 h. *C. jejuni* was cultured on Kamali agar plates (bioTRADING Benelux B.V., Mijdrecht, the Netherlands)

which were then incubated under the same conditions as in the enrichment procedure. The initial concentration of *C. jejuni* was approximately 7 $\log_{10}$ CFU ml^{-1}.

M. synoviae was enriched in Mycoplasma experience (ME) broth (Mycoplasma Experience, Reigate, UK) for two days at 37°C. *M. synoviae* decimal dilution was plated onto an ME agar plate (Mycoplasma Experience, Reigate, UK). The plates were incubated at 37°C under ambient air conditions until visible colonies appeared (around the seventh day of incubation). The initial concentration of *M. synoviae* in suspension was approximately 8 $\log_{10}$ CFU ml^{-1}.

Uranine test

The uranine (CAS#518-47-8, Fisher Scientific, Landsmeer, the Netherlands) was used as the tracer in this study mainly based on its many advantages. The uranine is an inert stable compound compared to microbiological tracers and it is safer than radio-labeling method (Verreault et al. 2008). Also, it can be detected as a very low dose. In this study, the uranine was quantified by a fluorescent detector (HP 1046 A, HP, US) with a detection limit of 0.002 µg ml^{-1}. The excitation and emission wavelengths for uranine are 494 nm and 521 nm, respectively.

To test whether the bacterial culturability may be affected by the uranine, 10 ml suspensions were prepared in duplicate for all four bacterial species. Uranine was added to one of each duplicate suspension, so that the uranine concentration in the suspension was 0.02%; the other suspension served as control. The culturable bacterial concentrations were determined in both suspensions at 0h, 1h, and 2 h after the addition of uranine. These times were chosen because in the efficiency assessment in Part II (Zhao et al. 2011), all bacterial suspensions were to be prepared less than 2 h before aerosolization. The bacterial suspensions were stored at 4°C during the test. The test was done twice.

The effect of uranine on the culturability of *E. faecalis* in aerosol state was investigated by aerosolizing this bacterial species with and without 0.02% uranine. In both cases, 20 ml of suspension was aerosolized in duplicate into a 1.38 m^3 HEPA isolator using Walther Pilot I spray-head (Walther Spritz-und Lackiersysteme Wuppertal, Germany) with a nozzle of 0.5 mm diameter coupled to an air compressor (Mecha Concorde, type 7SAX, 100l, 10 bar/max; SACIM, Verona, Italy) at a pressure of two bars. A schematic drawing of the isolator and samplers was given in Part II (Zhao et al. 2011). The aerosolization period lasted for approximately 1.2 min, and the final temperature was 21-23°C and the relative humidity 90-95%. Immediately after aerosolization, the Andersen impactor, AGI-30, OMNI-3000 and Airport MD8 started sampling for two min. The liquid air samples from the AGI-30 (20 ml BPW with 0.005% silicone antifoam) and OMNI-3000 (10 ml phosphate buffer saline) were directly diluted and analyzed for culturable *E. faecalis* and uranine concentrations. The samples of the Andersen impactor and the Airport MD8 were transferred into liquid media as described in section 2.4, and then analyzed similarly to the other air samples.

The effect of *E. faecalis* on uranine was investigated too, by aerosolizing 20 ml uranine dilutions of 0.02% eight times and sampling the uranine aerosols with the four bioaerosol samplers exactly as described for *E. faecalis*. The measured concentrations of uranine aerosolized from its solutions were compared with those in the two times aerosolization of uranine with *E. faecalis*.

Effect of pre-sampling process on bacterial culturability

The bacterial suspension was aerosolized into the isolator. A Petri dish containing 20 ml of BPW was put on the floor of the isolator and exposed during 1.2 min of aerosolization. The bioaerosols settled gently on the BPW, presumably without stress to the bacteria. The Petri dish was covered immediately after aerosolization. The liquid sample was stirred thoroughly, and sent to the laboratory to determine the concentrations of bacteria and uranine. The *E. faecalis* suspensions with or without uranine were aerosolized in duplicate. *E. coli*, *C. jejuni*, and *M. synoviae* suspensions without uranine were aerosolized in triplicate. Uranine dilutions of 0.02% were aerosolized eight times, and the uranine aerosols were collected by the Petri dish in exactly the same way as the bacteria. The ratio of bacteria/uranine was used to calculate the percentage of bacteria that remained culturable during aerosolization (*A*, %), as shown in Equation 1.

$$A = \frac{C_{petridish,culturable} / C_{suspension,culturable}}{C_{petridish,tracer} / C_{suspension,tracer}} \times 100\% \tag{1}$$

$C_{petridish,culturable}$: culturable bacterial concentration in the sample of the Petri dish (CFU ml^{-1});

$C_{suspension,culturable}$: culturable bacterial concentration in the bacterial suspension (CFU ml^{-1});

$C_{petridish,tracer}$: uranine concentration in the air sample of the Petri dish (µg ml^{-1});

$C_{suspension,tracer}$: uranine concentration in the aerosolized bacterial suspension (µg ml^{-1}).

When *E. faecalis* suspensions with uranine were aerosolized, *A* was calculated with the bacterial and tracer concentrations obtained simultaneously from the suspensions and Petri dish samples. When bacterial suspensions without uranine were aerosolized, the tracer concentrations in the denominator ($C_{petridish,tracer}$ / $C_{suspension,tracer}$) in Equation 1 were obtained from the aerosolization of 0.02% uranine dilution.

Effects of post-sampling processes on recovery of culturable bacteria and uranine

"Recovery", here, is defined as the ratio of the "quantity of culturable bacteria (or uranine) collected from a source" to "its original quantity in the source".

In the efficiency assessment in Part II (Zhao et al. 2011), the Andersen impactor was used with sheep blood agar for sampling aerosolized *E. faecalis*, MacConkey agar for *E. coli*, Charcoal Cefoperazone Deoxycholate (CCD) agar for *C. jejuni*, and ME agar for *M. synoviae*. After sampling, the collected bacteria (and also the uranine) were transferred into liquid media with rinsing method. In

this study, the recoveries of culturable bacteria and uranine obtained by using this method were investigated by plating 0.1 ml of bacterial suspension containing 0.02% uranine onto an agar plate. After five-min exposure in ambient air at room temperature, each agar plate was rinsed three times with 2 ml BPW by carefully scraping the agar surface with a plastic spreader (TS30C, bioTRADING Benelux BV, Mijdrecht, the Netherlands). The five-min exposure was chosen because it would take about five min to transport the air samples to the lab for the efficiency assessment described in Part II (Zhao et al. 2011). The three times rinsed-off liquid media for the same plate were mixed together. The volume of the final sample and the concentrations of culturable bacteria and uranine were measured. In order to ensure that all bacteria and uranine had been rinsed off the plate was rinsed a fourth time with 2 ml BPW. The test was done in duplicate for all bacterial species.

In a Petri dish, a disposable gelatin filter (17528-80-ACD, Sartorius, Nieuwegein, the Netherlands) was topped with 0.5 ml of a bacterial suspension of known concentration containing 0.02% uranine. After five-min exposure in ambient air at room temperature, the gelatin filter was dissolved in 20 ml BPW kept at 37ºC. The liquid sample was pipetted into a small flask containing 30 ml BPW (final sample volume was 50 ml). The test was repeated four times for all bacterial species.

The recoveries of culturable bacteria and uranine from the two post-sampling processes were calculated with Equation 2.

$$R = \frac{C_{liquidsample} \cdot V_{liquidsample}}{C_{suspension} \cdot V_{suspension}} \times 100\% \tag{2}$$

R: recovery of culturable bacteria or uranine (%);

$C_{liquidsample}$: concentration of culturable bacteria (CFU ml^{-1}), or uranine (µg ml^{-1}) in liquid sample;

$V_{liquidsample}$: volume of liquid sample (ml);

$C_{suspension}$: concentration of culturable bacteria (CFU ml^{-1}) or uranine (µg ml^{-1}) in original suspension;

$V_{suspension}$: volume of original suspension added onto the agar plate of Andersen impactor (0.1 ml), or onto the gelatin filter of Airport MD8 (0.5 ml).

Data analysis

To test the effect of uranine on the culturability of bacteria in suspensions, concentrations of bacteria in suspensions were transformed to log scales (log$_{10}$ CFU ml^{-1}) and treated as the response in the following model for each bacterial species (Equation 3).

$$Y_{ijk} = \mu + U_i + T_j + U_i \cdot T_j + e_{ijk} \tag{3}$$

Y_{ijk}: concentration of bacteria in suspension (log$_{10}$ CFU ml^{-1});

μ: overall mean;

U_i: effect of uranine (i = with uranine, without uranine);

T_j: effect of uranine with time (j = 0 h, 1 h, 2 h);

$U_i \cdot T_j$: effect of the interaction between uranine and time;

e_{ijk}: residual error.

Equation 4 was used to analyze the effect of uranine on aerosolized *E. faecalis*. The concentrations of airborne bacteria in the isolator were calculated by dividing the total CFU collected in the air samples by the volumes of air sampled:

$$Y_{ijk} = \mu + U_i + S_j + U_i \cdot S_j + \beta_b + e_{ijk} \tag{4}$$

Y_{ijk}: concentration of airborne *E. faecalis* ($\log_{10}$ CFU m^{-3});

μ: overall mean;

U_i: effect of uranine (i = with uranine, without uranine);

S_j: effect of bioaerosol sampler (j = Andersen impactor, AGI-30, OMNI-3000, Airport MD8);

$U_i \cdot S_j$: effect of the interaction between uranine and bioaerosol sampler;

β_b: concentration of *E. faecalis* in the suspension before aerosolization ($\log_{10}$ CFU ml^{-1});

e_{ijk}: residual error.

Similarly, the effect of *E. faecalis* on uranine in aerosol state was tested (Equation 5). The concentrations of uranine in the air, in µg m^{-3}, were calculated by dividing the total amount of uranine collected in the air samples by the volumes of air sampled.

$$Y_{ijk} = \mu + EF_i + S_j + EF_i \cdot S_j + \beta_u + e_{ijk} \tag{5}$$

Y_{ijk}: concentration of uranine in the air ($\log_{10}$ µg m^{-3});

μ: overall mean;

EF_i: effect of *E. faecalis* (i = with *E. faecalis*, without *E. faecalis*);

S_j: effect of bioaerosol sampler (j = Andersen impactor, AGI-30, OMNI-3000, Airport MD8);

$EF_i \cdot S_j$: effect of the interaction between *E. faecalis* and bioaerosol sampler;

β_u: concentration of uranine in the suspension before aerosolization ($\log_{10}$ µg ml^{-1});

e_{ijk}: residual error.

The difference in percentages of the four species of bacteria that remained culturable during aerosolization was tested with the following equation:

$$Y_{ij} = \mu + B_i + e_{ij} \tag{6}$$

Y_{ij}: bacteria that remained culturable during aerosolization (%);

μ: overall mean;

B_i: species of bacteria (i = *E. faecalis*, *E. coli*, *C. jejuni*, *M. synoviae*);

e_{ij}: residual error.

All the statistical analyses were performed with the SAS program (SAS 9.1.3 Service Pack 4, SAS Institute Inc., Cary, US). The effects of the different factors on the response variables were estimated with the general linear model (GLM) procedure. The percentages of bacteria that remained culturable during aerosolization, and the recoveries of bacteria and uranine from samples of the Andersen impactor and the Airport MD8 were compared against 100% with pooled standard error (se) with the one-sample T-test procedure.

RESULTS

Effect of uranine on the culturability of bacteria in suspensions and on E. faecalis in aerosol state

Table 1. Bacterial culturability in suspensions with or without 0.02% uranine (each value is the mean of two measurements).

Bacteria	Suspension	Concentration ($\pm$ SE[a], $\log_{10}$ CFU ml^{-1})			GLM analysis	
		0 h after adding uranine	**1 h after adding uranine**	**2 h after adding uranine**	**Factor**	**P[b]**
E. faecalis	BPW	8.9^{A,a} ($\pm$ 0.0)	9.0^{A,a} ($\pm$ 0.1)	8.9^{A,a} ($\pm$ 0.0)	Uranine	0.55
	BPW + uranine	9.0^{A,a} ($\pm$ 0.1)	8.8^{A,a} ($\pm$ 0.0)	8.9^{A,a} ($\pm$ 0.1)	Time	0.74
					Interaction	0.14
E. coli	BPW	8.9^{A,a} ($\pm$ 0.1)	9.2^{A,a} ($\pm$ 0.1)	8.9^{A,a} ($\pm$ 0.1)	Uranine	0.69
	BPW + uranine	9.0^{A,a} ($\pm$ 0.1)	9.0^{A,a} ($\pm$ 0.1)	8.9^{A,a} ($\pm$ 0.0)	Time	0.01
					Interaction	0.10
C. jejuni	HI Broth	7.1^{A,a} ($\pm$ 0.3)	6.5^{A,a} ($\pm$ 0.2)	6.8^{A,a} ($\pm$ 0.2)	Uranine	0.01
	HI Broth + uranine	7.0^{A,a} ($\pm$ 0.1)	6.3^{B,a} ($\pm$ 0.1)	5.2^{B,b} ($\pm$ 0.2)	Time	< 0.01
					Interaction	0.01
M. synoviae	ME Broth	8.1^{A,a} ($\pm$ 0.0)	8.2^{A,a} ($\pm$ 0.0)	8.1^{A,a} ($\pm$ 0.0)	Uranine	0.94
	ME Broth + uranine	8.1^{A,a} ($\pm$ 0.1)	8.1^{A,a} ($\pm$ 0.0)	8.2^{A,a} ($\pm$ 0.0)	Time	0.26
					Interaction	0.50

[a] Standard error.

[b] Probability that there is no significant effect of the factor on bacterial concentration.

A,B For each bacterial species, means within a row with a common superscript letter are not significantly different (P > 0.05).

a,b For each bacterial species, means within a column with a common superscript letter are not significantly different (P > 0.05).

Table 1 shows the results of bacterial culturability in suspensions with and without addition of 0.02% uranine. Small differences in the concentrations of *E. faecalis*, *E. coli* and *M. synoviae* were observed in all suspensions at 0h, 1h and 2h. Statistical analysis showed there was neither significant effect of uranine on these three bacterial species, nor was there an interaction effect of uranine over time. However, after the addition of 0.02% uranine there was a marked decrease in *C. jejuni* concentrations: from 7.0 $\log_{10}$ CFU ml^{-1} at 0 h to 5.2 $\log_{10}$ CFU ml^{-1} at 2 h. Statistical analysis showed

that uranine ($P = 0.01$), time ($P < 0.01$) and their interaction ($P = 0.01$) had significant effects on the culturability of *C. jejuni*. There was also a significant effect of time ($P = 0.01$) on *E. coli* culturability ($P = 0.01$).

Table 2. Concentrations of airborne *E. faecalis* aerosolized with and without uranine (each value is the mean of two measurements).

Sampler	Concentration of *E. faecalis* ($\pm$ SE[a], $\log_{10}$ CFU m^{-3})		GLM analysis	
	With uranine	**Without uranine**	**Factor**	**P[b]**
Andersen	9.4[ab] ($\pm$ 0.1)	9.4[ab] ($\pm$ 0.0)	Uranine	0.59
AGI-30	9.2[ab] ($\pm$ 0.2)	9.2[ab] ($\pm$ 0.2)	Bioaerosol sampler	0.02
OMNI-3000	9.0[a] ($\pm$ 0.0)	9.1[a] ($\pm$ 0.0)	Interaction	0.90
Airport MD8	9.5[b] ($\pm$ 0.1)	9.6[b] ($\pm$ 0.0)		

[a] Standard error.

[b] Probability that there is no significant effect of the factor on *E. faecalis* concentration.

a,b Means within a column with a common superscript letter are not significantly different ($P > 0.05$).

Table 2 shows the concentrations of airborne *E. faecalis* collected by four bioaerosol samplers after aerosolization of bacterial suspensions with or without uranine. The size distribution of the *E. faecalis* aerosols is given in Part II (Zhao et al. 2011). Most of the bioaerosols were between 1 and 10 μm (in volume). Using the same bioaerosol sampler, the concentrations of *E. faecalis* were almost identical regardless of whether the bacterial suspensions had been aerosolized with or without uranine ($P = 0.59$). The type of bioaerosol sampler had a significant effect on the concentration of *E. faecalis* ($P = 0.02$), but there was no significant interaction between uranine addition and bioaerosol sampler ($P = 0.90$).

Effect of E. faecalis on airborne uranine

The concentrations of airborne uranine (with or without *E. faecalis*) collected by the four bioaerosol samplers are listed in Table 3. The statistical analysis showed that *E. faecalis* did not interfere with uranine concentrations ($P = 0.96$). The type of bioaerosol sampler was a significant factor on uranine concentration ($P < 0.01$), but no significant effect was found for the interaction between *E. faecalis* and bioaerosol sampler ($P = 0.89$).

Effect of pre-sampling process on bacterial culturability

The percentages of bacteria that remained culturable during aerosolization are listed in Table 4. The GLM analysis showed that aerosolization had a significant effect on the culturability of the bacteria. The percentages of bacteria remaining culturable during aerosolization were 88% for *E. faecalis*, 68% for *E. coli* and 84% for *M. synoviae*. One-sample T-test showed that the culturability of these three bacterial species during aerosolization was not statistically different from 100%. However, only 29% of *C. jejuni* remained culturable and this was significantly lower than 100% ($P = 0.02$).

Table 3. Concentrations of airborne uranine aerosolized with or without *E. faecalis* collected by the bioaerosol samplers 0 min after aerosolization (values of uranine with *E. faecalis* are the means of two measurements, values of uranine without *E. faecalis* are the means of eight measurements).

Sampler	Concentration of uranine ($\pm$ SE[a] $\log_{10}$ µg m^{-3})		Coefficient of variance[b]	GLM analysis	
	With *E. faecalis*	Without *E. faecalis*		Factor	P[c]
Andersen	2.3^a ($\pm$ 0.0)	2.2^a ($\pm$ 0.1)	0.53	*E. faecalis*	0.96
AGI-30	3.1^b ($\pm$ 0.1)	3.0^b ($\pm$ 0.1)	0.13	Bioaerosol sampler	< 0.01
OMNI-3000	3.0^b ($\pm$ 0.1)	2.8^b ($\pm$ 0.1)	0.16	Interaction	0.89
Airport MD8	3.2^b ($\pm$ 0.0)	3.0$^{b,[d]}$ ($\pm$ 0.1)	0.36		

[a] Standard error.

[b] Coefficient of variance = SD/Mean. SD: standard deviation of relative uranine concentrations (ratios of the concentrations in the air and the concentrations in the suspensions for aerosolization); Mean: mean of relative uranine concentrations.

[c] Probability that there is no significant effect of the factor on uranine concentration.

[d] Based on six tests in which the gelatin filter did not become severely clogged.

a,b Means within a column with a common superscript letter are not significantly different (P > 0.05).

Table 4. The percentages of bacteria that remain culturable during aerosolization (each value is the mean of triple measurements).

Bacteria	Percentage ($\pm$ SE[a], %)	P[b]
E. faecalis	88$^{a,[c]}$ ($\pm$ 29)	0.53
E. coli	68^{ab} ($\pm$ 7)	0.10
C. jejuni	29^b ($\pm$ 8)	0.02
M. synoviae	84^a ($\pm$ 4)	0.28
Pooled SE	11$^{[d]}$	

[a] Standard error.

[b] Probability that the bacterial culturability during aerosolization is not different from 100%.

[c] Mean of two measurements with uranine, due to equipment dysfunction.

[d] Pooled se for *E. faecalis* is 13 due to n = 2.

a,b Means within a column with a common superscript letter are not significantly different (P > 0.05).

Effects of post-sampling processes on recovery of culturable bacteria and uranine

Andersen

The results in Table 5 show that the recoveries of bacteria from the agar plates by rinsing three times with BPW after five-min exposure to the ambient air at room temperature were between 87%– 124%. The recoveries did not differ significantly between the bacterial species and did not differ significantly from 100%. The recoveries of uranine from all agar types were significantly less than 100%: the lowest recovery was from CCD agar. Uranine recoveries from CCD agar (33%) and ME agar (48%) were significantly lower than the corresponding bacterial recoveries. The additional

(fourth) rinsing recovered only a small number of bacteria (1 - 2%) but an appreciable amount of uranine (5 - 12%).

Table 5. Recoveries of bacteria and uranine by rinsing the agar plate surfaces of Andersen impactor (each value is the mean of two measurements).

Bacteria	Agar type	Recovery by the first three times of rinsing ($\pm$ SE[a], %)				Recovery by the fourth rinsing ($\pm$ SE, %)	
		Bacteria	P[b]	Uranine	P[b]	Bacteria	Uranine
E. faecalis	Sheep blood agar	107[A,a] ($\pm$ 27)	0.74	60[A,a] ($\pm$ 2)	0.04	1 ($\pm$ 0)	10 ($\pm$ 3)
E. coli	MacConkey no.3 agar	87[A,a] ($\pm$ 13)	0.57	53[A,a] ($\pm$ 1)	0.04	1 ($\pm$ 0)	5 ($\pm$ 5)
C. jejuni	CCD agar	124[A,a] ($\pm$ 6)	0.37	33[B,b] ($\pm$ 3)	0.03	2 ($\pm$ 2)	8[c]
M. synoviae	ME agar	98[A,a] ($\pm$ 9)	0.92	48[B,ab] ($\pm$ 4)	0.03	1 ($\pm$ 0)	12 ($\pm$ 3)
Pooled SE		16		3			

[a] Standard error.

[b] Probability that the recoveries of culturable bacteria or uranine is not significantly different from 100%.

[c] One value was obtained.

[A,B] Means within a row with a common superscript letter are not significantly different (P > 0.05).

[a,b] Means within a column with a common superscript letter are not significantly different (P > 0.05).

Gelatin filter of Airport MD8

Table 6 shows the recoveries of bacteria and uranine after dissolving the gelatin filters, which were loaded with 0.5 ml bacterial suspensions containing 0.02% uranine, in 37°C BPW. The recoveries of bacteria varied, depending on the species. The recoveries of *E. faecalis*, *E. coli*, and *M. synoviae* did not differ significantly from 100%. However, 172% of *C. jejuni* was recovered, which was significantly higher than 100% (P = 0.01). Uranine was recovered without significant loss. The recovery of *E. faecalis* was lower than that of uranine from the same filter. For the other three species, there was no significant difference between bacteria and uranine recoveries.

Table 6. Recoveries of bacteria and uranine from gelatin filters of Airport MD8 (each value is the mean of four measurements).

Bacteria	Recovery ($\pm$ SE[a], %)			
	Bacteria	P[b]	Uranine	P[b]
E. faecalis	63[A,a] ($\pm$ 4)	0.06	95[B,a] ($\pm$ 1)	0.56
E. coli	87[A,a] ($\pm$ 8)	0.47	97[A,a] ($\pm$ 2)	0.71
C. jejuni	172[A,b] ($\pm$ 21)	0.01	113[A,a] ($\pm$ 3)	0.08
M. synoviae	73[A,a] ($\pm$ 6)	0.37	114[A,a] ($\pm$ 7)	0.07
Pooled SE	13[c]		5[d]	

[a] Standard error.

[b] Probability that the bacterial and uranine recoveries is not significantly different from 100% (P > 0.05).

[c] Pooled se for *M. synoviae* is 18 due to n = 2.

[d] Pooled se for uranine in *E. faecalis* and *E. coli* is 6 due to n = 2.

[A,B] Means within a row with a common superscript letter are not significantly different (P > 0.05).

[a,b] Means within a column with a common superscript letter are not significantly different (P > 0.05).

DISCUSSION

Uranine test

The use of tracer enabled the physical efficiency and bioaerosol deposition to be evaluated. It is preferable to aerosolize microbial suspensions in combination with the tracer. This is because, when they are aerosolized separately, the size distributions of microbial aerosols and tracer aerosols might be different, resulting in different aerodynamic characteristics. Aerosolizing a microbial suspension in combination with a tracer requires that the aerosolized microorganisms should remain unaffected by the tracer, whether they are in suspensions or in aerosol state. The results in Tables 1 and 2 clearly show that uranine had no significant effect on the culturability of *E. faecalis* in suspensions and in aerosol state. It is concluded that *E. faecalis* can be aerosolized with addition of 0.02% uranine. The culturability of *E. coli* and *M. synoviae* in suspensions was not affected by the uranine either. The effects of uranine on both bacteria in aerosol state remain to be investigated. In contrast, the culturability of *C. jejuni* in suspension was negatively affected; therefore, this bacterial species cannot be aerosolized in combination with uranine. In Part II (Zhao et al. 2011), we report that the size distributions for bacterial aerosols and uranine aerosols which were sprayed separately were identical. Furthermore, ten aerosolization events of uranine yielded coefficients of variance of 0.13 for uranine concentrations determined with the AGI-30 and of 0.16 for uranine concentrations determined with OMNI-3000. The low coefficients of variance reflected the high reproducibility of the uranine aerosols (Table 3), therefore uranine can be aerosolized separately. The coefficients of variance of the Andersen impactor (0.53) and the Airport MD8 (0.36) were greater, probably because the recovery of uranine by the rinsing method was instable (Andersen) and also because the gelatin filters of the Airport MD8 became clogged at high humidity (90-95%).

Effect of pre-sampling process on bacterial culturability

The bacterial species with the highest mean culturability during aerosolization (88%) and thus low culturability losses was *E. faecalis*. The result is consistent with other studies which have reported low losses of Gram-positive bacteria during aerosolization (Theunissen et al. 1993; Landman et al. 2004). The culturability of the Gram-negative bacteria *E. coli* and *C. jejuni* remained 68% and 29%, respectively. The poor recovery of *C. jejuni* (significantly lower than 100%) is probably because the aerosolization was done in an isolator with an oxygen content similar to that of ambient air, where *C. jejuni* cannot survive well as it is a micro-aerophilic bacterial species (Goodhew et al. 1988). Furthermore, the outer membranes of Gram-negative bacteria are more susceptible to rupture during aerosolization (Hambleton 1970). *M. synoviae,* the bacterial species without cell wall, was not affected by aerosolization stress.

Effects of post-sampling processes on recovery of culturable bacteria and uranine

The Andersen impactor has been widely used for sampling airborne microorganisms and has been recommended as a reference sampler (Brachman et al. 1964). The advantage of the Andersen impactor is that it classifies the collected microorganisms by their aerodynamic sizes, which simulate the particle deposition in the human respiratory tract. A major disadvantage of the Andersen is that agar plates may become overloaded in a heavily contaminated environment. Although the overloading can be mitigated by reducing sampling duration to minutes or even seconds (Predicala et al. 2002; Zhao et al. 2011), such a short sampling duration would diminish the accuracy and the representativeness of the sample (Agranovski et al. 2004). To avoid the overloading problem, we rinsed the colonies from the agar surfaces with a liquid medium, instead of the traditional method (culturing the loaded agar plates directly). The aggregated bacteria in colonies on the agar plates of the Andersen impactor may also be disagglomerated in the final liquid samples. This allows the true number of culturable bacteria to be counted. The results showed that all four species of bacteria could be recovered without significant loss with three rinsing steps. The recoveries of bacteria after an additional rinsing step were low, indicating that the fourth rinsing was unnecessary. The recoveries of uranine were lower than those of bacteria, especially when recovered from CCD agar and ME agar. This might be because uranine binds more strongly with the agar than bacteria, a hypothesis supported by the finding that 5-12% uranine could still be recovered by the fourth rinsing. Furthermore, uranine is very soluble and small, so it might penetrate into the agar with the water, making the rinsing more difficult.

Li and Lin (2001) found decay of *E. coli* increased concomitantly with the storage time of gelatin filters. However, we could not detect the decay for any of the four bacterial species. Li and Lin (2001) used a storage time of 1 h, whereas we exposed all the gelatin filters with bacteria to the ambient air for a maximum of five min. Our results show that as long as the microbially loaded gelatin filter is dissolved and analyzed directly after the exposure, the culturability of the bacteria will remain unaffected. The possible reasons that the recovery of *C. jejuni* was significantly higher than 100% are replication of this bacterial species in the 37°C BPW used for dissolving the gelatin filters, or variations in the counting. Landman et al. (2004) also found more *Mycoplasma* in dissolved filters than originally present. We recovered all the uranine from the gelatin filters.

Our study has confirmed that in pre- and post-sampling processes, bacteria may lose culturability or multiply. When air samples are taken in practice, there is no pre-sampling process, so in an aerosolization lab-scale experiment, the loss of bacteria in this process should be excluded from the efficiency calculation. However, the post-sampling processes are always performed. Therefore when sampling in practice, account must be taken both for the sampling efficiency and for bacterial recovery in the corresponding post-sampling process.

CONCLUSIONS

Carried out prior to assessing the sampling efficiency of bioaerosol samplers, the losses of bacterial culturability in three non-sampling processes (tracer-induced loss, loss in the pre-sampling process, and loss in the post-sampling process) were investigated in this study. We conclude that:

- Uranine affected the culturability of *C. jejuni* and should not be aerosolized together with the suspensions of this bacterial species in efficiency assessment.

- During aerosolization, the culturability of *E. faecalis*, *E. coli* and *M. synoviae* remained unaffected, but the culturability of *C. jejuni* was negatively affected.

- All four species of bacteria were recovered without significant losses from samples of the Andersen impactor by rinsing the agar plates, but uranine was not fully recovered. As long as the microbially loaded gelatin filters of Airport MD8 were dissolved and cultured soon after exposure (five min in this experiment), the culturability of bacteria remained unaffected. The possible reasons more *C. jejuni* were recovered are bacterial multiplication in the warm liquid medium used for dissolving the filter, or counting error.

ACKNOWLEDGEMENT

INVESTIGATION OF THE EFFICIENCIES OF BIOAEROSOL SAMPLERS FOR COLLECTING AEROSOLIZED BACTERIA USING A FLUORESCENT TRACER. II: SAMPLING EFFICIENCY, AND HALF-LIFE TIME

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ABSTRACT. Using uranine as a physical tracer, this study assessed the sampling efficiencies of four bioaerosol samplers (Andersen 6-stage impactor, all glass impinger "AGI-30", OMNI-3000 and Airport MD8 with gelatin filter) for collecting Gram-positive bacteria (*Enterococcus faecalis*), Gram-negative bacteria (*Escherichia coli* and *Campylobacter jejuni*), and bacteria without cell wall (*Mycoplasma synoviae*) which were aerosolized in a HEPA isolator. In addition, the half-life times of these bacteria in aerosols were estimated. The uranine concentrations collected by the samplers were used for calculating the physical efficiencies, and the bacteria/uranine ratios were used for calculating the biological efficiencies.

The results show the Airport MD8 had the highest physical efficiency. Compared with the Airport MD8, the physical efficiencies of the AGI-30 and the OMNI-3000 were 74% and 49%, respectively. A low physical efficiency of the Andersen impactor (18%) was obtained, but it was mainly caused by the incomplete recovery of uranine when handling the air samples, so could not be ascribed to the sampler efficiency. Both the Andersen impactor and the AGI-30 showed high biological efficiencies for all four bacterial species. The biological efficiencies of the OMNI-3000 for *C. jejuni* (1%) and of the Airport MD8 for *E. coli* (38%) and *C. jejuni* (2%) were significantly lower than 100%, indicating that their sampling stresses inactivated the bacterial culturability. The half-life times at 21-23°C temperature and 80-85% relative humidity were 43.3 min for *E. faecalis,* 26.7 min for *M. synoviae*, 21.2 min for *E. coli,* and 4.0 min for *C. jejuni* in the air.

Keywords. Sampler, Microorganism, Survival, Aerial, Aerosol, Fluorescein Sodium, Nebulizing.

INTRODUCTION

Concentrations of airborne microorganisms in occupational and home environments are increasingly being measured for hazard evaluations. Bioaerosol samplers with known sampling efficiencies are required in order to get reliable assessments on the microbial concentrations. Bioaerosol samplers that are commonly used include Andersen 6-stage impactor, all glass impinger (AGI-30) and Airport MD8, while others such as the OMNI-3000 with a high sampling flow rate have been introduced more recently. However, the efficiencies of all the devices mentioned above, when sampling *Enterococcus faecalis*, *Escherichia coli*, *Mycoplasma synoviae* and *Campylobacter jejuni*, have not been established so far.

The sampling efficiency of a bioaerosol sampler includes its physical efficiency and biological efficiency. The physical efficiency describes how well airborne particles are aspirated by the device's inlet and transported to the collection medium, and how well the bioaerosol sampler retains these particles in its medium (Nevalainen et al. 1992; Griffiths and Stewart 1999). If the particles are living organisms, they may be inactivated by sampling stresses, e.g. impaction stress (Stewart et al. 1995), impingement stress (Shipe et al. 1959; Tyler and Shipe 1959; Tyler et al. 1959) and/or dehydration (Li et al. 1999). Therefore, in order to indicate how well a bioaerosol sampler prevents cell damage and maintains the microbial viability during sampling, the concept of biological efficiency has been introduced (Griffiths and Stewart 1999).

The efficiencies of different bioaerosol samplers have been evaluated previously. In some studies the efficiencies of bioaerosol samplers were assessed by operating them side-by-side in an environment with an unknown microbial concentration (Henningson et al. 1982; Thorne et al. 1992; Engelhart et al. 2007; Zhen et al. 2009). This method easily ranks the performances of different samplers; however, it does not reveal whether the amounts of microorganisms collected in the air samples accurately represent the microbial content of the air, nor does it distinguish between the physical efficiency and biological efficiency.

In order to overcome the previously mentioned shortcomings, the efficiencies of bioaerosol samplers can be evaluated in an aerosol experiment, in which a known amount of microorganisms together with a tracer are nebulized in an isolator. In this way, the physical efficiency can be determined by comparing the amount of tracer collected by a bioaerosol sampler with that collected by the reference sampler (a sampler that has high physical efficiency); the biological efficiency is subsequently indicated by the change in the ratio of microorganism/tracer.

The use of any kind of tracer is only possible if it does not influence the culturability of microorganisms both in suspension and in aerosol state. A commonly used tracer is uranine, which was previously found to be virus-friendly (Ijaz et al. 1985; Ijaz et al. 1985; Auckenthaler et al. 2002). In another study, the influences of uranine on the culturability of *E. faecalis*, *E. coli*, *M. synoviae* and *C. jejuni* in suspensions and for *E. faecalis* in aerosol state were assessed (Zhao et al. 2011). The

results showed that uranine did not significantly influence the culturability of mentioned bacteria, in suspensions and for *E. faecalis* in aerosol state. An exception was *C. jejuni*, which was influenced by uranine. Therefore, this bacterial species must be aerosolized separately, which is possible due to the high repeatability of the uranine aerosolization (low coefficient of variance between subsequent uranine aerosolization events) (Zhao et al. 2011).

Bacteria that differ in their biological properties have different resistances to sampling stresses. Airborne Gram-positive bacteria have been found to be more resistant to the impaction stress of an Andersen impactor than Gram-negative bacteria (Stewart et al. 1995), probably because the rigid structure of the cell wall of Gram-positive bacteria protects them against injury during sampling. Airborne *Mycoplasma* without a cell wall appeared more vulnerable to decay than *E. faecalis* (Landman et al. 2004). Until now, the biological efficiencies of bioaerosol samplers based on different sampling principles have not been comprehensively investigated by quantifying the ability to collect Gram-positive bacteria, Gram-negative bacteria and bacteria without cell wall.

Our study aimed to assess the physical and biological efficiencies of four bioaerosol samplers based on different principles (Andersen impactor, AGI-30, OMNI-3000 and Airport MD8), after aerosolizing suspensions of four bacterial species in a HEPA isolator. The bacteria studied were: one Gram-positive bacterial species (*E. faecalis*); two Gram-negative bacterial species (*E. coli*, and a micro-aerophilic bacterial species *C. jejuni*), and one bacterial species without cell wall (*M. synoviae*). The efficiencies were corrected by the losses of bacterial culturability in non-sampling processes, which were presented in Part I (Zhao et al. 2011). Based on these data, the suitable bioaerosol samplers for these bacteria were identified when the bacteria were collected with high physical and biological efficiencies. In addition, the half-life times of these four species of airborne bacteria were determined at 21-23°C and 80-85% relative humidity (RH).

MATERIALS AND METHODS

Bioaerosol samplers

The Andersen impactor (TE-10-800, Pacwill Environmental Ltd., Beamsville, Ontario, Canada) consists of six stages, in each of which a plate with agar is placed under a screen with 400 perforations. The number of perforations is the same for each stage, but at each consecutive stage their diameter is smaller. When taking samples at an air flow of 28.3 l min^{-1}, the air speed through the perforations increases from the first stage to the sixth stage. The particles are impacted and are grouped by their aerodynamic diameters. The largest particles are retained on the plate of the first stage, and the smallest on the plate of the sixth stage.

The AGI-30 (7540, Ace glass Inc., Vineland, US) impinges airborne bacteria into buffered peptone water (BPW, bioTRADING Benelux B.V., Mijdrecht, the Netherlands) with 0.005% silicone antifoam. The air flow of AGI-30 is 12.5 l min^{-1}.

The OMNI-3000 (Evogen Inc., Kansas City, US) operates at a high air flow rate of 300 l min^{-1}. The collection fluid of 10 ml phosphate buffered saline (PBS) is sucked from a cartridge into the contactor, where PBS rotates and is exposed to the incoming air. The aerosols are impinged into PBS. After sampling, PBS containing the collected airborne bacteria is drained back to the cartridge automatically. Bacterial count is subsequently performed on a sample from the cartridge.

The Airport MD8 (Sartorius, Göttingen, Germany) collects bacteria on a gelatin filter (8 cm in diameter, 17528-80-ACD, Sartorius, Göttingen, Germany) by filtration at a sampling flow rate of 30 l min^{-1}. The loaded filter is dissolved in the liquid medium, which is then used to make decimal dilutions and inoculate agar plates for bacterial analysis.

Isolator

A stainless steel negative pressure HEPA isolator (Beyer and Eggelaar, Utrecht, the Netherlands) 1.94 m long, 0.75 m wide, and 0.95 m high was used as the aerosolization space. The climatic conditions were monitored with a temperature and humidity sensor (HygroClip2, ACIN Instrumenten BV, Rijswijk, the Netherlands) installed in the centre of the isolator.

Aerosolization, sampling and sample processing

The bacterial species for aerosolization included *E. faecalis*, *E. coli*, *C. jejuni*, and *M. synoviae*. Their physical sizes, shapes, and categories according to Gram staining identification are tabulated in Table 1. Gram staining technique is used to differentiate the bacteria into two large groups, Gram-positive and Gram-negative, based on their physical properties of cell wall. We chose bacteria from both groups in this study, because they were thought to differ in resistance to sampling stresses (Stewart et al. 1995). The bacterial species without cell wall was also chosen for comparison.

Table 1. Physical sizes, shapes and categories of the bacteria.

Bacteria	Size[a] (µm)	Shape	Category
E. faecalis	1[b]	Sphere	Gram +
E. coli	2 × 0.5	Rod	Gram -
C. jejuni[c]	3 to 5 × 0.5	Spiral	Gram -
M. synoviae	0.3 to 0.8	Sphere	No cell wall

[a] Diameter for spheres and length × width for other shapes.

[b] Roughly estimated.

[c] *C. jejuni* is a micro-aerophilic bacterial species.

The preparation of the bacterial suspensions were described in Part I (Zhao et al. 2011). The only difference was that in this study the concentration of culturable *C. jejuni*, a micro-aerophilic bacterial species, in suspensions was about 9 log$_{10}$ CFU ml^{-1}. *E. faecalis* suspensions were aerosolized in duplicate with or without 0.02% uranine as a physical tracer. *E. coli*, *C. jejuni* and *M. synoviae* were aerosolized in triplicate without uranine. Dilutions with only uranine were aerosolized eight times. In

total, 21 aerosolization events were randomly scheduled in 11 days (a maximum of two aerosolization events per day), see Table 2. The volumes of the bacterial suspensions and uranine solutions for aerosolization were the same (20 ml).

Table 2. Overview of aerosolization of bacterial suspensions and uranine solutions.

Bacteria	With or without uranine	Replication
E. faecalis	With	2
E. faecalis	Without	2
E. coli	Without	3
C. jejuni	Without	3
M. synoviae	Without	3
Uranine	-	8

A Walther Pilot spray-head with a nozzle of 0.5 mm diameter (Walther Spritz-und Lackiersysteme, Wuppertal, Germany) was used for the aerosolization. It was connected to an air compressor (Mecha Concorde type 7SAX, 1001, SACIM, Verona, Italy) set at an air pressure of 2 bar. The aerosol size distribution of the spray-head was characterized by laser diffraction (Mastersizer-S long bed, Malvern Instruments, Malvern, UK); the volume median diameter D (v, 0.5) of the sprayed aerosol near the spray-head was approximately 10 µm. To account for evaporation in this study, the actual size distributions of aerosols collected by the bioaerosol samplers were measured by a portable spectrometer (GRIMM 1.109, GRIMM Aerosol Technik Gmbh & Co. KG, Ainring, Germany). In this experiment, the spray-head was positioned close to the right side wall of the isolator and the bacterial suspensions and uranine solutions were sprayed to the left.
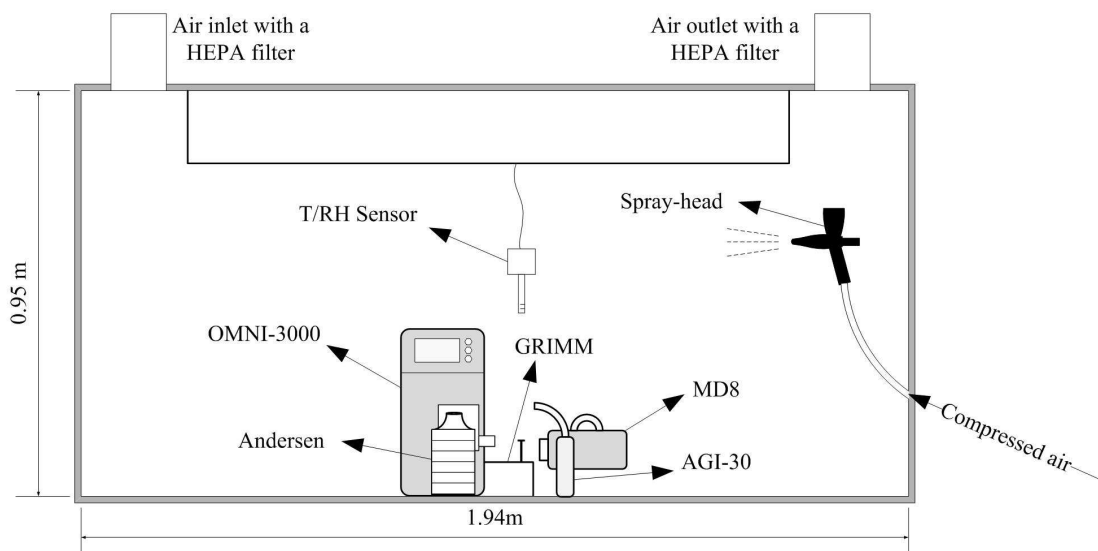

Figure 1. Schematic drawing of the isolator with bioaerosol samplers, T/RH sensor and spray-head.

The schematic drawing of the isolator is made in Figure 1. All bioaerosol samplers were positioned in duplicate inside the isolator before aerosolization. One of each type of bioaerosol sampler sampled

the air directly after aerosolization, and the others took samples 20 min after aerosolization. For both sampling moments, the four bioaerosol samplers and the spectrometer were located in the same area of 0.4 m × 0.4 m in the middle of the isolator. The height of the inlets of the bioaerosol samplers and the spectrometer was approximately 20 cm above the isolator floor. The sampling duration for all bioaerosol samplers was two min. During sampling, the outlet of the isolator was closed, while the inlet of the isolator was kept open to allow air to enter in order to compensate for the negative pressure induced by the bioaerosol samplers. Monitoring revealed that during the experiments the air temperature in the isolator was 21-23°C. The RH was 90-95% immediately after aerosolization, decreased rapidly in the next one to two min, and then remained constant at 80-85%. The isolator was ventilated for at least two hours between two aerosolization events, to clean the inside air from bacteria.

Liquid air samples from the AGI-30 and the OMNI-3000 were processed for bacteriological and uranine analyses without further treatment. Samples from the Andersen impactor and the Airport MD8 were transferred into liquid form in the way described in Part I (Zhao et al. 2011). The analysis of culturable bacteria followed the standard procedure (ISO 7402 1985). Uranine was analyzed by a fluorescent detector (HP 1046 A, HP, US) with a detection limit of 0.002 μg ml^{-1}. All the final concentrations of bacteria and uranine were corrected with the losses of bacterial culturability and uranine in the non-sampling processes obtained in Part I (Zhao et al. 2011).

Data analysis

Physical efficiency

The physical efficiencies at 0 (E_p, %) and 20 min (E_p', %) were calculated with Equations 1 and 2.

$$E_p = \frac{C_{sampler,tracer}}{R_t \cdot \left(\sum C_{MD8,tracer}\right)/n} \times 100\% \tag{1}$$

$$E_p' = \frac{C_{sampler,tracer}'}{R_t \cdot \left(\sum C_{MD8,tracer}'\right)/n} \times 100\% \tag{2}$$

$C_{sampler,tracer}$: airborne uranine concentration sampled with a bioaerosol sampler at 0 min after aerosolization (μg m^{-3});

$C_{sampler,tracer}'$: airborne uranine concentration sampled with a bioaerosol sampler at 20 min after aerosolization (μg m^{-3});

$C_{MD8,tracer}$: airborne uranine concentration sampled with the Airport MD8 at 0 min after aerosolization (μg m^{-3});

$C_{MD8,tracer}'$: airborne uranine concentration sampled with the Airport MD8 at 20 min after aerosolization (μg m^{-3});

n: numbers of uranine measurements with the Airport MD8 ($n = 8$: six were from aerosolization of uranine dilutions and two were from aerosolization of uranine with *E. faecalis*);

R_t: recovery of uranine in post-sampling processes for the Andersen impactor, 33-60%, and the Airport MD8, 95-114%, see Part I (Zhao et al. 2011).

The physical efficiencies at 0 and 20 min were pooled and analyzed with the following model:

$$Y_{ijk} = \mu + S_i + M_j + S_i \cdot M_j + e_{ijk} \tag{3}$$

Y_{ijk}: physical efficiencies of bioaerosol samplers (%);

μ: overall mean;

S_i: effect of bioaerosol sampler (i = Andersen impactor, AGI-30, OMNI-3000, Airport MD8);

M_j: effect of sampling moment (j = 0 min, 20 min);

$S_i \cdot M_j$: interaction effect between bioaerosol sampler and sampling moment;

e_{ijk}: residual error.

Biological efficiency

The biological efficiency (E_b, %) was calculated with the following equation:

$$E_b = \frac{C_{sampler,culturable} \Big/ (\sum C_{suspension,culturable} / m)}{\sum C_{sampler,tracer} \Big/ \sum C_{suspension,tracer}} \times \frac{1}{A} \times 100\% \tag{4}$$

$C_{sampler,culturable}$: airborne culturable bacterial concentration sampled with a bioaerosol sampler, at 0 min after aerosolization (CFU m^{-3}).

$C_{suspension,culturable}$: culturable bacterial concentration in the bacterial suspension (CFU ml^{-1});

$C_{suspension,tracer}$: uranine concentration in the bacterial suspension (µg ml^{-1});

A: percentage of bacteria remained culturable during aerosolization, 88% for *E. faecalis*, 68% for *E. coli*, 29% for *C. jejuni*, and 84% for *M. synoviae*, see Part I (Zhao et al. 2011);

m: number of aerosolization events (four for *E. faecalis*, and three for other bacterial species).

The effects of bioaerosol sampler and bacterial species on the biological efficiency were tested with the following model:

$$Y_{ijk} = \mu + S_i + B_j + S_i \cdot B_j + e_{ijk} \tag{5}$$

Y_{ijk}: biological efficiencies of bioaerosol samplers (%);

μ: overall mean;

S_i: effect of bioaerosol sampler (i = Andersen impactor, AGI-30, OMNI-3000, Airport MD8);

B_j: effect of bacterial species (j = *E. faecalis*, *E. coli*, *C. jejuni*, *M. synoviae*);

$S_i \cdot B_j$: interaction effect between bioaerosol sampler and bacterial species;

e_{ijk}: residual error.

Detection limit

The detection limits of the bioaerosol samplers on the bacteria were calculated with Equation 6.

$$DL = \log\left[\frac{(1/V_{plated}) \cdot V_{liquid}}{R \cdot V_{air} \cdot E_p \cdot E_b}\right] \tag{6}$$

DL: detection limit, $\log_{10}$ CFU m^{-3};

1: 1 CFU;

V_{plated}: volume of decimal dilution plated onto agar plate (ml);

V_{liquid}: volume of sample liquid (ml);

V_{air}: air volume sucked by a bioaerosol sampler in two min sampling (m^3);

R: recovery of bacteria in post-sampling processes for the Andersen impactor (107% for *E. faecalis*, 87% for *E. coli*, 124% for *C. jejuni*, and 98% for *M. synoviae*) and the Airport MD8 (63% for *E. faecalis*, 87% for *E. coli*, 172% for *C. jejuni*, and 73% for *M. synoviae*), see Part I (Zhao et al. 2011).

Half-life time

Half-life is the time taken for the concentration of culturable bacteria in the air to decrease by half. The half-life times of the bacteria ($t_{1/2}$, min) were calculated with Equation 7 (Weesendorp et al. 2008).

$$t_{1/2} = \frac{(\log_{10} 2) \times T}{\log_{10}\left(C_{sampler,culturable} / \alpha C'_{sampler,culturable}\right)} \tag{7}$$

T: time interval ($T = 20$ min);

$C_{sampler,culturable}$: airborne bacterial concentration at 0 min (CFU m^{-3});

$C'_{sampler,culturable}$: airborne bacterial concentration at 20 min (CFU m^{-3});

α: correction factor for physical loss of bacteria.

As the air inlet of the isolator was open during sampling, the bacterial concentration at 20 min was physically diluted due to air passively entering the isolator. Assuming this dilution effect was linear, Weesendorp et al. (2008) proposed calculating *α* using Equation 8:

$$\alpha_v = (V_{isolator} + t \cdot v_e)/V_{isolator} \tag{8}$$

$V_{isolator}$: volume of the isolator (1.38 m^3);

v_e: rate of air extraction by the four bioaerosol samplers per min ($v_e = 0.37$ m^3 min^{-1});

t: sampling duration ($t = 2$ min).

α was also calculated on the basis of the tracer concentrations collected by the Airport MD8, using Equation 9. In this way, *α* took account of both the dilution due to air passively entering and the physical deposition of bioaerosols.

$$\alpha_t = \sum\left(C_{MD8,tracer} / C'_{MD8,tracer}\right)/n \tag{9}$$

The effects of bacterial species and bioaerosol sampler on $\log_{10}(t_{1/2})$ were tested with the following model:

$$Y_{ijk} = \mu + S_i + B_j + S_i \cdot B_j + e_{ijk} \qquad\qquad (10)$$

Y_{ijk}: half-life time of bacteria ($\log_{10}$ min);

μ: overall mean;

S_i: effect of bioaerosol sampler (i = Andersen impactor, AGI-30, OMNI-3000, Airport MD8);

B_j: effect of bacterial species (j = *E. faecalis*, *E. coli*, *C. jejuni*, *M. synoviae*);

$S_i \cdot B_j$: interaction effect between bioaerosol sampler and bacterial species;

e_{ijk}: residual error.

Data were analyzed with the SAS program (SAS 9.1.3 Service Pack 4, SAS Institute Inc., Cary, US). The effects of factors were tested with the general linear model (GLM) procedure; the biological efficiencies were compared to 100% with the one-sample T-test.

RESULTS

Aerosol size distribution

Figure 2 and Figure 3 show the size distributions in terms of aerosol counts and volumes at 0 min and 20 min after aerosolization. The patterns of size distributions of all aerosolization events (for either bacterial suspensions or uranine solution) were very high similar. In count, most of the aerosols had droplet sizes in the range from 0.25 µm to 3.0 µm. In volume, the aerosols in the range from 1.0 µm to 10.0 µm dominated. On average, at 20 min the aerosol count was 70% of that at 0 min, and the aerosol volume had been reduced to 10% of the volume at 0 min.
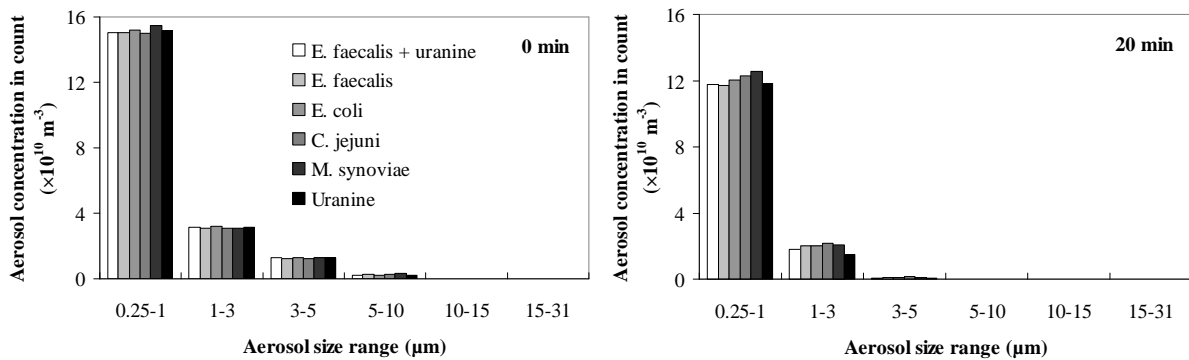
Figure 2. Size distributions in terms of aerosol counts at 0 and 20 min after aerosolization (A value bar of 0 min is the average count of particles from 0 to 2 min, and a value bar of 20 min is the average count of particles from 20 to 22 min measured by the spectrometer at intervals of 6 s).
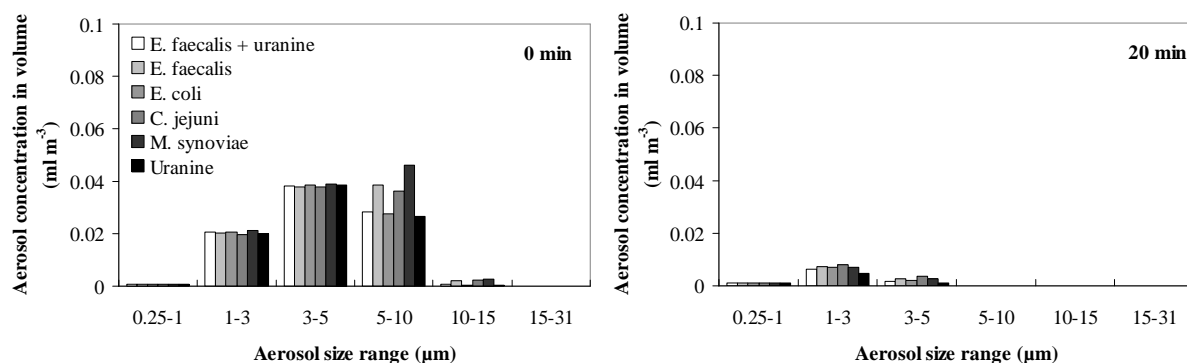
Figure 3. Size distributions in terms of aerosol volumes at 0 and 20 min after aerosolization (A value bar of 0 min is the average volume of particles from 0 to 2 min, and a value bar of 20 min is the average volume of particles from 20 to 22 min measured by the spectrometer at intervals of 6 s).

Physical efficiency

Table 3 shows the physical efficiencies of the four bioaerosol samplers. Because the sampling moment was an insignificant factor ($P = 0.76$), the physical efficiencies at 0 min and 20 min were pooled. The physical efficiencies of the bioaerosol samplers were significantly different ($P < 0.01$). Multiple comparisons showed the efficiency of the OMNI-3000 was lower than those of the AGI-30 and the Airport MD8. Based on the amount of uranine recovered, the Andersen impactor showed the lowest physical efficiency (18%). The physical efficiency of the AGI-30 was similar to that of the Airport MD8. No interaction effect was found between sampler and sampling moment ($P = 0.66$).

Table 3. Physical efficiencies of the four bioaerosol samplers.

Sampler	n[a]	Physical efficiency (±se[b], %)			GLM analysis	
		0 min	20 min	Mean	Factor	P[c]
Andersen	19[d]	_[e]	_[e]	_[e]	Bioaerosol sampler	< 0.01
AGI-30	20	77 ± 11	72 ± 11	74[a] ± 8	Moment	0.76
OMNI-3000	20	58 ± 9	41 ± 9	49[b] ± 6	Interaction	0.66
Airport MD8[f]	16	100 ± 4	100 ± 10	100[a] ± 6		

[a] Number of replications.

[b] Standard error.

[c] Probability that there is no effect of the factor on physical efficiency.

[d] One value is missing due to dysfunction of the fluorescent detector.

[e] Low physical efficiency ($14 ± 1\%$ at 0 min, $23 ± 8\%$ at 20 min, and $18 ± 4\%$ for mean) was obtained due to the low uranine recovery from agar plates.

[f] Physical efficiency of the Airport MD8 was set to 100%.

a,b Means within a column with a common superscript letter are not significantly different ($P > 0.05$).

Biological efficiency

Table 4 shows the biological efficiencies of the four bioaerosol samplers for the four species of bacteria. The statistical analysis showed that biological efficiency was significantly affected by

sampler type (P < 0.01) and bacterial species (P < 0.01). The calculated biological efficiencies of the Andersen impactor were far higher than 100% due to the low recovery of uranine from agar plates by the rinsing method. The AGI-30 showed high biological efficiencies for all bacterial species. The efficiencies of the OMNI-3000 (P = 0.05) and the Airport MD8 (P < 0.01) differed, depending on the bacterial species. The Airport MD8 had biological efficiencies significantly lower than 100% for sampling *E. coli* (38%) and *C. jejune* (2%), which was similar to the biological efficiency of the OMNI-3000 when sampling *C. jejuni* (1%). There was a significant interaction effect between bioaerosol sampler and bacterial species (P < 0.01).

Table 4. Biological efficiencies of the four bioaerosol samplers.

Sampler	Biological efficiency (±se[a], %)				P[b]	GLM analysis	
	E. faecalis	*E.coli*	*C. jejuni*	*M. synoviae*		Factor	P[c]
Andersen	_[d]	_[d]	_[d]	_[d]	-	Bioaerosol sampler	< 0.01
AGI-30	128[A,a] ± 34	65[A,a] ± 28	147[A,a] ± 29	113[A,a] ± 15	0.32	Bacterial species	< 0.01
OMNI-3000	102[A,a] ± 5	107[A,a] ± 51	1[B,b,*] ± 1	67[AB,a] ± 14	0.05	Interaction	< 0.01
Airport MD8	134[A,a] ± 16	38[B,a,*] ± 10	2[B,b,*] ± 1	56[B,a] ± 27	< 0.01		
P[e]	0.58	0.40	< 0.01	0.18			

[a] Standard error.

[b] Probability that there is no difference in the biological efficiencies of a bioaerosol sampler between the four bacterial species.

[c] Probability that there is no effect of the factor on biological efficiency.

[d] Very high biological efficiencies (1519 ± 73% for *E. faecalis*, 205 ± 4% for *E. coli*, 1408 ± 450% for *C. jejuni*, 1458 ± 400% for *M. synoviae*) were obtained due to the low uranine recovery from agar plates.

[e] Probability that there is no difference in the biological efficiencies of AGI-30, OMNI-3000 and Airport MD8 for sampling one of the bacterial species.

[A,B,C] Means within a row with a common superscript letter are not significantly different (P > 0.05).

[a,b,c] Means within a column with a common superscript letter are not significantly different (P > 0.05).

[*] Mean is significantly different from 100% based on one-sample T-test (P < 0.05).

Table 5 shows the airborne bacterial concentrations measured by the four bioaerosol samplers at 0 min after aerosolization. The Andersen impactor and the AGI-30 showed comparable bacterial concentrations for all four species, and they recovered more *C. jejuni* than the other two samplers. The concentrations of *E. faecalis* (P < 0.01) and *C. jejuni* (P = 0.01) sampled with different bioaerosol samplers were significantly different.

Detection limit

Table 6 shows the detection limits of the bioaerosol samplers on airborne *E. faecalis*, *E. coli*, *C. jejuni* and *M. synoviae* for a sampling period of two min. The detection limits of the Andersen impactor, the AGI-30 and the Airport MD8 are comparable for airborne *E. faecalis*, *E. coli* and *M. synoviae*. The OMNI-3000 has low detection limits for these three bacterial species due to its high

flow rate. For airborne *C. jejuni*, the detection limits of the Andersen impactor and the AGI-30 are lower than those of the OMNI-3000 and the Airport MD8.

Table 5. Bacterial concentrations measured by the four bioaerosol samplers at 0 min after aerosolization.

Sampler	Concentration ($\pm se^{[a]}$, $\log_{10}$ CFU m^{-3})			
	E. faecalis	*E.coli*	*C. jejuni*	*M. synoviae*
Andersen	$9.39^{ab} \pm 0.03$	$8.97^{a} \pm 0.02$	$9.10^{a} \pm 0.13$	$8.25^{a} \pm 0.13$
AGI-30	$9.21^{ab} \pm 0.12$	$9.35^{a} \pm 0.17$	$9.27^{a} \pm 0.08$	$7.89^{a} \pm 0.06$
OMNI-3000	$9.05^{a} \pm 0.03$	$9.32^{a} \pm 0.33$	$4.88^{b} \pm 1.36$	$7.37^{a} \pm 0.13$
Airport MD8	$9.55^{b} \pm 0.05$	$9.29^{a} \pm 0.11$	$7.15^{ab} \pm 0.21$	$7.70^{a} \pm 0.23$
P$^{[b]}$	< 0.01	0.48	0.01	0.10

[a] Standard error.

[b] Probability that there is no difference in the measured bacterial concentrations between bioaerosol samplers.

a, b, c Means within a column with a common superscript letter are not significantly different (P > 0.05).

Table 6. Detection limits of the four bioaerosol samplers for airborne bacteria (two min sampling duration).

Sampler	Detection limit ($\log_{10}$ CFU m^{-3})			
	E. faecalis	*E.coli*	*C. jejuni*	*M. synoviae*
Andersen	3.9	3.9	3.7	3.8
AGI-30	3.9	4.2	3.8	4.0
OMNI-3000	2.5	2.5	4.5	2.7
Airport MD8	4.1	4.4	5.4	4.3

Half-life time

Table 7 shows that the half-life times of the airborne bacteria (corrected for dilution due to air passively entering during sampling). *C. jejuni* was not detected by OMNI-3000 at 20 min after aerosolization. Statistical analysis showed that the half-life time was significantly affected by the bacterial species (P < 0.01) and by the type of bioaerosol sampler (P = 0.01), with a profound interaction effect (P < 0.01). There was no significant difference in half-life times between *E. faecalis* and *M. synoviae*, or between *E. coli* and *C. jejuni*. However, the half-life times of *E. faecalis* and *M. synoviae* were significantly longer than those of *E. coli* and *C. jejuni*.

In almost all the cases, the half-life time corrected on the basis of the tracer method (Table 8) was longer than that corrected for dilution due to air passively entering (Table 7), with the exception of the half-life time of *M. synoviae* collected by the OMNI-3000 and the Airport MD8. Similarly as in Table 7, the calculated half-life time of *E. faecalis* was longer than that of the two species of Gram-negative bacteria. The half-life time of *M. synoviae* did not differ from that of *E. coli*, but was significantly longer than that of *C. jejuni*. Two negative half-life time values for *E. faecalis* and five for *M. synoviae* were calculated, because after correction with the tracer, the bacterial concentrations at 20 min were

higher than those at 0 min. These values were excluded from the calculations of the average half-life times.

Table 7. Half-life times of airborne bacteria determined by using bioaerosol samplers to measure the bacteria concentrations at 0 and 20 min after aerosolization and corrected for dilution due to air passively entering during sampling.

Sampler	Half-life time (±se[a], min)				GLM analysis	
	E. faecalis	*E.coli*	*C. jejuni*	*M. synoviae*	Factor	P[b]
Andersen	8.6 (± 0.5)	2.5 (± 0.2)	1.9 (± 0.1)	8.3 (± 0.9)	Bioaerosol sampler	0.01
AGI-30	9.3 (± 2.2)	7.3 (± 2.0)	1.6 (± 0.0)	9.8 (± 1.9)	Bacterial species	< 0.01
OMNI-3000	8.7 (± 1.0)	1.9 (± 0.5)	-[c]	30.6 (± 12.3)	Interaction	< 0.01
Airport MD8	8.4 (± 0.3)	7.0 (± 1.7)	4.5 (± 1.2)	15.8 (± 6.9)		
Mean	8.8[A] (± 0.3)	4.7[B] (± 0.8)	2.7[B] (± 0.5)	16.1[A] (± 3.5)		

[a] Standard error.

[b] Probability that there is no effect of the factor on half-life time.

[c] Half-life time cannot be determined because *C. jejuni* was undetectable by OMNI-3000 at 20 min.

[A,B] Means within a row with a common superscript letter are not significantly different (P > 0.05).

DISCUSSION

The vast majority of bioaerosols originate as wet aerosols. In humans and animals, sneezing, coughing, urine splashes, etc. represent important sources of wet aerosols (Wainwright et al. 2009). The water shells around the microorganisms will partly or totally evaporate during airborne transmission, similar as in this study. Wet aerosols of live attenuated microorganisms are also used for mass vaccination of animals, especially in the poultry industry (Schuijffel et al. 2005; Marangon and Busani 2007). Given their importance, we chose to generate wet bioaerosols in this study to determine the sampling efficiencies of samplers.

In this experiment, uranine was used as a physical tracer to evaluate the physical efficiencies of samplers and deposition of bioaerosols. This requires that the bacteria/uranine ratios in all aerosols of different sizes should be identical (or that the size distributions of bacterial and uranine aerosols are identical). In order to achieve this, all the suspensions were sprayed as wet aerosols with an average diameter of 10 µm, which is much larger than the size of the bacteria (< 5µm). This allows a uniform distribution of bacteria in these wet aerosols. As uranine is small (0.15 µm, measured with a Mastersizer 2000, Malvern Instrument Ltd., Worcestershire, UK) and very soluble (600 g l^{-1}), it would also be uniformly dissolved and distributed in the wet aerosols. Therefore, it can be assumed that wet aerosols with an identical bacteria/uranine ratio were obtained. Water evaporates from the wet aerosols after aerosolization. The particles within the aerosol will coagulate and it is reasonable to believe that they stay coagulated in the air. This conclusion is supported by a study by Ijaz et al. (1987), who sampled the uranine aerosols with an Andersen 6-stage impactor at different moments at three RH levels (30%, 50% and 80%). The results showed neither a difference in the uranine proportion in

larger aerosols (larger than 1 µm) at all RH levels, nor a decreasing uranine proportion in these larger aerosols over time. Furthermore, in our study the evaporation had no significant effect (P = 0.76) on the physical efficiencies of the samplers (Table 3). In Figure 2, there is a peak in counts of small aerosols (0.25 - 1 µm). The main source of these aerosols seems to be the solutes in the suspensions, as the size distributions in the counts for all treatments (i.e. bioaerosols with uranine, bioaerosols without uranine and uranine aerosols) showed a similar pattern. Uranine would also have been present in these small aerosols, however, its amount was negligible because it accounted for only a very small proportion in volume (Figure 3). Therefore, it can be concluded that uranine was a suitable tracer for this study.

The samplers were placed together, but not so closely that they could influence each other. The OMNI-3000 with air flow rate of 300 l min^{-1} was most likely to influence other samplers. The inlet of the OMNI-3000 was placed at least 30 cm from the inlets of the other samplers. Assuming a sucking angle of 90°, the OMNI-3000 could merely generate an air velocity of approximately 0.02 m s^{-1}.

The results of our study showed that the Airport MD8 with gelatin filter had the highest physical efficiency of all the bioaerosol samplers tested. This was according to expectations, as from another laboratory experiment Burton et al. (Burton et al. 2007) reported that gelatin filters had the highest physical efficiency (over 93% for 0.08 µm particles). Both our data and the work by Burton et al. (2007) prompted us to choose the Airport MD8 as the reference for determining the physical efficiencies of the other samplers. A study by Terzieva et al. (1996) showed that the inlet efficiency and collection efficiency of the AGI-30 were approximately 100% for particles in the size range of 0.7 to 1.0 µm. Grinshpun et al. (1997) reported a collection efficiency of 80-90%. Compared to these figures, our result showed a slightly lower physical efficiency (a combination of inlet and collection efficiency) of 74% for the AGI-30, which, however, did not significantly differ from that for the reference sampler (Airport MD8). According to the uranine data, the physical efficiency of the Andersen impactor was very low, 18%, even after the correction for uranine recovery during the agar plate rinsing process. This might be explained by the fact that the uranine particles were probably impacted at a high velocity during air sampling, forcing them into the agar and impairing their release at rinsing (Stewart et al. 1995). As can be deduced from Table 5, the Andersen impactor sampled similar bacterial concentrations as the AGI-30. Moreover, previous studies showed that the 50% cut-off diameter of the Andersen impactor is as low as 0.65 µm (Andersen 1958). It is therefore probable that its actual physical efficiency is much higher, but due to the low recovery of uranine from the agar plates the calculated physical efficiency appeared low. The physical efficiency of OMNI-3000 was 49%, which was significantly lower than those of the Airport MD8 and the AGI-30. Kesavan and Schepers (2006) reported that the physical efficiency of OMNI-3000 showed an inversed "U" shape depending on the particle sizes. For 0.5 (solid particle), 1 (solid particle), 2.9 (oil aerosol), 5.8 (oil aerosol) and 8 µm (oil aerosol) aerosols these authors found efficiencies of 36%, 43%, 69%, 49%, and 29%, respectively. When these values are combined with the volume size distribution in Fig 2, in our

experiment 53% of the aerosols are expected to be collected by OMNI-3000 at 0 min and 58% at 20 min. Both percentages are close to the efficiency that we found.

The biological efficiencies of the Andersen impactor (Table 4) were higher than 100% due to the low recovery of uranine from agar plates. In terms of the bacterial concentrations collected, the Andersen impactor proved to perform well when sampling all four bacterial species (Table 5), which means both its physical efficiency and biological efficiencies were high. Decreasing efficiency of impactors has been reported with increasing sampling duration (Mainelis and Tabayoyong 2010). The sampling duration of Andersen impactor in our experiment was short - two min – and the biological efficiencies were not compromised. The biological efficiencies of the AGI-30 were high for all four bacterial species. Prior to our experiment, the biological efficiency of the OMNI-3000 had not been investigated. This bioaerosol sampler had high biological efficiencies when sampling airborne *E. faecalis*, *E. coli* and *M. synoviae*. However, only 1% of *C. jejuni* survived during sampling, probably due to the cell structure being damaged by the high air flow rate. The biological efficiencies of the Airport MD8 for *E. faecalis* and *M. synoviae* were not significantly different from 100%. Its biological efficiencies for sampling *E. coli* and *C. jejuni* were low (38% and 2%, respectively) and attributed to loss of culturability, probably by dehydration (Cox 1989; Li et al. 1999).

The biological efficiency may reflect the resistance of airborne bacteria to sampling stresses. It has been shown in previous studies that airborne Gram-positive bacteria are more resistant to sampling stresses than Gram-negative bacteria and bacteria without cell wall (Stewart et al. 1995; Landman et al. 2004). In this study *E. faecalis*, a Gram-positive bacterial species, retained its culturability during sampling by all bioaerosol samplers. The lower biological efficiencies for the Gram-negative bacteria (*E. coli* and *C. jejuni*) indicated that they were less resistant to sampling stresses compared to the Gram-positive one. This difference can be partly explained by the fact that the cell wall of the Gram-negative bacteria are not as rigid as that of the Gram-positive ones (Stewart et al. 1995). If the theory that the bacterial cell wall is the only protection to sampling stress, one may expect a bacterial species without cell wall is more readily to be inactivated during sampling compared to both Gram-positive and Gram-negative bacteria. However, the bacterial species without cell wall in our study, *M. synoviae*, preserved its culturability comparably to those with cell wall, and sometimes even better than the two Gram-negative ones. This finding indicates, besides the cell wall, other factors may affect the resistance of bacteria to sampling stresses, for instance, ambient climate condition (temperature, humidity, oxygen level etc), composition of aerosol suspension for microorganisms, and composition of collection liquid medium (Benbough 1971).

If the efficiencies of the bioaerosol samplers are known, their detection limits for bacteria can be calculated by assuming a minimum concentration of 1 CFU in 0.1 ml of undiluted liquid collection medium, or agar plate rinsing fluid, or gelatin filter solution medium (Table 6). The physical and biological efficiencies of the Andersen impactor are set at 100%. It appears that the Andersen impactor and the AGI-30 have similar detection limits: about 4 $\log_{10}$ CFU m^{-3}, for all four bacterial species. The

detection limits of the Airport MD8 for *E. faecalis*, *E. coli*, and *M. synoviae* are similar to the Andersen impactor and the AGI-30, but the detection limit of *C. jejuni* is higher (5.4 log$_{10}$ CFU m^{-3}). Due to its high air flow rate, the OMNI-3000 can detect concentrations of *E. faecalis*, *E. coli*, and *M. synoviae* as low as 2.5-2.7 log$_{10}$ CFU m^{-3}. However, the high air flow rate does not benefit the sampling of *C. jejuni*. The high detection limits of the Airport MD8 and the OMNI-3000 for sampling *C. jejuni* are mainly due to their low biological efficiencies, which are caused by the inactivation of viable bacterial during sampling.

Table 8. Half-life times of airborne bacteria determined by using bioaerosol samplers to measure the bacteria concentrations at 0 and 20 min after aerosolization and corrected both for dilution due to air passively entering and for bioaerosol deposition and impaction (with tracer method).

Sampler	Half-life time (±se[a], min)				GLM analysis	
	E. faecalis	*E.coli*	*C. jejuni*	*M. synoviae*	Factor	P[b]
Andersen	35.9 (± 9.2)	3.2 (± 0.2)	2.2 (± 0.1)	33.0 (± 10.9)	Bioaerosol sampler	0.27
AGI-30	22.3[c] (± 8.8)	47.0 (± 35.5)	1.8 (± 0.0)	72.1[c] (± 59.5)	Bacterial species	< 0.01
OMNI-3000	77.2 (± 50.9)	2.4 (± 0.7)	_[d]	13.4[c]	Interaction	0.04
Airport MD8	29.2[c] (± 3.6)	32.2 (± 20.7)	8.7 (± 4.0)	12.1[c]		
Mean	43.3[A] (± 14.7)	21.2[B,C] (± 10.6)	4.0[B] (± 1.3)	26.7[A,C] (± 16.5)		

[a] Standard error.

[b] Probability that there is no effect of the factor on half-life time.

[c] Negative half-life time values (caused by a higher bacteria concentration at 20 min after correction compared to that of 0 min) were not included in the calculation of the mean.

[d] Half-life time cannot be determined because *C. jejuni* was undetectable by OMNI-3000 at 20 min.

[A,B,C] Means within a row with a common superscript letter are not significantly different (P > 0.05).

The half-life time of the airborne bacteria at 21-23°C temperature and 80-85% RH was calculated by assuming that the physical efficiencies and biological efficiencies of bioaerosol samplers were the same at 0 min and 20 min (Table 8). Of the four bacterial species tested, airborne *E. faecalis* and *M. synoviae* survived the best while *C. jejuni* survived the least. The half-life time of *E. faecalis* (8.8 min) was similar to, or lower than that reported in previous aerosolization studies (Landman and van Eck 2001; Landman et al. 2004). Using the gelatin filter, *M. synoviae* could not be detected 25 min after aerosolization by other investigators (Landman et al. 2004). In our study, the second air sampling occurred 5 min earlier, i.e. at 20 min after aerosol generation, resulting in *M. synoviae* recoveries by all bioaerosol samplers and a measurable half-life time of 16.1 min. The difference may be caused by different climate conditions and droplet sizes (Marthi et al. 1990). In addition, Benbough (1971) reported that microbial survival was affected by the composition of the aerosolized suspension. In our study, *M. synoviae* was aerosolized in ME broth, but in the study by Landman et al. (2004) it was aerosolized in 1:1 ME broth and BPW.

The survival of airborne *E. coli* has been widely investigated (Cox 1968; Cox 1970; Wathes et al. 1986) and found to be affected by climate conditions, oxygen content in the air and composition of the

aerosolized suspension. Wathes et al. (1986) found a half-life time of airborne *E. coli* of 83 min at 15°C and of 14 min at 30°C with RH higher than 50%. When *E. coli* was aerosolized in three different liquid media at 26.5°C and 80% RH (Cox 1966), its survival 15 min after aerosolization ranged from 14% to 84%, corresponding to a half-life time from 5.3 to 59.6 min. We found a shorter half-life time of *E. coli* (4.7 min) at 21-23°C and 80-85% RH.

The survival of *C. jejuni* in aerosol state was studied here for the first time. The half-life time of airborne *C. jejuni* (2.7 min) was shorter than the other three bacterial species, which is not surprising because *C. jejuni* is a strictly micro-aerophilic bacterial species.

Inevitably, particles are lost by sedimentation and impaction during the 20 min between the first and second air sampling after aerosol generation. Therefore, besides correcting for the volume of air allowed to enter the isolator during air sampling, corrections must be made for the above mentioned losses. This was done by calculating the half-life time based on the tracer concentrations. By doing so, the correction factor (α) increased from 1.5 to 5.0 resulting in higher half-life times for all four bacterial species. In seven instances (two for *E. faecalis* and five for *M. synoviae*), the half-life time was infinite. These values were obtained because the bacterial concentrations at 20 min after correction exceeded the concentrations at 0 min, presumably because of variations in the counting technique.

CONCLUSIONS

The physical and biological efficiencies of four bioaerosol samplers for collecting *E. faecalis*, *E. coli*, *C. jejuni* and *M. synoviae* were determined using uranine as a physical fluorescent tracer. From these efficiency data we conclude that the Andersen impactor and the AGI-30 are suitable for sampling all four species of airborne bacteria, because of their abilities to efficiently collect and retain bacteria in the air samples and preserve their culturability during sampling. Although the physical efficiency of the OMNI-3000 is lower than the other samplers, it still can be used for sampling airborne bacteria in environments with low bacterial concentrations, because its high air flow rate gives low detection limits. The OMNI-3000 is not suitable for sampling *C. jejuni*, the culturability of which can be profoundly inactivated during sampling. The Airport MD8 is suitable for sampling *E. faecalis* and *M. synoviae*, but is less suitable for sampling *E. coli* and *C. jejuni*. The method of efficiency assessment described in this paper and in Part I (Zhao et al. 2011) can be applied to evaluate other bioaerosol samplers for collecting other microorganisms. The outcomes are valuable for the selection of suitable sampling techniques for airborne microorganisms in practices.

The half-life times of the four species of aerosolized bacteria were determined, either by correcting for dilution due to air passively entering the isolator during sampling, or from the tracer concentrations. The latter correction, the tracer method, produces more accurate half-life time as it also takes physical deposition and impaction into account.

EVALUATION OF THE EFFICIENCIES OF FOUR BIOAEROSOL SAMPLERS FOR COLLECTING AEROSOLIZED GUMBORO VACCINE VIRUS

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ABSTRACT. This study evaluated the sampling efficiencies of four samplers (Andersen six-stage impactor, all glass impinger "AGI-30", OMNI-3000 and MD8 airscan with gelatin filter) for collecting Gumboro vaccine virus, which was aerosolized from suspensions combined with a fluorescent tracer to determine the physical efficiency and the aerosol deposition. Losses of viral infectivity in three non-sampling processes - tracer-induced loss, loss during aerosolization and loss in air sample handling procedure - were determined and used as correction factors when calculating the sampling efficiencies. Half-life time of airborne Gumboro vaccine virus was also investigated.

The results showed that an addition of 0.1% uranine (the tracer) did not induce infectivity loss of Gumboro vaccine virus. The loss of viral infectivity during aerosolization was profound, only 5% of virus remained infective after this process. In the sample handling procedures, the loss of infective virus increased further when the samples of the Andersen impactor were stored for a longer period. However, no loss of virus was noticed in the sample handling procedure for the MD8, as long as its gelatin filters were dissolved within 60 min after loaded with virus. The physical and biological efficiencies of the Andersen impactor, the AGI-30 and the MD8 were not significantly different from 100% for collecting the aerosolized virus. However, both physical efficiency (62%) and biological efficiency (23%) of the OMNI-3000 were significantly lower than 100%. The half-life time of the Gumboro vaccine virus was 11.9 min at 20°C and 70% relative humidity.

It is concluded that viral infectivity losses in non-sampling processes may cause underestimation of the sampler efficiencies when these losses are not excluded from the efficiency calculation. The Andersen impactor, the AGI-30 and the MD8 have both high physical and biological efficiencies for collecting Gumboro vaccine virus. Despise its relatively low sampling efficiency, the OMNI-3000 is suitable to be used in environments with low viral concentrations because of its high flow rate giving a low detection limit.

Keywords. *Aerial, Fluorescein Sodium, Nebulizing, Stress, Infectious bursal disease virus.*

INTRODUCTION

Airborne transmission of viruses has been epidemiologically implied to be responsible for the transmission of several viral diseases, e.g. foot-and-mouth disease (Gloster et al. 2003; Mikkelsen et al. 2003; Gloster and Alexandersen 2004), Newcastle disease (Hugh Jones et al. 1973) and porcine reproductive and respiratory syndrome disease (Kristensen et al. 2004). Gumboro disease (infectious bursal disease) is recently suspected to be also airborne transmittable in poultry. For bio-security assessments, efficient bioaerosol samplers with low detection limits are required to accurately measure airborne viruses like the Gumboro virus.

The commonly used bioaerosol samplers include Andersen six-stage impactor which works based on the impaction principle, all glass impinger (AGI-30) which works based on the impingement principle, and MD8 using a gelatin filter (MD8) for sampling based on the filtration principle. The flow rates of these samplers are relatively low, resulting in high detection limits. For sampling at low microbial concentration conditions, some high flow rate samplers were developed, e.g. OMNI-3000 with a flow rate of 300 l min^{-1}. In general, it is assumed that none of the samplers can recover all the microorganisms from the sampled air (Eduard and Heederik 1998; Li et al. 1999; Dart and Thornburg 2008). This is because, physically, a sampler is imperfect in aspirating the airborne microorganisms into the inlets, transport them in the airstreams, and retaining them in the collection mediums; biologically, microorganisms may lose viability due to sampling stresses, which have been reported to be caused, for instance, by impaction when microorganisms are collected by the Andersen impactor (Stewart et al. 1995), and by dehydration when they are collected by a filter (Lin and Li 1999). Besides the sampling principle as a factor, the physical efficiency of a bioaerosol sampler depends on the size of airborne particles as it influences the aspiration (through the sampler inlet), transportation (in the air stream) and retention (in the collection medium) of particles in a sampling process, yet the biological efficiency depends on the microbial resistance to the sampling stress.

Previous studies investigated the sampling efficiencies of samplers by operating them in environments of unknown microbial concentrations in the air (Henningson et al. 1982; Thorne et al. 1992; Engelhart et al. 2007). This allows to compare the relative amounts of collected microorganisms between sampler types, but not linking them to the microbial concentrations in the air. Consequently, the real concentrations of microorganisms in the air can never be obtained with the samplers whose efficiencies were evaluated in this way. To overcome the above mentioned problem, efficiencies of bioaerosol samplers were studied in environments of known microbial concentrations which were created by aerosolizing specified amounts of microorganisms from suspensions (Thompson et al. 1994; Zhao et al. 2011; Zhao et al. 2011). By doing this, the correlation between the collected amount of microorganisms by the samplers and their concentrations in the air can be established. The obtained efficiencies, therefore, can be used either as a standard for selecting suitable samplers or as correction factors in practical measurements.

For evaluating physical efficiencies in an aforementioned experiment by aerosolization, an optical particle counter was applied to measure the bioaerosol concentrations upstream and downstream of a sampler (Mainelis et al. 2002). However, the optical counter can only provide a good estimation when the number of microorganisms in aerosols in each size range is known, which is generally difficult. A more handy way is to aerosolize the microbial suspension with an inert tracer. The physical efficiency of a bioaerosol sampler can be calculated by comparing the amount of tracer collected to a reference sampler (with very high physical efficiency), and the biological efficiency is calculated by the changes in microorganism/tracer ratios. It is important to assure that the applied tracer does not affect the microbial survival. Among the tracers, uranine (Fluorescein Sodium) was reported to be harmless to some viruses and has been widely used in microbial survival studies as a tracer to indicate bioaerosol deposition (Ijaz et al. 1987). However, the effect of uranine on the Gumboro virus is not clear.

In the process of bioaerosol generation, microorganisms suffer stresses and may be inactivated during aerosolization (Griffiths et al. 1996; Reponen et al. 1997). The loss of viable microorganisms in this process creates an environment, in which the efficiencies of samplers are evaluated, with airborne microbial concentration lower than expected. After sampling, microorganisms may also lose viability due to the handling of the air samples. For instance, the time between sampling and the analysis of air samples decrease the recovery of microorganisms from liquid mediums and on gelatin filers (Li and Lin 2001). The losses in microbial viability, both during aerosolization and in the air sample handling process, are not ascribed to the sampler inefficiency, therefore should be investigated and excluded from the efficiency calculation.

The objective of this study was to investigate the sampling efficiencies (both physical and biological) of the Andersen impactor, the AGI-30, the OMNI-3000 and the MD8 with gelatin filter for collecting aerosolized Gumboro virus in a HEPA filtered isolator, by excluding the losses of viral infectivity in three non-sampling processes (tracer-induced, during aerosolization, and in air sample handling procedures). Wet aerosols were used in this study because this model represents best the mass vaccination of animals, especially in the poultry industry (Schuijffel et al. 2005; Marangon and Busani 2007), and also represents the aerosols originated from coughing, sneezing and urine splashing in human beings and animals. The half-life time of Gumboro virus in the air was also investigated with these samplers at 20°C of temperature and 70% relative humidity (RH). For safety considerations, a vaccine strain was used instead of a verogenic Gumboro virus.

MATERIALS AND METHODS

General experimental set-up

Figure 1 shows the overview of the experimental set-up. For investigating the tracer-induced loss of viral infectivity, the amounts of infective virus in the air samples obtained by aerosolization of viral suspensions with and without tracer addition were compared (see "Tracer-induced loss" section). The

loss during aerosolization was assessed by comparing the virus/tracer ratio in the sample of a Petri dish to the virus/tracer ratio in the viral suspension (see "Loss during aerosolization" section). The Petri dish contained liquid medium and was exposed to the air during aerosolization for collecting deposited aerosols. The loss in air sample handling procedures was determined prior to the aerosolization events (see "Loss in air sample handling procedure" section). The efficiency of a sampler was calculated based on the amount of virus in the aerosol suspension and in the final liquid sample, by excluding the losses in non-sampling processes (see "Sampling efficiency" section). The half-life time of airborne virus was determined by comparing the amounts of infective virus in the air samples at 0 min and those at 20 min after aerosolization (see "Half-life time" section).
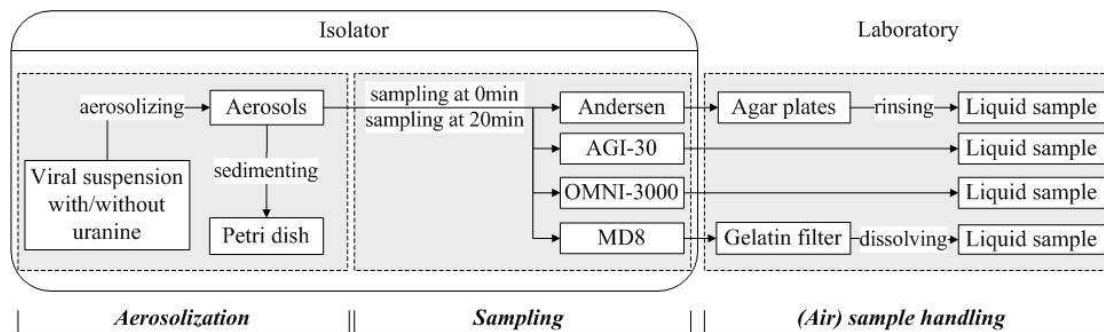
Figure 1. Overview of the experimental set-up showing the three main processes involved.

Viral suspension, viral titration, and uranine analysis

Gumboro vaccine virus (Gallivac IBD, L258577, expiry date Nov-17-2010) was provided by Merial B.V. Velserbroek in the Netherlands. One vial contained approximately 7 $\log_{10}$ 50% egg infectious dose (EID_{50}). Vaccine virus of each of the eight vials was diluted in 20 ml Hanks' Balanced Salt Solution (HBSS, GIBCO, Breda, the Netherlands), which was either with or without 0.1% uranine addition (CAS#518-47-8, Fisher Scientific, Landsmeer, the Netherlands).

The concentration of infective virus in a suspension was determined with an egg embryonic death test. The suspension was firstly decimal diluted (10^{-1}, 10^{-2}…). A volume of 0.5 ml of each decimal dilution was injected into allantoic cavity of five 9-day-old specific pathogen free (SPF) embryonated eggs. The inoculated eggs were incubated at 37°C for 7 days, and the viral concentration was calculated based on death of the embryos and specific abnormalities of the living embryos using the formula of Spearman and Karber (Spearman 1908). The final viral concentrations in the viral suspensions for aerosolization were approximate 6 to 7 $\log_{10}$ EID_{50} ml^{-1}. The viral concentrations in all air samples were determined in the same way. The airborne viral concentrations, ($\log_{10}$) EID_{50} m^{-3}, were obtained by dividing the amounts of collected virus by the air volumes that were sucked through the bioaerosol samplers. The uranine concentrations were analyzed by a fluorescent detector (HP 1046 A, HP, US). The detection limit of the fluorescent detector was 0.002 µg ml^{-1}. The excitation and emission wavelengths for uranine were 494 nm and 521 nm, respectively.

Bioaerosol samplers

The four bioaerosol samplers investigated were Andersen impactor (TE-10-800, Pacwill Environmental Ltd. Canada), AGI-30 (7540, Ace glass Inc., US), OMNI-3000 (Sceptor Industries Inc., US) and MD8 (airscan, Sartorius, Goettingen, Germany) with gelatin filter.

The Andersen impactor (1958) collects microorganisms and differentiates them in different stages according to their particle sizes. From the first stage to the sixth stage, particles with sizes of > 7.1 µm, 4.7-7 µm, 3.3-4.7 µm, 2.1-3.3 µm, 1.1-2.1 µm and 0.65-1.1 µm are respectively collected, when the Andersen impactor is operated with an airflow rate of 28.3 l min^{-1}. In this experiment, plates with Mycoplasma Experience (ME) agar (Mycoplasma Experience, Reigate, UK) were used in the Andersen impactor as the medium for collection. The collected vaccine virus on the agar plates was rinsed with HBSS as described in "Loss of viral infectivity and uranine in air sample handling procedures".

The AGI-30 impinges airborne microorganisms into 20 ml liquid medium at an air flow of 12.5 l min^{-1}. In this experiment, HBSS with 0.005% Silicone antifoam (85390, Sigma-Aldrich Inc., the Netherlands) was used as collection medium for the Gumboro vaccine virus.

The OMNI-3000 is operated at a high airflow rate of 300 l min^{-1}. When sampling, the collection fluid of 10 ml Phosphate Buffered Saline (PBS) is sucked from a cartridge into a contactor where PBS rotates and contacts with the incoming air. The microorganisms in the incoming air are collected in the PBS. The PBS containing microorganisms is drained back to the cartridge automatically after sampling.

The MD8 collects microorganisms on a gelatin filter (17528-80-ACD, Sartorius, Germany) by air filtration. In this study, the viruses-loaded filter was dissolved in 20 ml 37°C HBSS medium after sampling as described in "Loss of viral infectivity and uranine in air sample handling procedures".

Isolator

A stainless steel isolator (Beyer and Eggelaar, Utrecht, the Netherlands) of 1.38 m^3 was used for aerosolization. HEPA filters were installed at both inlet and outlet. A temperature and humidity sensor (HygroClip2, ACIN Instrumenten BV, Rijswijk, NL) was installed in the middle of the isolator.

Aerosolization and air sampling

A Walther Pilot spray-head (Walther Spritz- und Lackiersysteme, Wuppertal, DE) connected to an air compressor (Mecha Concorde type 7SAX, 1001, SACIM, Verona, IT) was used to aerosolize 20 ml of viral suspension at each time. The duration of aerosolization was about 1.2 min with an air pressure of 2 bars.

The efficiency of a sampler is particle size-dependent (Nevalainen et al. 1992); therefore, it is important to know the size distribution of the aerosols that are to be sampled. The aerosol size distribution of the spray-head was characterized by laser diffraction (Mastersizer-S long bed, Malvern

Instruments, Malvern, UK); the volume median diameter D (v, 0.5) of the sprayed aerosol near the spray-head was approximately 10 µm. To account for evaporation in this study, a spectrometer (Model 1.109, Grimm Aerosol Technik GmbH & Co. KG, Ainring, DE) was used to measure the aerosol size distribution near the samplers during air sampling. The viral suspensions were aerosolized eight times: five times with 0.1% uranine addition and three times without.

Each type of the four bioaerosol samplers was pre-installed in duplicate in the middle of the isolator. Four of them were installed close to each other in an area of 0.4 m × 0.4 m and took samples at 0 min after aerosolization. After the sampling, these samplers were replaced by the other four samplers, which took air samples at 20 min after aerosolization. The sampling duration was 2 min for all four bioaerosol samplers and the two measurement moments.

The inlet of the isolator was kept open during sampling to allow filtered air entering in order to compensate for the negative pressure in the isolator induced by the bioaerosol samplers. The isolator was ventilated (70 m^3 h^{-1}) for 2 h between two aerosolization events.

Tracer-induced loss

To test the effect of tracer on viral infectivity, the viral concentrations at 0 min after aerosolizing suspensions with uranine were compared to those without uranine. The air samples of the AGI-30 and the MD8 were used for this purpose. Only when uranine did not affect virus infectivity, the samples of the Andersen impactor and the OMNI-3000 obtained from aerosolization with uranine were determined for viral concentrations.

Loss during aerosolization

A Petri dish containing 20 ml of HBSS positioned on the floor of the isolator was exposed to the air to collect the virus and uranine during the 1.2 min aerosolization (five aerosolization events of suspensions containing uranine). The viral aerosols gently settled down and immersed in the HBSS. It was assumed that this sampling procedure did not cause any stress to the virus infectivity. Immediately after aerosolization, the Petri dish was covered. The liquid sample was vortexed before viral and uranine analysis. The loss of viral infectivity during aerosolization (*La*) is calculated with equation 1.

$$L_a = (1 - \frac{C_{petridish,virus} / C_{suspension,virus}}{C_{petridish,tracer} / C_{suspension,tracer}}) \times 100\% \qquad (1)$$

$C_{petridish,virus}$: viral concentration in the sample of the Petri dish (EID$_{50}$ ml^{-1});

$C_{suspension,virus}$: viral concentration in the suspension (EID$_{50}$ ml^{-1});

$C_{petridish,tracer}$: uranine concentration in the air sample of the Petri dish (µg ml^{-1});

$C_{suspension,tracer}$: uranine concentration in the suspension (µg ml^{-1}).

Loss in air sample handling procedures

The AGI-30 and the OMNI-3000 collect microorganisms into liquid media which can directly be diluted and analyzed. However, the collected microorganisms in the samples of the Andersen impactor and the MD8 have to be transferred into liquid mediums before analysis. Whether or not the viral infectivity is affected by the effects of both handling procedure and delay in handling of the air samples was determined. The air sample handling procedures for the Andersen impactor and the MD8 were described in the following two paragraphs.

Four Andersen impactor plates with ME agar were each added with 0.1 ml of viral suspension with uranine. At 0 min after adding the suspensions, two of the agar plates were rinsed by 2 ml HBSS for 3 times with a plastic spreader (TS30C, bioTRADING Benelux BV, Mijdrecht, the Netherlands). The rinsing-off liquid (in total about 5 to 6 ml) was mixed and the viral concentrations were analyzed. The other two agar plates were rinsed in the same way, but 60 min after exposure to the ambient air at room temperature.

Four MD8 gelatin filters positioned in Petri dishes were added each with 0.5 ml of viral suspension with uranine. At 0 min after adding the suspensions, two of the filters were dissolved in 20 ml 37°C HBSS, and the other two were dissolved 60 min after exposure to the ambient air at room temperature.

The losses of viral infectivity in these two air sample handling procedures (L_h) were calculated with equation 2:

$$L_h = (1 - \frac{C_{liquid} \times V_{liquid}}{C_{suspension} \times V_{suspension}}) \times 100\%$$

(2)

C_{liquid}: viral concentration in the liquid sample (EID$_{50}$ ml^{-1});

V_{liquid}: volume of the liquid sample (ml);

$C_{suspension}$: viral concentration in the suspension (EID$_{50}$ ml^{-1});

$V_{suspension}$: volume of the suspension added on an agar plate (0.1 ml) or a gelatin filter (0.5 ml).

For the air samples of the Andersen impactor and the MD8 in this study, the same methods as above mentioned were used to transfer collected microorganisms into liquid mediums.

Sampling efficiency

The physical efficiencies were determined by comparing the amounts of uranine collected by the bioaerosol samplers to a reference sampler. The MD8 was set as the reference sampler because it was reported to have very high physical efficiency (Burton et al. 2007). Combining the data in this study with data collected in a previous study (Zhao et al. 2011), a larger dataset for calculating the physical efficiency was obtained. Because the uranine concentration in the previous study was lower (0.02%) , we introduced the relative concentration (RC) for all the uranine data, see equation 3. The physical efficiency at 0 (E_p) and 20 min (E_p') was calculated with equation 4 and equation 5.

$$RC_{sampler,tracer} = \frac{C_{sampler,tracer}}{C_{suspension,tracer}}$$

(3)

$C_{sampler,tracer}$: uranine concentration measured by a bioaerosol sampler, µg m^{-3};

$$E_p = \frac{RC_{sampler,tracer}}{\left(\sum RC_{MD8,tracer}\right)/n} \times 100\%$$

(4)

$$E_p^{'} = \frac{RC_{sampler,tracer}^{'}}{\left(\sum RC_{MD8,tracer}^{'}\right)/n} \times 100\%$$

(5)

$RC_{sampler,tracer}$: relative uranine concentration in the air sampled with a bioaerosol sampler at 0 min after aerosolization (ml m^{-3});

$RC_{sampler,tracer}^{'}$: relative uranine concentration in the air sampled with a bioaerosol sampler at 20 min after aerosolization (ml m^{-3});

$RC_{MD8,tracer}$: relative uranine concentration in the air sampled with the MD8 at 0 min after aerosolization (ml m^{-3});

$RC_{MD8,tracer}^{'}$: relative uranine concentration in the air sampled with the MD8 at 20 min after aerosolization (ml m^{-3});

n: number of uranine aerosolization ($n = 15$).

The biological efficiency (E_b) was determined based on the virus/uranine ratios, with equation 6. The number of repetitions of measurements with the Andersen impactor and the OMNI-3000 was five, and the number of repetitions of measurements with the AGI-30 and the MD8 was eight. The latter was higher because the measurements with three times aerosolization of viral suspensions without uranine addition were also included.

$$E_b = \frac{C_{sampler,virus}\big/\left(\sum C_{suspension,virus}/m\right)}{\sum C_{sampler,tracer}\big/\sum C_{suspension,tracer}} \times \frac{1}{1-L_a} \times \frac{1}{1-L_h} \times 100\%$$

(6)

$C_{sampler,virus}$: viral concentration in the air measured with a bioaerosol sampler at 0 min after aerosolization (EID$_{50}$ m^{-3});

$C_{suspension,virus}$: viral concentration in the suspension (EID$_{50}$ ml^{-1});

$C_{sampler,tracer}$: uranine concentration in the air measured with a bioaerosol sampler at 0 min after aerosolization (µg m^{-3});

$C_{suspension,tracer}$: uranine concentration in the suspension (µg ml^{-1});

m: number of aerosolization ($m = 5$ for the Andersen impactor and the OMNI-3000, and $m = 8$ for the AGI-30 and the MD8).

Detection limit

The lowest detectable viral concentration in liquid samples by viral titration is calculated by using the fact that three out of five eggs (3 out of 5) that were inoculated with undiluted liquid samples were infected by the virus. This equaled to 0.4 $\log_{10}$ EID$_{50}$ ml^{-1} (2.5 EID$_{50}$ ml^{-1}) with Spearman and Karber method (1908). Therefore, the detection limit of the bioaerosol samplers for sampling the airborne Gumboro vaccine virus was calculated with equation 7.

$$DL = \log\left[\frac{\beta \cdot V_{liquid}}{(1 - L_h) \cdot V_{air} \cdot E_p \cdot E_b}\right]$$

(7)

DL: detection limit ($\log_{10}$ EID$_{50}$ m^{-3});

β: viral concentration calculated by assuming that an undiluted sample successfully infects 3 eggs (out of 5), ($\beta = 2.5$ EID$_{50}$ ml^{-1});

V_{liquid}: volume of sample liquid (ml);

V_{air}: air volume sucked by bioaerosol samplers in 2 min sampling (m^3).

Half-life time

The half-life time ($t_{1/2}$) evaluates the survival abilities of microorganisms in the air. In this study, it was defined as the duration required for the concentration of infective virus to decrease by half. The half-life time was calculated with equation 8 (Weesendorp et al. 2008). Because the viral aerosols were also diluted during sampling and deposited in the 20 min between the first and the second air sampling, the concentrations of the infective virus were corrected by the tracer (α).

$$t_{1/2} = \frac{(\log_{10} 2) \times T}{\log_{10}\left(C_{sampler,virus} / \alpha C'_{sampler,virus}\right)}$$

(8)

T: time interval ($T = 20$ min);

$C'_{sampler,virus}$: viral concentration in the air at 20 min, EID$_{50}$ m^{-3};

α: correction factor for dilution due to ventilation and deposition ($\alpha = \Sigma(C_{MD8,tracer}/C'_{MD8,tracer})/5$).

Table 1. Responses and tested factors with GLM procedure.

Description	Response	Factor	Symbol	Level
Tracer-induced loss	C_a [a]	Uranine presence	U_i	With uranine, without uranine
		Sampler type	S_j	AGI-30, MD8
		Interaction	$U_i \cdot S_j$	-
Loss in air sample handling procedures	L_h	Handling moment	M_i	0 min, 60 min
Physical efficiency	E_p, E'_p	Sampler type	S_i	Andersen, AGI, OMNI, MD8
		Sampling moment	M_j	0 min, 20 min
		Interaction	$S_i \cdot M_j$	-
Biological efficiency	E_b	Sampler type	S_i	Andersen, AGI, OMNI, MD8
Half-life time	$t_{1/2}$	Sampler type	S_i	Andersen, AGI, OMNI, MD8

[a] log-scale concentration of virus in air ($\log_{10}$ EID$_{50}$ m^{-3}).

[b] log-scale concentration of virus in suspension ($\log_{10}$ EID$_{50}$ ml^{-1}).

Data analysis

All data were analyzed with the SAS program (SAS 9.1.3 Service Pack 4, SAS Institute Inc., Cary, NC, US). The effects of concerned factors on responses were calculated with a general linear model. The tested responses and the factors are listed in Table 1. The losses of viral infectivity during aerosolization and in air sample handling procedures were compared to "no loss" (0%) with a one-sample T-test. The physical and biological efficiencies were compared with 100% with a one-sample T-test.

RESULTS

Aerosol size distribution

Due to the importance of the aerosol size for interpreting the sampler efficiency, size distribution of the aerosols was measured with a spectrometer near the inlet of the bioaerosol samplers. The volumetric concentrations of aerosols in different size ranges are shown in Figure 2. The aerosols were mostly in the size range between 1 – 10 µm at 0 min after aerosolization. The aerosols in range of 1 – 5 µm dominated at 20 min. The aerosols of 3 – 10 µm pronouncedly deposited and/or evaporated after 20 min, and the total volume of aerosols reduced to 13% from that at 0 min.
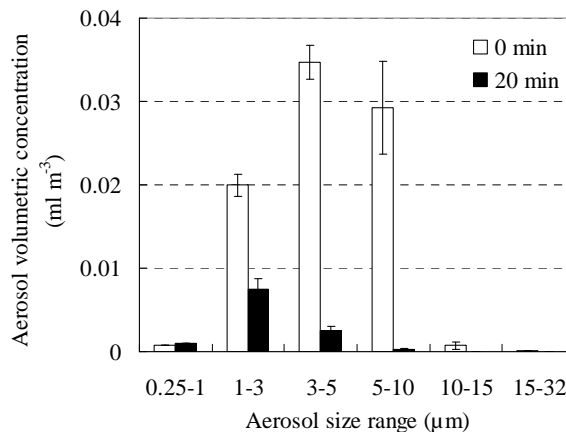

Figure 2. Mean aerosol size distribution at 0 and 20 min after aerosolization (n = 5).

Losses of viral infectivity in non-sampling processes

Table 2 shows the concentrations of airborne virus measured by AGI-30 and MD8 at 0 min after aerosolization. The concentrations of virus that were aerosolized from suspensions either with or without uranine were approximately 6 $\log_{10}$ EID_{50} m^{-3}. Statistical analysis showed that both uranine (P = 0.19) and bioaerosol sampler (P = 0.76) did not significantly affect virus infectivity; no interaction effect was found either (P = 0.56).

A large proportion of virus, 95 ± 2%, was inactivated due to the aerosolization stress. Statistical analysis showed that this loss was significantly different from 0% (which meant no loss, P < 0.01). Tables 3 and 4 show the losses of viral infectivity in the air sample handling procedures. Compared to

that was previous added onto the agar plates (0.1 ml of viral suspension), no loss of viral infectivity was found (-8 ± 10%) when the agar plates of the Andersen impactor were rinsed immediately after addition. The loss increased to 89 ± 8% when the agar plates were rinsed 60 min after the viral suspension was added, which was not statistically significant (P = 0.06). Statistical analysis showed a significant effect of rinsing moment on the loss of viral infectivity (P = 0.02).

Table 2. Concentrations of Gumboro vaccine virus aerosolized with or without uranine.

Bioaerosol sampler	Concentrations[a] (± SE[b], $\log_{10}$ EID$_{50}$ m^{-3})		GLM analysis	
	With uranine	Without uranine	Factor	P[c]
AGI-30	5.9 ± 0.6	6.1 ± 0.6	Uranine presence	0.19
MD8	5.8 ± 0.5	6.1 ± 0.6	Sampler type	0.76
			Interaction	0.56

[a] n = 5 for treatment "with uranine" and n = 3 for treatment "without uranine".

[b] SE: standard error.

[c] Probability that there was no effect of the factor on the virus concentration.

The viral infectivity was not significantly affected by the handling procedure for MD8 samples, regardless whether the gelatin filter was dissolved immediately after adding 0.5 ml viral suspension (P = 0.92), or when the filter was dissolved 60 min after (P = 0.62) this addition. The dissolving moment, therefore, showed no effect on the viral infectivity loss (P = 0.65).

Table 3. Loss of viral infectivity in handling procedure for Andersen impactor samples by rinsing method.

Moment	Loss (± SE[a], %)	n	One-sample T-test	GLM analysis	
			P[b]	Factor	P[c]
0 min	-8 ± 10	2	0.57	Moment	0.02
60 min	89 ± 8	2	0.06		

[a] SE: standard error.

[b] Probability that the loss was not different from "no loss" (0%).

[c] Probability that there is no effect of the factor on the infectivity loss.

Table 4. Loss of viral infectivity in handling procedure for MD8 samples by dissolving gelatin filters.

Moment	Loss (± SE[a], %)	n	One-sample T-test	GLM analysis	
			P[b]	Factor	P[c]
0 min	4 ± 18	4	0.92	Moment	0.65
60 min	-21 ± 19	4	0.62		

[a] SE: standard error.

[b] Probability that the loss was not different from "no loss" (0%).

[c] Probability that there is no effect of the factor on the infectivity loss.

Sampling efficiency and detection limit

Table 5 shows the physical efficiencies of the four bioaerosol samplers. No effect was found (P = 0.79) of sampling moment on the physical efficiency thus the efficiency data at 0 and 20 min were pooled. The Airport MD8 with a gelatin filter was used as the reference sampler and its efficiency was

set to 100%. The physical efficiencies of the other samplers were compared to that of the Airport MD8. Statistical analysis showed that different samplers significantly differed in their physical efficiencies (P < 0.01). Multiple comparisons showed that the MD8 and the AGI-30 had higher physical efficiencies than the other two sampler types. The physical efficiency of the Andersen impactor was lowest (28 ± 8%). The interaction effect of sampler type and sampling moment was not significant (P = 0.78).

Table 5. Physical efficiencies of the four bioaerosol samplers.

Bioaerosol sampler	Physical efficiency (± SE[a], %)	n	One-sample T-test	GLM analysis	
			P[b]	Factor	P[c]
Andersen	_[d]	30	< 0.01	Sampler type	< 0.01
AGI-30	112[a] ± 4	30	0.21	Moment	0.79
OMNI-3000	62[b] ± 6	30	< 0.01	Interaction	0.78
MD8	100[a] ± 9	26	1.00		

[a] SE: standard error.

[b] Probability that the physical efficiency was not different from 100%.

[c] Probability that there is no effect of the factor on the physical efficiency.

[d] Low physical efficiency of the Andersen impactor was obtained (28 ± 8), probably because of the difficulty in rinsing off uranine from the agar plates.

a,b,c Means with a com

mon superscript letter are not significantly different (P > 0.05).

Table 6. Biological efficiencies of the four bioaerosol samplers.

Bioaerosol sampler	Biological efficiency (± SE[a], %)	n	One-sample T-test	GLM analysis	
			P[b]	Factor	P[c]
Andersen	61[a,b] ± 30	5	0.27	Sampler type	0.03
AGI-30	90[a,b] ± 36	8	0.78		
OMNI-3000	23[b] ± 10	5	< 0.01		
MD8	163[a] ± 30	8	0.07		

[a] SE: standard error.

[b] Probability that the biological efficiency was not different from 100%.

[c] Probability that there is no effect of the factor on the biological efficiency.

a,b,c Means with a common superscript letter are not significantly different (P > 0.05).

Table 7. Detection limits of the bioaerosol samplers for collecting Gumboro vaccine virus for two min.

Bioaerosol sampler	Detection limit ($\log_{10}$ EID_{50} m^{-3})
Andersen	4.1
AGI-30	3.3
OMNI-3000	2.5
MD8	2.9

Table 6 shows the biological efficiencies of the four bioaerosol samplers for collecting Gumboro vaccine virus. The biological efficiency was significantly different between the samplers (P = 0.03).

The efficiency of the MD8 (163%) tended to be higher than 100% (P=0.07). The efficiency of the OMNI-3000 (23%) was significantly lower than 100% (P < 0.01), but not those of the Andersen impactor (P = 0.27) and the AGI-30 (P = 0.78).

The detection limits of the different bioaerosol samplers on sampling (two min) airborne Gumboro vaccine virus are listed in Table 7. The detection limits ranged from 2 to 4 $\log_{10}$ EID_{50} m^{-3}. The OMNI-3000 had the lowest detection limit.

Half-life time

The half-life time of airborne Gumboro vaccine virus at 20°C and 70% RH determined by sampling with the different bioaerosol samplers is shown in Table 8. There was no significant difference in the half-life time determined by the different bioaerosol samplers. The mean half-life time was 11.9 ± 2.0 min. Four negative half-life time values (two with AGI-30 samples, one with OMNI-3000 samples, and one with MD8 samples) were obtained because a higher viral concentration at 20 min, after correction for dilution due to ventilation and deposition (formula 8), compared to that of 0 min. These negative values were excluded from calculating of the half-life times.

Table 8. Half-life time of Gumboro vaccine virus.

Bioaerosol sampler	Half-life time[a] (± SE[b], min)	n	GLM analysis	
			Factor	**P[c]**
Andersen	15.5 ± 7.0	5	Bioaerosol sampler	0.57
AGI-30	11.3 ± 2.1	6		
OMNI-3000	14.6 ± 5.7	4		
MD8	8.2 ± 1.9	7		
Mean	11.9 ± 2.0	22		

[a] Negative values (due to a higher virus concentration at 20 min, after correction, compared to that of 0 min) was not included in the calculation of the half-life time.

[b] SE: standard error.

[c] Probability that there is no effect of the factor on the half-life time.

DISCUSSION

Tracers have been widely used to evaluate the dilution and deposition of bioaerosols in investigations of microbial survival (Marthi et al. 1990; Ijaz et al. 1994). It is preferable to aerosolize a tracer combined with the microorganisms; however several requirements need to be fulfilled in order to do so. Firstly, the tracer should not induce the loss of microbial viability. The common concentrations of uranine are lower than 0.1% in microbial suspensions. When the suspensions are aerosolized, the concentrations of uranine in aerosols increase due to water evaporation, which is suspected to increase the inactivation risk for microorganisms. Therefore, it is better to investigate the tracer-induced loss of microorganisms after they are aerosolized. In this experiment, the concentrations of Gumboro vaccine virus aerosolized either with or without uranine addition were similar, with an insignificant tracer effect (P = 0.19). Secondly, the ratios of microorganism/tracer in

all aerosols should be identical when using the tracer to evaluate the physical efficiencies of the bioaerosol samplers. The aerosols were produced in this experiment with a cut-off diameter of 10 µm, which is much larger than the sizes of the Gumboro vaccine virus (0.06-0.09 µm) and uranine particles (0.15 µm). This allows a uniform distribution (in the aerosols) of both virus and uranine that will coagulate during water evaporation (Ijaz et al. 1987; Zhao et al. 2011). Thirdly, the tracer should be well dissolved in microbial suspensions and accurately analyzed. The uranine has a high solubility of 600 g l^{-1} in the water, and can be detected at a tiny concentration of 0.002 µg ml^{-1}. For the above mentioned advantages, the uranine is evaluated as a safe and reliable tracer for this study.

In a previous study, *Enterococcus faecalis*, *Escherichia coli* and *Mycoplasma synoviae* were not significantly inactivated when they were nebulized by the Walther Pilot spray-head , but the *Campylobacter jejuni* was inactivated for 71% (Zhao et al. 2011). A large proportion of Gumboro vaccine virus, 95%, lost its infectivity during aerosolization by the spray-head in this study. The loss of infective virus is probably due to be the shock of a sudden climate change immediately after aerosolization, in which the virus undergoes dehydration and oxidization stresses (Tang 2009). The initial infectivity loss in this process can be much more profound compared to the loss in the following atmospheric equilibrium state (Harper 1961; Schaffer et al. 1976). Other studies reported that the bacteria were subjected to shear force, which deagglomerates the suspensions into small aerosols and causes bacteria to fragment (Reponen et al. 1997; Mainelis et al. 2005). However, it seemed not to be the main reason for the inactivation of virus in this study, because the aerosol size that was produced by the spray-head was much larger than the virus itself. Noticing that microorganisms can be inactivated during aerosolization, the calculated sampling efficiency should be corrected for this aerosolization loss.

When the Andersen impactor is used in a highly microbially contaminated environment, it can easily become overloaded. An Andersen impaction plate becomes overloaded when more than 400 colonies are formed on the agar plate. In highly contaminated environments this can already be the case after a very short sampling period, of e.g. more than 10 seconds (Zhao et al. 2011). To avoid the overloading problem, the collected microorganisms can be transferred into liquid mediums that may be diluted and analyzed at will. By doing this, the true number of microorganisms can be obtained because agglomerated microorganisms in particles are detached in liquid. Two methods have been used for transferring so far, i.e. homogenizing the microbial-loaded agar into liquid (Lundholm 1982) and rinsing the microorganisms off the agar surface (Zhao et al. 2011). The losses of four bacterial species with the latter method were previously found insignificant (Zhao et al. 2011). In this study, Gumboro vaccine virus was also recovered without significant loss from agar plates by the rinsing method immediately after adding the viral suspension. However, about 89% of infective virus was lost when the agar plates were rinsed 60 min after storage in ambient air at room temperature. The statistical analysis showed a significant effect of storage time on the loss of virus (P = 0.02). Compared to the bacteria, more virus was lost in this air handling procedure. This was probably

caused by the relatively small size of the Gumboro vaccine virus as compared to bacteria allowing it to penetrate deeper into the agar layers with the liquid streams that hampered the rinsing efficiency. The virus was stored on the agar plates for a longer time (60 min) before rinsing than the bacteria (5 min), which may result in an extensive penetration of virus into the agar. Li and Lin (2001) reported that approximately 60-80% of *E. coli* lost viability after 60 min storage on a gelatin filter. Loss was not found in this study for Gumboro vaccine virus up to 60 min storage on the gelatin filter before dissolving. The insignificant loss of infective virus on the gelatin filter suggests that the loss on the agar plate at 60 min after adding viral suspension is not likely to be the inactivation of virus due to the storage in the room environment.

The physical efficiencies of the AGI-30 and the MD8 with gelatin filter were higher compared to the OMNI-3000, which is according expectation. Our results were consistent with the study of Terzieva et al.(1996), who reported the AGI-30 could collect almost all particles of 0.7 - 1.0 µm. Actually, Nevalainen et al.(1992) calculated that the cut-off diameter of the AGI-30 is as low as 0.31 µm (Nevalainen et al. 1992). In this study, most aerosols (in volume) were larger than this cut-off diameter (Figure 1), indicating that they could be effectively collected by the AGI-30. The sampling duration of the AGI-30 was short (2 min), therefore, a decrease in physical efficiency of the AGI-30 due to the evaporation of liquid collection medium was not a severe problem (Lin et al. 1997). By measuring particle concentrations upstream and downstream of a gelatin filter of the MD8 with an optical particle counter, its physical efficiency was found >93% for collecting nano-particles (0.08 µm) (Burton et al. 2007). The efficiency of the MD8 for collecting bacterial aerosols was also high (Zhao et al. 2011). Therefore the MD8 was selected as the reference sampler for physical efficiency in this study. The physical efficiency of the OMNI-3000 ($62 \pm 6\%$) in our study fell within the range reported by Kesavan and Schepers (2006), who found that its efficiency was 22-92% for aerosols (either solid or liquid) of 0.5-8 µm. The cut-off diameter of the sixth stage of the Andersen impactor is 0.65 µm. This sampler was expected to be effective for collecting the aerosols; however a low efficiency of $28 \pm 8\%$ of the Andersen impactor was found. Considering that the efficiency data were obtained based on the amounts of collected uranine which could attach firmly with the agar (Zhao et al. 2011), the low efficiency of the Andersen impactor in this study was probably not due to the inefficiency of the sampler, but to the low recovery of uranine from the agar plates by the rinsing method.

Various samplers differ in their ability to preserve microbial survival during sampling, which is confirmed in this study for airborne Gumboro vaccine virus (P = 0.03). A biological efficiency significantly lower than 100% indicates that the microorganisms are inactivated due to the sampling stress of a bioaerosol sampler. Less stress was posed to Gumboro vaccine virus when it was collected by the Andersen impactor and the AGI-30. These two samplers were previously found to also well preserve the culturability of several bacterial species (Zhao et al. 2011). The biological efficiency of the OMNI-3000 ($23 \pm 10\%$) was significantly lower than 100%. This was probably because of its high flow rate during sampling, which produces high shear forces to the microorganisms. This sampler has

a slit inlet of 3 cm long and approximate 0.2 cm wide. When sampling at an airflow rate of 300 l min^{-1}, the microorganisms are sucked into the sampler and impinged into the rotating collection liquid at a very high speed. Gumboro vaccine virus was not significantly inactivated when filtered by the MD8 with a gelatin filter. The biological efficiency of the MD8 was found to be (not significantly) higher than 100%. It is not likely that the virus may replicate without the living cells of organisms. Therefore, the high biological efficiency can only be explained the high variations generally found in viral titrations.

The detection limits of the bioaerosol samplers for colleting Gumboro vaccine virus were calculated based on their known physical and biological efficiencies. The detection limits of these samplers ranged from 2.5 - 4.1 log$_{10}$ EID$_{50}$ m^{-3}. Lower detection limits can be achieved by increasing the sampling duration of the samplers (equation 7). However, sampling duration can not be extended too much because the amount of the microorganisms and the sampling duration are not linearly related due to either a decrease in physical efficiency (e.g. evaporation of collection medium of the AGI-30 compromises its physical efficiency) or in biological efficiency (Lin et al. 1997). Although the sampling efficiency of the OMNI-3000 is relatively low, its high air flow rate still causes the detection limit to be lower than of the other samplers. Therefore, the OMNI-3000 can be used best in environments where the viral concentrations are low.

The half-life times of many airborne viruses have been studied at different temperature and RH. The results showed a big variation in the viral half-life time ranging from several minutes to days (Larson et al. 1980; Sattar et al. 1984; Ijaz et al. 1985; Landman and van Eck 2001). With the bioaerosol samplers evaluated in this study, we investigated the half-life time of airborne Gumboro vaccine virus, a RNA non-enveloped virus. The mean half-life time was 11.9 min. Although the bioaerosol samplers were different in their efficiencies, the half-life times that were determined by these samplers did not show a significant difference (P = 0.57). This is probably because the efficiency of each sampler was identical between the sampling at 0 min and that at 20 min. Four negative half-life time values were found due to higher virus concentrations at 20 min (after correction with the tracer) compared to those at 0 min. These negative values were excluded from the calculation of the half-life times, however, a negative value means infinity half-life time.

CONCLUSIONS

Using a fluorescent tracer, this study evaluated the physical and biological efficiencies of four bioaerosol samplers for collecting Gumboro vaccine virus aerosolized from viral suspensions. It is concluded that the virus may lose infectivity in non-sampling processes, which would lead to underestimation of sampling efficiency if they are not excluded from the efficiency calculation. The physical and biological efficiencies of the Andersen impactor, the AGI-30 and the MD8 with gelatin filter are high for collecting the virus. Although the OMNI-3000 has lower efficiencies, its high air

flow rate causes a low detection limit, nominating it as a suitable sampler for detecting the virus at low concentrations.

DETECTION OF AIRBORNE *CAMPYLOBACTER* WITH THREE BIOAEROSOL SAMPLERS FOR ALARMING BACTERIA TRANSMISSION IN BROILERS

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ABSTRACT. In an airborne transmission experiment, the *Campylobacter* in the air was sampled by three types of bioaerosol samplers (All Glass Impinger "AGI-30", Andersen six-stage impactor, and OMNI-3000) in four broiler rooms. In each room, five out of 15 broilers were kept in one central cage and inoculated with *Campylobacter jejuni* at day 0 (when the broilers were 14 day old). The other ten broilers, as susceptible animals, were kept individually in ten cages at a distance of approximately 75 cm, surrounding the central cage. Air samples were taken on eight days, i.e. the day before inoculation (BI) as a negative control, and 1, 3, 6, 9, 14, 21, and 29 days post-inoculation (PI). Presence of *C. jejuni* was investigated both with the culture method for culturable bacteria and with the PCR test for a bacterial DNA. Results showed that *Campylobacter* infection of susceptible broilers occurred in all four rooms; however, no culturable *C. jejuni* could be detected in all the air samples. This might have been the result of the low number of broilers in the room and the unfavorable conditions for *Campylobacter* survival, leading to *Campylobacter* concentrations below the detection limits of the bioaerosol samplers. The PCR tests showed there was DNA of *C. jejuni* in the air on day 1 PI days, but no bacterial DNA on following days. It is concluded that the three samplers used in this study are not capable to alarm the *Campylobacter* outbreaks through the air route when low bacterial concentrations are present. Developments of new sampling techniques with low detection limits are required for bio-security assessment.

Keywords. *Aerial, Bacteria, Campylobacter, Poultry, Transmission.*

INTRODUCTION

Campylobacter species are recognized as an important cause of human illness, such as diarrhea, Guillain-Barré syndrome and sporadic morbidity (Keener et al. 2004; Humphrey et al. 2007). It was reported that 50% to 70% of *Campylobacter* infections are caused by consumption of poultry products in United States, Europe and Australia (Allos 2001). The prevalence of *Campylobacter* is high in the poultry industry. Approximate 20% of the broiler flocks in 1999-2002 (Van de Giessen et al. 2006), and 35% of the organic broiler flocks in 2003 (Rodenburg et al. 2004) were *Campylobacter*-positive in the Netherlands. For bio-security, an obligatory monitoring program for *Campylobacter* in poultry has been implemented to identify the presence and to develop control strategies (European Union 2003).

Campylobacter transmission in poultry was thought to be caused mainly by contacting contaminated feed, water, infected animals and insects (Jacobs-Reitsma et al. 1995). Recently, airborne transmission of *Campylobacter* is suspected as another mechanism for disease spreading, along with the recovery of culturable bacteria from the air in poultry processing plants and an infection case of a poultry worker via the air (Berrang et al. 2004; Wilson 2004; Johnsen et al. 2007). If this mechanism is true, the detection of airborne bacteria with bioaerosol samplers is required to be included in the monitoring program for *Campylobacter*. However, it is not clear whether or not an effective monitoring, with respect to alarming the airborne transmission of *Campylobacter*, can be achieved by sampling using the bioaerosol samplers.

The techniques for sampling airborne microorganisms include impingement, impaction, filtration and cyclone (Eduard and Heederik 1998). In practical measurements, none of these techniques can fully recover all the microbial species from the air, due to the non-isokinetic sampling and the sampling stresses to the target organisms (Stewart et al. 1995). These limitations make a precise quantification of airborne micro-organisms always a challenge, especially for the stress-sensitive species. So far, All Glass Impinger (AGI-30) and Andersen six-stage impactor are the most commonly used bioaerosol samplers, which are recommended as standard samplers for airborne micro-organisms. Because the air flow rates of these two samplers are relative low (12.5 l min^{-1} for AGI-30 and 28.3 l min^{-1} for Andersen impactor), their utility is limited in aerial environments where low concentrations of micro-organisms are present. In order to perform the air sampling in these environments, some high volume samplers have been developed, e.g. OMNI-3000 (300 l min^{-1}), which may sample a large amount of air in a short time.

Previous studies on airborne transmission of pathogenic microorganisms have been performed with infected and healthy animals in lab-scale experiments (Mars et al. 1999; Berthelot-Herault et al. 2001; Brockmeier and Lager 2002). An airborne infection can be shown when healthy susceptible animals become infected by infected ones, that are physically, but not aerially, separated from each other. A similar experimental set-up could also be applied to study the airborne transmission of *Campylobacter*.

To analyze *Campylobacter*, a culture method or the polymerase chain reaction (PCR) technology can be used. They both have their advantages and disadvantages. With the culture method, culturable *Campylobacter* which is capable to multiply themselves can be quantified, but this method is time and labor consuming. A PCR test is fast compared to the culture method; however, due to the fact that the test detects only a part of a certain gene, *Campylobacter* DNA is determined without differentiation between culturable and dead bacteria.

The objective of this study was to test three bioaerosol samplers (AGI-30, Andersen impactor and OMNI-3000) on detecting the *C. jejuni* (culturable and DNA) in the air for alarming its transmission within an airborne experimental set-up in broilers.

MATERIALS AND METHODS

Experimental Rooms

The study was conducted in 4 climate controlled (temperature and relative humidity) rooms of 7.40 m length and 4.75 m width. Room temperature gradually decreased from 25 ºC on the day before inoculation (BI) (13 bird age day) to 18 ºC on 17 post-inoculation (PI) day, then it remained constant until the end of the experiment (35 PI day). Relative humidity was set at a constant level of 55%. The rooms were ventilated by an overpressure system in the roofs of the building. The incoming air was filtered by an absolute filter to prevent interference from outside. The air outlet was installed on one side wall. The average ventilation rate of each room was 1000 m^3 h^{-1}.

Before the experiment, all rooms were disinfected with formaldehyde gas then high pressure water. Samples from 12 different surfaces were taken to insure absence of both culturable *C. jejuni* and the bacterial cell.
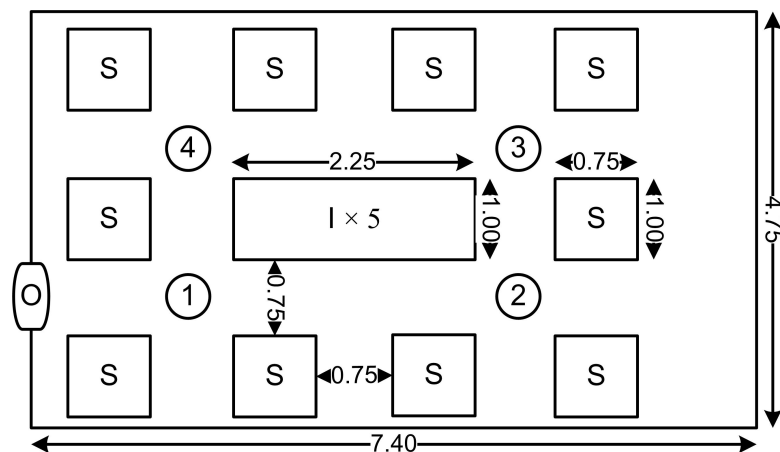

Figure 1. Schematic drawing of the room plan. (I: inoculated broilers central cage, S: susceptible broiler cage, O: air outlet, 1-4: Air sampling locations).

Animals and Cages

Fifteen 12 day-old *Campylobacter*-free broilers were introduced into each room. Five of them were reared in one central cage (2.25 m in length and 1.00 m in width) in the middle of the room. They were

orally inoculated with 10^6 colony forming unit (CFU) *C. jejuni* strain 356 two day after introducing. The other ten broilers were separately reared as susceptible animals in 10 individual cages surrounding the central one. The individual cages (1.00 m in length and 0.75 m in width) for susceptible broilers were placed about 0.75 m away from the central cage. A schematic drawing of the room plan is depicted in Figure 1. All broilers were reared on wood shavings on the ground. The water was supplied by a nipple drinking system, using separate systems for the center cage and the other cages.

From 1 PI day until 35 PI day, cloacae swabs were taken on a daily basis. When the inoculated broilers were tested positive for *C. jejuni* on three consecutive post-inoculation days, swabs were taken weekly instead of daily. The susceptible broilers were tested every day. Whenever a susceptible broiler turned positive for *C. jejuni*, it was ruled out from the room.

Bioaerosol Samplers

Three bioaerosol samplers were used: AGI-30, Andersen impactor and OMNI-3000. The AGI-30 and the Andersen impactor were able to preserve viability of *C. jejuni* due to their low sampling stresses, therefore, were suggested as culturable samplers for airborne *C. jejuni* (Zhao et al. 2011; Zhao et al. 2011). When the bacteria DNA (could be from both culturable and dead bacteria) were of concern, the AGI-30 and the Andersen impactor may be not suitable because of their low air flow rate 12.5 l min^{-1} for the AGI-30 and 28.3 l min^{-1} for the Andersen impactor. A new type of high air flow rate sampler, the OMNI-3000 (300 l min^{-1}), was used in this study to be able to detect bacterial DNA at low concentrations in the air.

The AGI-30 (7540, Ace glass Inc., Vineland, US) collects bacteria into liquid medium by impingement. It is operated at an airflow rate of 12.5 l min^{-1}. Physiological salt water (PSW, bioTRADING Benelux B.V., Mijdrecht, the Netherlands) of 20 ml was used as collection medium in this study.

The Andersen impactor (TE-10-800, Pacwill Environmental Ltd., Beamsville, Ontario, Canada) consists of six stages in each of which a glass Petri dish was placed under a screen with 400 holes. The number of holes is the same for each stage but the diameter of the holes becomes smaller every next downward stage. Providing an airflow rate of 28.3 l min^{-1}, the air speed through the holes increases from the first stage to the sixth stage. The bacterial particles in the air stream are impacted on the Petri dishes and are differentiated according to their size. From the first stage to the sixth stage, bacterial particles in size of > 7.1 μm, 4.7 – 7.1 μm, 3.3 – 4.7 μm, 2.1 – 3.3 μm, 1.1 – 2.1 μm, and 0.65 – 1.1 μm are collected respectively. Normally, a Petri dish with agar is positioned underneath each stage. In this study, 2.5 ml buffered peptone water (BPW, bioTRADING Benelux B.V., Mijdrecht, the Netherlands) with a gelatin filter (17528-80-ACD, Sartorius, Göttingen, Germany) was used as the collection medium in each Petri dish. This is because the airborne *Campylobacter* was successfully recovered from the air in slaughter houses by this adaption (Jacobs-Reitsma 2002).

The OMNI-3000 (Evogen Inc., Kansas City, US) is operated at a high airflow rate of 300 l min^{-1}. It collects bacteria in 10 ml phosphate buffered saline (PBS, Evogen Inc., Kansas City, US). Before sampling, the PBS is sucked from a cartridge into the contactor. Air flow provided by an inner pump forces the PBS to rotate and to contact with the incoming air in the contactor. In this way the bacteria are trapped in the PBS. To compensate for the evaporation of PBS during sampling, sterilized water is continuously supplemented into the contactor to keep the sample volume always at 10 ml. After sampling, the PBS is drained from the contactor back to the cartridge.

Sampling Schedule

The bioaerosol samplers were used for sampling from 10 to 30 mins at a level of approximately 0.25 m above the floor in the broiler rooms. The sampling location and date are indicated in Table 1 and Figure 1.

Table 1. Air sampling duration, airflow rate, sample volume, sampling location and date in broiler rooms.

Sampler	Sampling duration (min)	Airflow rate (l min^{-1})	Sample volume (ml)	Location[a]	Sampling date (PI day)[b]
AGI-30	30	12.5	20	1, 2, 3, 4	Control[c], 1, 3, 6, 9, 14, 21, 29
Andersen	10	28.3	12.5 × 6	1	3, 9, 21[d], 29
OMNI-3000	10	300.0	10	1, 3	3, 9, 14, 21, 29

[a] See Figure 1 for the locations. Samples from location 1 and 3 were analyzed by PCR.

[b] PI Day = post-inoculation day.

[c] Air sampling that was taken on the day before inoculation (13 bird-age day).

[d] The day on which air samples were not analyzed by PCR.

Sample Processing and Bacterial Analysis

After air sampling with the Andersen impactor, the gelatin filter in each Petri dish was moved to a 15 ml sterilized vial. The Petri dish was carefully washed with 10 ml of BPW, which was pipetted into the vial with the gelatin filter. These vial samples were centrifuged at 2500×g for 10 min. The samples of the AGI-30 and the OMNI-3000 were sent directly for analysis.

The culturable and DNA of *C. jejuni* was analyzed with culture method and PCR test, respectively. For culturable *C. jejuni*, 1 ml of each liquid sample was used to make decimal dilutions (10^{-1} to 10^{-6}) in PSW. 0.1 ml from each dilution was plated on Charcoal Cefoperazone Deoxycholate agar (CCDA) plate, which was incubated at 41.5℃ for 48 h under micro-aerial condition (6% O_2, 10% CO_2 and 84% N_2). If colonies were formed after incubation, the CFU on the agar plate (normally with 30-300 colonies) was counted and the CFU in the liquid sample could be calculated (International Organization for Standardization (ISO) 2006). Another 1 ml liquid sample was first added into 10 ml of Bolton broth (bioTRADING Benelux B.V., Mijdrecht, the Netherlands) for enrichment at 37 ℃ for 4-6h, and then at 41.5 ℃ for 48 h at micro-aerial conditions. The culturable *C. jejuni* count in the enriched sample was also analyzed with the culture method.

Half of the air samples of the AGI-30 (those from location 1 and 3, see Figure 1) and all samples of the Andersen impactor and the OMNI-3000 were analyzed for DNA of *C. jejuni* with the PCR test. The liquid air sample was filtrated by a Polytetrafluoroethylene (PTFE) filter (pore size 0.5 μm, Sartorius, Nieuwegein, the Netherlands). The filter and the filtrated bacteria, then, were immersed totally in 0.4 ml Lysis buffer in a sample tube, and stored at -80°C before the PCR test.

Airborne Dust Sampling

The PM_{10} (particulate matter smaller than 10 µm of aerodynamic diameter) and $PM_{2.5}$ were respectively determined for 24 hours with DustTrak (TSI Inc., Shoreview, US), which measured the mass concentration of aerosols with photometric principle, in two of the four rooms on 28 PI day. The two DustTrak samplers were put at the same level as the bioaerosol samplers at sampling location 1.

Data Analysis

The non-100% specificity of PCR test reported also positive results for the air samples of control (1 BI day), which were taken by the AGI-30. Thus, the amounts of DNA of *C. jejuni* sampled by each type of bioaerosol samplers on different sampling days were separately compared to control samples with non-parametric test (Mann Whitney U test, SPSS 15, SPSS Inc.). The reason to use non-parametric test is due to the skew distribution of the data. Positive samples were recognized as those whose mean rank values were higher than the control samples and the P values were < 0.05.

RESULTS AND DISCUSSION

Infection in Broilers

Test of cloacae swabs showed that all the broilers in the central cages of four rooms got infected within two days after orally inoculated with *C. jejuni* (Van Bunnik et al. 2009). This result was comparable with other studies (Beery et al. 1988; Shanker et al. 1988), in which chickens were positive on 1 PI day. In total 13 susceptible broilers turned positive between 15 PI day an 35 PI day, 10 of which were infected during our air sampling period (1 to 29 PI day). In details, six susceptible broilers were infected between 15 PI day and 29 PI in one room, one broiler was infected on 30 PI day in one room, one broiler was infected on 35 PI day in one room, and two broilers were infected on 16 PI day and 34 PI day in the last room (Van Bunnik et al. 2009). The infections of susceptible broilers revealed the airborne transmission of *Campylobacter* occurred.

Detection of Airborne *C. jejuni*

The PCR test showed that DNA of *C. jejuni* DNA was collected by the AGI-30 on 1 PI day (Table 2). No bacterial DNA was detected in the air by any of the samplers from 3 to 29 PI day. All the air samples were negative for culturable *C. jejuni*, no matter whether they were directly cultured or cultured after enrichment in broth. The different result between the PCR and the culture tests shows

only dead *C. jejuni* was present in the air. Moreover, the PCR test seems more sensitive to indicate an airborne transmission of *Campylobacter*.

Table 2. Culturable *C. jejuni* (tested by culture method) and DNA of *C. jejuni* (tested by PCR) in air samples of four replicated broiler rooms.

Sampler	*C. jejuni* count (Culturable/DNA)							
	Control	**1 PI day**	**3 PI day**	**6 PI day**	**9 PI day**	**14 PI day**	**21 PI day**	**29 PI day**
AGI-30	Neg/Neg	Neg/Pos	Neg/Neg	Neg/Neg	Neg/Neg	Neg/Neg	Neg/Neg	Neg/Neg
Andersen	-[a]/-	-/-	Neg/Neg	-/-	Neg/Neg	-/-	Neg/-	Neg/Neg
OMNI-3000	-/-	-/-	Neg/Neg	-/-	Neg/Neg	Neg/Neg	Neg/Neg	Neg/Neg

[a] Not done.

The outcome that susceptible broilers were infected, but no culturable airborne *C. jejuni* was recovered, is probably to the low concentrations of the bacteria in the air which were below the detection limits of the three bioaerosol samplers. Five infected broilers were reared in the experimental rooms and therefore a low amount of *C. jejuni* was shed. Only the air samples on day 1 PI were positive in the PCR test and the chance of recovering culturable *C. jejuni* was even less. Some researchers assumed micro-organisms could be transmitted by the dust particles; therefore, they were somehow positively related with the dust concentration in the air (Cambra-Lopez et al. 2010). Our dust measurements showed that the average PM_{10} and $PM_{2.5}$ concentrations in the rooms were 118 µg m^{-3} and 43 µg m^{-3}, respectively, on 28 PI day. These concentrations were much lower than those reported in commercial poultry farms (Takai et al. 1998). Furthermore, *Campylobacter* has difficulties to survive in the air in the experimental broiler rooms (Luechtefeld et al. 1981; Doyle and Roman 1982). The optimal temperature for *Campylobacter* multiplication is approximately 42 ºC (Keener et al. 2004), and the optimal temperature for preservation of viability is from 4 to 10ºC (Buswell et al. 1998). In this experiment, the room temperature was controlled at 25-30 ºC, which is neither within the optimal growing temperature nor within the optimal survival temperature. The relative humidity in the broiler room, 55%, was lower than the preferred humidity of 98.7% for bacteria (Koop et al. 2000; NZFSA 2001).

The bioaerosol samplers may miss-collect the bacteria, and the *Campylobacter* may lose culturability due to the sampling stresses. When the standard Petri dishes with agar were used, the Andersen impactor has a cut-off diameter (D_{50}, defined as the particle diameter corresponding to 50% physical collection efficiency) of 0.65 µm for the sixth stage (Andersen 1958). In this experiment, we replaced the agar with a gelatin filter with 2.5 ml BPW because a previous study reported the airborne *Campylobacter* was successfully recovered with this adaption (Jacobs-Reitsma 2002). However, this adaption might increase the cut-off diameter of the Andersen impactor, therefore reduce its sampling efficiency (Andersen 1958). From biological aspect, the AGI-30 seems a suitable sampler for airborne *C. jejuni* in that it collects the bacteria in to liquid medium which provides a humid environment for them. Zhao et al. (2011) proved that the sampling stress of the AGI-30 to *C. jejuni* was lower

compared to the OMNI-3000. As well, the AGI-30 has a low cut-off diameter down to 0.31 μm (Nevalainen et al. 1992), and therefore has a relatively high physical efficiency (Zhao et al. 2011). The problem of the AGI-30 is that it has the lowest sampling flow rate, 12.5 L min^{-1}, among the three bioaerosol samplers. This restricts its application in the environments with low bacteria concentrations. The OMNI-3000 was originally expected to have the greatest chance to capture *Campylobacter*, because it samples the air at a high flow rate (300 L min^{-1}) which provides 8 times and 10.6 times the air volume compared to the AGI-30 and the Andersen impactor, respectively. However, it failed to collect both culturable *C. jejuni* and its DNA from the air. This might be because the low physical efficiency of OMNI-3000 and its severe sampling stress to the micro-organisms. In another study, only 1% of *C. jejuni* could be recovered under the high sampling stress of the OMNI-3000 (Zhao et al. 2011). The detection limits of the three samplers used in this experiment for culturable *C. jejuni* were calculated with the physical and biological efficiency of the samplers (Zhao et al. 2011) and by assuming 1 CFU on the cultured Petri dish with undiluted sample (Table 3).

Table 3. Detection limit of AGI-30, Andersen impactor, and OMNI-3000 for culturable airborne *C. jejuni*.

Sampler	Detection limit (CFU m^{-3})
AGI-30	5.3×10^2
Andersen	4.4×10^2
OMNI-3000	6.8×10^3

Might airborne transmission occurs by susceptible broilers inhaling particles with viable but non-culturable (VNC) *Campylobacter* (Cappelier et al. 1999)? Two questions need to be taken into consideration for this case. Firstly, can VNC bacteria exist in airborne particles? The viable but non-culturable state for some other bacteria in aerosols has been proven (Heidelberg et al. 1997). Federighi et al., (1998) also verified the presence of VNC in microcosm water with plate culturing, staining and microscope scanning methods. However, currently there is lack of evidence of VNC *Campylobacter* in airborne particles. Secondly, can VNC infect animals? This remains a controversial issue. Some studies successfully infected eggs, chickens and mice with VNC *Campylobacter* (Cappelier et al. 1999; Talibart et al. 2000). The disputable point of their findings is that the authors were not one-hundred-percent sure if the inoculation medium was viable *Campylobacter* free. The other studies failed to infect birds with VNC *Campylobacter*, which suspended in water at room temperature for 7, 10, or 14 days (Ziprin et al. 2003). The conclusion that VNC inoculation did not produce infection however, seems also doubtable as in an investigation by Thomas et al. (2002), it was indicated that there were no VNC , but only dead bacteria, in the aqueous medium after 7 days suspension.

CONCLUSIONS

In this experiment, none of the bioaerosol samplers could recover culturable *C. jejuni* from the air, despite the fact that susceptible broilers were infected by the infected broilers by airborne transmission,

which is probably due to the bacterial concentrations were lower than the detection limits of the bioaerosol samplers. It is suggested that negative results from these bioaerosol samplers cannot assure a safe air environment. Sampling techniques with low detection limits requires to be developed in order to detect low concentrations of microorganisms for bio-security assessment.

CHAPTER 9

GENERAL DISCUSSION

The growing world population and increasing economic prosperity have increased demand for livestock products, and this is intensifying livestock production considerably in different parts of the world. The drawback of these developments is the high pressure of the increasing number of animals on the environment. One of the main problems is air pollution. It has been shown that livestock production systems emit large amounts of aerial pollutants, e.g. ammonia (leading to eutrophication and acidification), methane, nitrous oxide, carbon dioxide (greenhouse gases responsible for global warming), odor (giving nuisance) and dust (affecting respiratory health) into ambient air. For example, 80% of ammonia in the US and Europe (van der Hoek 1998; Liang et al. 2005; US EPA 2005), 20% of PM_{10} in the Netherlands (Chardon and Van der Hoek 2002), and 18% of greenhouse gas worldwide (Steinfeld et al. 2006) originate from livestock production.

Another issue with intensive livestock production is the emission of microorganisms. Although the contribution of livestock sector to microbial emission in ambient air is not clear, there is no doubt that emissions of microorganisms from livestock houses are extremely high compared to those from human environments (Seedorf et al. 1998). These emitted microorganisms and their transmission could pose health risks to people and animals on the farm, and to those living in the vicinity of the farm. After several outbreaks of infectious diseases in livestock production (Gloster et al. 2003) that could not be attributed solely to common transmission mechanisms (e.g. by direct contact or a vector), there was increased research interest in the possible airborne transmission of pathogenic microorganisms between farms. Some zoonotic pathogens have been recovered from the air of livestock production environments, e.g. *Coxiella burnetii* (Welsh et al. 1958), methicillin-resistant *Staphylococcus aureus* (Harper et al. 2010) and Avian Influenza virus (Power 2005). However, the extent to which airborne transmission may play a role in these outbreaks remains unclear, because of the lack of knowledge about the whole process of transmission (Figure 1), as well as about the air measuring protocols (sampling strategy and devices) .
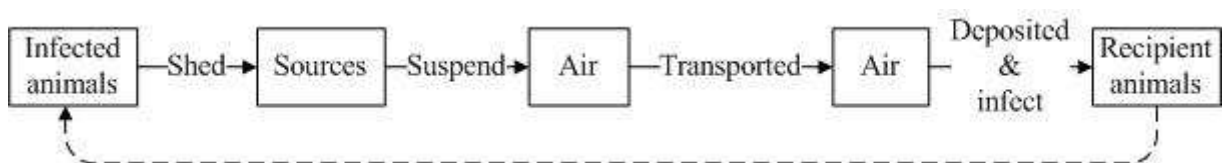

Figure 1. Illustration of the processes of airborne transmission.

AIRBORNE TRANSMISSION

Airborne transmission has been defined in different ways. Some researchers have tried to characterize airborne transmission as a long distance transmission that occurs only when the distance for infection is larger than 1 m (Brankston et al. 2007). This definition is to distinguish airborne transmission from short-distance droplet transmission, which represents an infection resulting from inhaling droplets containing microorganisms expelled by infected animals/humans (through coughing, sneezing, speaking or exhalation) within 1 m. However, the boundary of 1 m is rather arbitrary,

because the evaporation of the droplet greatly depends on its original size and specific environmental situation (temperature and humidity), which affect the distance it can be transmitted. In Chapter 1 of this thesis we therefore defined "airborne transmission" more generically, as "a transmission mechanism that causes infection in animals or humans via the inhalation of aerial pathogenic microorganisms".

Airborne transmission has been investigated with animal-to-animal models in lab-scale experiments and with farm-to-farm models in epidemiological studies. In lab-scale experiments, healthy and infected animals are separated physically but not aerially. The infection is attributed to airborne transmission when the healthy animals get infected. In this way, several microbial species have been proved to be airborne -transmittable (Berthelot-Herault et al. 2001; Brockmeier and Lager 2002).

It is difficult to extrapolate the findings from lab-scale experiments to interpret the farm-to-farm transmission, as meteorological and topological conditions may play important roles. For reasons of safety, is almost impossible to upscale animal-to-animal experiments and to investigate farm-to-farm airborne transmission empirically using pathogenic microorganisms and artificially infected and healthy farms. The information currently available on farm-to-farm transmission comes mainly from retrospective epidemiological studies. Although many of these studies have pointed out the possibility that transmission via airways could cause outbreaks of infectious diseases (Alexandersen et al. 2003; Gloster et al. 2003; Mikkelsen et al. 2003), airborne transmission is still an optional mechanisms for explaining certain outbreaks if commonly understandable mechanisms are not applicable. For instance, in the case of the epidemic of FMD in UK in 2001, Mikkelsen et al. (2003) reported that no means of disease spread other than airborne transmission could be identified as the route by which the disease could have been spread to the sixth outbreak farm, because there was no history of movement of animals, people or vehicles. However, their epidemiological model (RIMPUFF) also failed to predict an airborne viral concentration high enough to infect the animals on that farm. For lack of better alternative sources of knowledge, the culling of animals during the FMD outbreaks in 2001 was based on the epidemiological models. This strategy was sharply criticized by the veterinarians, who questioned the justification of using the predictive models as a basis for the governmental decision (Kitching et al. 2006).

It is difficult to judge objectively whether the culling strategy derived from these epidemiological models was wrong or not, because the epidemic was eventually successfully controlled. What can be concluded is that epidemiological models are still widely distrusted because they have never been convincingly validated. It is therefore essential carry out research to see whether models of airborne transmission can be validated by experiments combined with taking samples in real-life situations.

REDUCTION OF EMISSIONS OF AIRBORNE MICROORGANISMS AND DUST FROM LIVESTOCK HOUSES

Reduction techniques

The reduction techniques for emissions of airborne microorganisms and dust can be categorized according to which of two principles they are based on: control at source, or air purification. Techniques that aim to control particles at source are thought to be the most attractive solutions because in this way the particle concentrations inside livestock houses are also reduced, and thus the living and working conditions for animals and farmers are improved (Pearson and Sharples 1995). For instance, coating the feed with oil minimizes the likelihood of feed particles going into suspension. Gore et al. (1986) reported that adding 5% soybean oil to pig feed reduced concentrations of airborne bacteria by 27%, and settled dust by about 46%. Similar reductions were reported by Welford et al. (1992), who found that coating feed with 2% oil reduced inhalable dust concentrations by 31%. Various substances are suitable for coating feed: tallow, lecithin and lignin have been all used for feed coatings in livestock houses (Dawson ; Pearson and Sharples 1995).

Oil spraying is another option to reduce airborne microorganisms and dust (Kim et al. 2006). Aarnink et al. (2009) reported that the concentrations of PM_{10} were reduced by between 55% and 85% when daily rapeseed oil application rates in broiler rooms were increased from 6 to 24 ml m^{-2}. The downside of oil spraying technique is that litter becomes stickier and the incidence of footpad lesions of animals rises at a high oil spraying rate. It has therefore been recommended to limit the amount of oil applied to 16 ml m^{-2} per day in broiler houses (Aarnink et al. 2009).In the past, the oil was generally sprayed directly into the air. This kind of oil application creates extremely high aerosol concentrations during spraying, which may adversely affect respiratory health. Furthermore, it is unlikely that microorganism and dust concentrations can be reduced further by capturing the particles in airborne oil droplets, because these particles are so tiny relative to the volume of air inside the animal house (Takai 2007). Nowadays, the tendency is to spray oil nearer ground level.

Major reductions of concentrations and emissions of airborne dust can be achieved by the above-mentioned techniques because the dust mainly originates from surfaces in response to disturbance from the animals (Chapter 2). This may not be the case for airborne microorganisms. Though airborne microorganisms can go into suspension from similar sources as dust, they may also be expelled from the respiratory tract of animals through coughing, sneezing and expiration and then remain in the air (Hermann et al. 2008). The reduction principle of source control is still applicable for the former case, but for the latter case, air purification techniques are required.

The air purification technique of ionization charges airborne particles with negative ions, which are removed by attraction to positively charged surfaces. In poultry houses, this technique can reduce total dust concentrations by 13%-61% (Lyngtveit and Eduard 1997; Mitchell et al. 2000; Richardson et al. 2003; Mitchell et al. 2004), and PM_{10} by 36% and $PM_{2.5}$ by 10% (Cambra-Lopez et al. 2009).

However, the ionization technique was found to have no effect on the concentrations of total bacteria in broiler rooms (Cambra-Lopez et al. 2009).

The air scrubbing technique is increasingly being considered as an end-of-pipe solution for reducing emissions from livestock houses. This is not only so that the regulations on lowering pollutant emissions (e.g. dust, ammonia and odor) are met, but also because of the technique's potential to control the airborne transmission of highly pathogenic microorganisms between farms. The performance of single-stage air scrubbers (with acidified recirculation water or with biological conversion of ammonia and odorous compounds) has been investigated previously. Reductions were reported to be between 22% and 88% for total dust (Seedorf and Hartung 1999; Marsh et al. 2003; Aarnink et al. 2005), between 35% and 99% for ammonia, and between 29% and 49% for odor (Melse and Ogink 2005). The reduction of microorganisms by single-stage biological scrubbers, however, was not consistent. In some cases, the concentrations of microorganisms measured in the outgoing air of the biological scrubbers were even higher than those in the incoming air (Seedorf and Hartung 1999). Recently, multi-stage scrubbers were developed by combining the acid and biological scrubbing principles in one device. In Chapter 3, the three multi-stage scrubbers studied effectively reduced emissions of airborne total bacteria by between 46% and 85%, emissions of PM_{10} by between 61% and 93%, emissions of $PM_{2.5}$ by between 47% and 90%, and emissions of ammonia by between 70% and 100%. The removal efficiency of multi-stage scrubbers for odor was not investigated in our study, but merits future research. Compared with single-stage scrubbers, multi-stage scrubbers achieved more consistent reductions of ammonia, dust and microorganisms.

Considerations of end-of-pipe technique

Despite their consistent performance, multi-stage scrubbers still need to be improved and their overall economic and environmental impacts are not yet clear (the energy requirement for ventilators and pumps and how to treat the waste water, for instance).

The reduction of emission (46%-85%) of airborne total bacteria achieved by the multi-stage scrubbers is statistically significant (Chapter 3). The question is whether these reduction rates are sufficient. The concentration of total bacteria in the exhaust air of a pig house before the scrubber was found to be 5 $\log_{10}$ CFU m^{-3} (Chapter 3). Even the biggest reduction (85%) found in this study would not cause this level to drop by 1 log. The threshold limit values (TLVs) of indoor concentration of total bacteria recommended by several health organizations are 2-3 $\log_{10}$ CFU m^{-3} (ACGIH 1989; WHO 1990). For the air emitted from livestock houses not to exceed these TLVs, the air scrubber must achieve at least 99% reduction when the $\log_{10}$ CFU bacterial concentration in the air entering the scrubber is 5 $\log_{10}$ CFU m^{-3}. This seems to be an impossible goal for the multi-stage scrubbers investigated in our study.

The additive used in air scrubbers requires investigation. At the moment, sulfuric acid is the only additive permitted in practical acid scrubbers under Dutch law. Only a few other additives have been

tested in scrubbers for their reduction of airborne microorganisms. Peracetic acid showed promising reduction effects on airborne *E. faecalis* (Aarnink et al. 2005). In principle, all chemical additives that effectively inactivate microorganisms in water are candidate additives for air scrubbers. However, there is a lack of information about of the performances of these additives on microbial reduction and therefore further study is needed. Priority should be given to establishing the costs and safety of additives, as well as the ease of handling the waste water.

Melse and Ogink (2005) estimated that the investment costs of a single-stage acid scrubber were $42 animal-place^{-1} for growing-finishing pigs and $1.3 animal-place^{-1} for broilers, whereas the investment costs of a biotrickling filter (a certain type of biological scrubbers) were $45 animal-place^{-1} for growing-finishing pigs and $1.5 animal-place^{-1} for broilers. They reported that to achieve 95% ammonia reduction, the total operational costs for an acid scrubber were $14.8 pig-place^{-1} year^{-1}, and $0.5 broiler-place^{-1} year^{-1}. With 70% ammonia reduction, the costs for a biotrickling filter were $14.3 pig-place^{-1} year^{-1} and $0.5 broiler-place^{-1} year^{-1}. The costs for the discharge water treatment were not included because the required amount of water and the characteristics of the water vary greatly, depending on the local situation. Melse and Ogink argued that given that most of the operational costs are incurred from the electricity and chemicals used, the options for reducing costs are more efficient use of electricity (e.g. powering down during periods of low pollutant load) and the use of cheaper but equally effective chemical additives. Multi-stage scrubbers are probably more costly than the single-stage scrubbers per animal-place, but exact cost figures are not available yet.

A shortcoming of the study reported in Chapter 3 was that all the measurements were done within a short period during the winter, which means that the scrubbers were not tested at high ventilation rates. Measurements during a longer time frame which includes periods with maximum ventilation rates will give a full picture of the efficiencies of the multi-stage scrubbers.

Controlling epidemics of highly contagious diseases through airborne transmission by air scrubbers

The massive animal slaughter in FMD epidemics emphasizes the need for effective control techniques to prevent or mitigate airborne transmission of pathogenic microorganisms from infected farms to healthy farms. In epidemiology, an epidemic is defined as outbreaks that affect a nonzero fraction of the population in the limit of large system (Newman 2002). Whether or not an epidemic occurs is determined by the reproductive ratio R, which is defined as the average number of secondary cases arising from the introduction of one typical primary case into a fully susceptible population (Diekmann et al. 1990). An epidemic occurs when R is higher than 1; otherwise, the disease dies out or becomes endemic. Therefore, successful prevention of an epidemic depends on whether a certain control technique can reduce the R ratio below 1. It is hypothesized here that a well-designed multi-stage air scrubber can reduce the emission of pathogenic microorganisms from an infected farm. This hypothesis is supported by the fact that many of the airborne microorganisms are associated with dust

particles which can be effectively reduced by the air scrubbers (Chapter 3). If the hypothesis is true, the spread of pathogenic microorganisms to recipient farms would be correspondingly reduced, and thus the probability of the healthy animals on these farms coming into contact with the microorganisms will also decrease, thereby reducing the chance of infection. When emission of pathogenic microorganisms is reduced by an air scrubber to a certain level that results in an $R < 1$, the epidemic can be prevented. However, little is known about the direct effectiveness of the air scrubber in reducing certain pathogenic microorganisms, and about the extent to which any such a reduction might prevent or mitigate the epidemic. More research on this is necessary.

DUST SAMPLING IN LIVESTOCK HOUSES

Dust in livestock houses is different from dust in working place/ambient air (WA) in at least two ways: concentration and particle size distribution. The dust concentration in livestock houses is very much higher than the dust concentrations in WA. In Chapter 4 we reported measuring 1126 µg m^{-3} of PM_{10} in livestock houses (poultry and pigs) and only 14 µg m^{-3} in WA. The average $PM_{2.5}$ concentration was 39 µg m^{-3} in livestock houses, and 11 µg m^{-3} in WA. The mass of small particles accounts for only low proportions of PM_{10} dust in livestock houses. In poultry and pig houses, 5%-9% of PM_{10} was $PM_{2.5}$, while this was about 80% in WA (Chapter 4). The specific characteristics of dust in livestock houses make it necessary to have a special sampling protocol, which should include description of the sampling strategy and of the validated sampler.

Sampling strategy

A good strategy for dust sampling establishes the location, duration and techniques of the sampling on the basis of the research purpose. Isokinetic sampling is the ideal sampling method. It requires the sampler inlet to be aligned to face their direction of the air flow and the air velocity within the sampler head to be the same as the ambient air velocity (Zhang 2004). In practice, isokinetic sampling is very difficult to achieve because of the instability of air flow patterns and imperfection of dust samplers, e.g. shallow cut-off characteristics (Liu and Pui 1981). The current recommendations aim to reduce the sampling bias in non-isokinetic sampling by specifying the range of conditions under which the sampling may be performed. When human health is of concern, the sampling should be carried out near the human breathing zone (Ouellette et al. 1999). For animals, the height above the ground that dust should be collected is 30-40 cm for pigs, 10-25 cm for poultry and for cattle, at animal shoulder height (Topisirovic 2003; Kim et al. 2007). When dust emissions are of interest, the best sampling location is in or near the air outlet. Twenty-four-hour measurements are required to be able to calculate the average daily dust concentration (European Commission 1999; European Commission 2005; US EPA 2006), because many studies have shown that dust concentrations fluctuate greatly throughout a day (Takai et al. 1998; Kim et al. 2005). The fluctuation of dust concentrations within a day may be measured in a successive sampling using an online optical sampler. Other aspects to be

considered in the sampling strategy are practical considerations, e.g. ease of handling, sampling reliability and costs.

Sampler

Unlike the US EPA, which has already prepared a list of reference and equivalent samplers, the European Commission has specified only the reference sampler for PM sampling and a validation method for equivalent samplers in ambient air. In Chapter 4 we reported that the EU reference sampler with an impaction pre-separator is not suitable for $PM_{2.5}$ sampling in livestock houses, due to the severe overloading problem, and therefore equivalent samplers that can cope with high dust loads should be recommended. We followed the EU validation method (European Commission 2005) to validate the sampler with a cyclone pre-separator with respect to its tolerance of high dust loads and its equivalence to the EU reference sampler. We found that when tested in layer rooms with high dust concentrations, the PM_{10} and $PM_{2.5}$ samplers with a cyclone pre-separator could cope with high loads of dust. Furthermore, both PM_{10} and $PM_{2.5}$ cyclone pre-separators were equivalent to the impaction pre-separator in WA. When these pre-separators were used in environments with high dust concentrations (> 100 µg m^{-3}), the sampler with the PM_{10} cyclone pre-separators consistently collected more PM_{10} particles than the sampler with the PM_{10} impaction pre-separator. These values, however, could be corrected with a calibration factor (0.83). The validation could not be performed for the $PM_{2.5}$ cyclone pre-separator in livestock houses, because the overloading problem made impaction sampler unsuitable as a reference sampler for these environments.

So far, no standard protocol has been formulated for PM measurements in livestock houses. Our results showed that the cyclone pre-separators were less vulnerable for high dust concentrations and were the reference equivalent to impaction pre-separators. They are therefore suggested as promising sampling principles for such applications. For a sampler to be suitable as a reference technique, it must have a sharp cut-off collection curve. More research needs to be done to check the size distribution of the particles collected by a sampler with cyclone pre-separator.

SAMPLING OF MICROORGANISMS IN LIVESTOCK HOUSES

When airborne microorganisms are to be sampled, several of the aforementioned strategies for dust sampling can be applied (e.g. location selection and practical considerations), but several additional factors must be addressed. Firstly, whereas dust is inert, microorganisms are alive and must therefore be collected in a way that preserves their viability during sampling, so as to avoid underestimating their aerial concentrations. However, it is generally thought that current sampling techniques (impaction, impingement, cyclone forces or filtration) cannot recover all microorganisms from the air due to physical loss and biological inactivation. As a consequence, the loss and inactivation of microorganisms during sampling with these techniques (i.e. the physical and biological efficiency of the techniques) have to be investigated, to obtain calibration factors for correcting to the true aerial

concentration of microorganisms. Secondly, it is necessary to sample specific microbial species. To date, microbial samplings have generally been done to quantify the concentrations of total bacteria or fungi in livestock houses. These samplings do indeed provide overview evaluations of the exposure hazard. However, such evaluations are too rough because the microbial species vary in their pathogenicity. Air sampling of specific microbial species provides a more precise evaluation, and is required for studies of airborne transmission of pathogenic microorganisms. The challenge in sampling one microbial species is that its concentration is probably so low that it becomes difficult to detect with a given sampling technique (Chapter 7). Thirdly, the interpretation of the results of the microbial counts may differ, depending on which sampling and analyzing techniques were used. For instance, samplers based on the impaction technique (e.g. Andersen six-stage impactor) collect microorganisms on agar plates which are cultured immediately after sampling. The microbial count obtained with this technique is "the number of particles each of which contains at least one culturable microorganism" (Zhao et al. 2011). Other techniques (e.g. All Glass Impinger, OMNI-3000) collect microorganisms in liquid media that are decimally diluted and spread on agar plates for culturing. The final microbial count is "the number of culturable microorganisms" (Zhao et al. 2011). Previous studies have presented the results in either way, and it remains unclear which method best indicates the risk to human and animal health (i.e. do recipients develop more severe health problems when they ingest more "microbial particles", or when they ingest more "microorganisms"?).

Assessing the efficiency of bioaerosol samplers

Some of the previous studies that have assessed the efficiency of bioaerosol samplers have compared the efficiencies of different samplers by operating them side-by-side in an environment of unknown microbial concentration (Henningson et al. 1982; Thorne et al. 1992; Engelhart et al. 2007; Zhen et al. 2009). This method only ranks the relative efficiencies of samplers. Whether the amounts of microorganisms in the samples correctly represent what is in the air is not known. To overcome these shortcomings, we aerosolized known amounts of microorganisms (*E. faecalis*, *E. coli*, *C. jejuni*, *M. synoviae*, and Gumboro vaccine virus) using a tracer in an air space, where samplers were operated (Chapters 5, 6 and 7). By doing this, their absolute efficiencies were obtained. In such an experimental setup, microorganisms were combined with the tracer, aerosolized into air (pre-sampling process), collected by the sampler (sampling process), and processed for microbial analysis (post-sampling process). Because only the loss in the sampling process is ascribed to the efficiency of a bioaerosol sampler, we investigated the losses of microbial viability in the non-sampling processes in order to formulate correction factors for calculating the absolute sampling efficiency in the aerosol experiment. The results showed that some microbial species were vulnerable to the non-sampling processes, e.g. *C. jejuni* culturability was affected by tracer.

Our experiments demonstrated that the physical tracer uranine was reliable for assessing sampler efficiency. When an external tracer is used it is necessary to do a survival test before tracer-

microorganisms are aerosolized in combination with suspension, to ensure that the tracer does not influence the microbial viability. The polymerase chain reaction (PCR) technique allows the microorganisms to be quantified by their genetic material. Therefore no additional tracer is required and safety control is no longer necessary. However, the suitability of using genetic material quantified by the PCR technique as microbial tracer has seldom been investigated. A problem when using genetic material as tracer might be the potential degradation of this material (Wang et al. 2001; Verreault et al. 2008). This needs to be studied before the PCR techniques can be used instead of a tracer.

A popular vaccination method in poultry industry is the wet aerosolization of vaccine suspension in bird houses. However, little research has been carried out on its effectiveness. In our study (Chapter 7), it was found that 95% of the Gumboro vaccine virus was inactivated due to the stress caused by aerosolization at 20°C (Chapter 5). The inactivation becomes more pronounced when the vaccine virus is aerosolized at a higher temperature (99.9% at 30°C: Zhao et al., unpublished data). These findings suggest that the effectiveness of current vaccination is probably greatly compromised, so new methods which may preserve the viral viability during aerosolization should be developed. One alternative vaccination method would be aerosolization of dry vaccine virus to the birds (Corbanie et al. 2007; Corbanie et al. 2008).

Efficiency and detection limit of bioaerosol samplers

The physical and biological efficiencies of the Andersen impactor, the AGI-30, the OMNI-3000 and the MD-8 on collecting aerosolized *E. faecalis*, *E. coli*, *C. jejuni*, *M. synoviae* and Gumboro vaccine virus were evaluated in Chapters 5, 6 and 7. In these experiments, the physical efficiencies of bioaerosol samplers were evaluated in a still air environment (air velocity = 0 m s^{-1}). Grinshpun et al. (1994) reported that increasing the air velocity from 0 to 500 cm s^{-1} did not significantly affect the physical efficiency of the AGI-30 for particles ranging from 1 to 10 µm. However, the entrance efficiency of the Andersen impactor on these particles increased if the sampler inlet faced into the air flow and decreased if the sampler inlet was perpendicular to the air flow. In their study, entrance efficiency was defined as a ratio between the concentration of particles that have passed the short entrance region of a sampler's inlet and the actual concentration of particles in the undisturbed environment. Therefore, one should be aware that when using the Andersen impactor in practice, its physical efficiency might differ depending on the velocity and direction of the ambient air flow.

The total sampling efficiency (combination of physical and biological efficiencies) and the detection limit were calculated from the efficiency data and are listed in Table 1. This information may be helpful for selecting samplers suitable for practical measurements. The Andersen impactor and the AGI-30 are suitable for sampling all microbial species (*E. faecalis*, *E. coli*, *C. jejuni*, *M. synoviae* and Gumboro vaccine virus) because their total efficiencies are high. The MD8 is suitable for sampling *E. faecalis*, *M. synoviae* and Gumboro vaccine virus, but not *E. coli* and *C. jejuni*. Although the OMNI-3000 has low sampling efficiencies of 49% for *E. faecalis*, *E. coli* and *M. synoviae*, and

14% for Gumboro vaccine virus, it could still be a suitable sampler because its high air flow rate gives a low detection limits. The OMNI-3000 cannot be used for *C. jejuni* because this species would be seriously inactivated by the sampling stress. Data on the recoveries of bacteria in the post-sampling processes can be combined with total sampling efficiency to yield measuring corrector, and this can also be used to guide the correcting the airborne microbial concentrations collected by the samplers.

For a 2 min sampling, the detection limits of the bioaerosol samplers to the five investigated microbial species are in the range from 2.5 to 5.4 $\log_{10}$ CFU/EID$_{50}$ m^{-3}. It cannot be concluded here whether these detection limits are low enough for practical measurements in livestock production systems because the concentration of specific microbial species is unpredictable. But a detection limit of 5.4 $\log_{10}$ CFU m^{-3} (for *C. jejuni*) might be too high because it is at a comparable level as the total microbial concentration in livestock production systems (Chapter 2). In order to reach a lower detection limit, one can operate the bioaerosol samplers for longer sampling duration. However, it should be noted that the detection limit might be not lowered with a factor 2 (e.g. decreasing from 5.4 to 3.4 $\log_{10}$ CFU m^{-3}) when the sampling duration is increased with a factor 2 (e.g. increasing from 2 to 200 min), because the sampling duration and the amount of collected microorganisms are not linearly related (Durand et al. 2002).

Table 1. Total sampling efficiency and detection limit of bioaerosol samplers.

	E. faecalis	*E. coli*	*C. jejuni*	*M. synoviae*	**Gumboro vaccine virus**
Sampling efficiency (%)[a]					
Andersen[b]	100	100	100	100	100
AGI-30	100	100	100	100	100
OMNI-3000	49	49	0.5	49	14
MD8	100	38	2	100	100
Detection limit[c]					
Andersen]	3.9	3.9	3.7	3.8	4.1
AGI-30	3.9	4.2	3.8	4.0	3.3
OMNI-3000	2.5	2.5	4.5	2.7	2.5
MD8	4.1	4.4	5.4	4.3	2.9

[a] 100% means that in our study the measured efficiency was not significantly different from 100%.

[b] Physical and biological efficiencies of the Andersen impactor were set to 100% because it collected similar amounts of culturable bacteria as the AGI-30.

[c] DL was calculated based on a 2 min sampling duration. The unit of DL is $\log_{10}$ CFU m^{-3} for bacteria, and $\log_{10}$ egg infective dose 50% (EID$_{50}$) m^{-3} for virus.

Air sampling for *Campylobacter*

In the study on the transmission of *Campylobacter* from physically separated, infected broilers to susceptible broilers, neither the Andersen impactor, nor the AGI-30, nor the OMNI-3000 collected any culturable *Campylobacter*, and therefore airborne transmission between broilers was not demonstrated (Chapter 7). The failure to prove transmission was probably because the minimum detection limits (at

sampling durations of 10-30 min) of the bioaerosol samplers exceeded the concentration of culturable *Campylobacter* in the air. Using the sampling scheme for *Campylobacter* as described in Chapter 7, the calculated detection limits were 530 CFU m^{-3} for the Andersen impactor and 440 CFU m^{-3} for the AGI-30. Assuming that the infection followed the single-hit model and only 9 CFU was reported to be able to infect the chickens (Ruiz-Palacios et al. 1981), these detection limits were possibly too high. Although the OMNI-3000 collected airborne bacteria at a high flow rate, the calculated detection limit (6800 CFU m^{-3}) was even higher than the other two samplers because the efficiency of the OMNI-3000 for *Campylobacter* was extremely low (0.5%). In order to avoid incorrect safety reports, the sampling scheme including the samplers should be improved by doing research to find a lower minimum detection limit.

HALF-LIFE OF AIRBORNE MICROORGANISMS

The half-life of microorganisms is a critical parameter in airborne transmission models and has been extensively studied for various microbial species in different climate conditions. The half-life has often been determined by comparing the amounts of airborne microorganisms collected at two different moments after aerosolization (Dinter and Muller 1988; Weesendorp et al. 2008). The interval between the two sampling moments should be set properly. If it is too long, the second sampling will not recover any microorganisms; if it is too short, the amounts of microorganisms collected at the two sampling moments will not differ, and in both cases it will be impossible to calculate the half-life. In Chapters 6 and 7, we set the sampling interval to 20 min for all microbial species when investigating their half-lives. The half-lives ranged from 4.0 (for *C. jejuni*) to 43.3 (for *E. faecalis*) min (Table 2). This means still half of the bacteria could reach the neighboring farm at 1200-13000 meters in a viable state, assuming a wind speed of 300 m min^{-1} (gentle breeze). However, these distances are estimated with the optimal scenario of airborne transmission. In reality, many other factors may play role on the survival of microorganisms in airborne transmission, e.g. open air factor, variation in wind direction, and dilution effect etc.

Table 2. Half-life of the airborne microorganisms.

Microorganisms	Half-life[a] (min)
E. faecalis	43.3 (± 14.7)
E. coli	21.2 (± 10.6)
C. jejuni	4.0 (± 1.3)
M. synoviae	26.7 (± 16.5)
Gumboro vaccine virus	11.9 (± 2.0)

[a] The number of replication is 14 for E. faecalis, 12 for E. coli, 8 for C. jejuni, 7 for M. jejuni, and 22 for Gumboro vaccine virus.

We calculated 13 negative half-lives for the microbial species which had high survival ability in the air (two for *E. faecalis*, five for *M. synoviae* and six for Gumboro vaccine virus). These negative

values were excluded from calculation of the average half-lives; however, they represented infinite survival time because no decay was detected during the 20 min interval. This implies that the survival of these microbial species might still be underestimated. No negative half-lives were found within the replicates for *E. coli* and *C. jejuni*. These findings support our abovementioned contention that in future research the sampling interval should be set properly and adjusted according to the survival ability of the microbial species.

In the experiments reported in this thesis, we studied the half-lives of microorganisms at 20°C temperature and 70%-90% relative humidity only. In real life, microorganisms may be subjected to various climate conditions during long distance airborne transmission between farms. Supplementary information is therefore needed on microbial survival at a wider range of temperature and humidity.

MAIN CONCLUSIONS

- Multi-stage air scrubbers reduce pig house emissions of total bacteria (by between 46% and 85%), PM_{10} (by between 61% and 93%), $PM_{2.5}$ (by between 47% and 90%), and ammonia (by between 70% and 100%).

- The EU reference impaction pre-separator used for sampling ambient air is not suitable for sampling $PM_{2.5}$ in livestock houses, because of a problem with overloading. The cyclone pre-separator proved to be equivalent to the impaction pre-separator in ambient air and it is more resistant to high dust concentration in livestock houses than the impaction pre-separators. It is therefore recommended to use the sampler with cyclone pre-separator for sampling PM in livestock houses.

- The efficiency of a bioaerosol sampler can be investigated by collecting the aerosolized microorganisms with a fluorescent tracer. In such an experiment, microbial viability (or multiplication) may be lost in several non-sampling processes, e.g. tracer-induced loss, loss during aerosolization, and loss in air sample handling. These losses in the non-sampling processes cannot be ascribed to the inefficiency of the sampler and therefore should be determined before the efficiency assessment and should be excluded when calculating the sampling efficiencies of bioaerosol samplers.

- Bioaerosol samplers vary in their sampling efficiencies for airborne microorganisms. Their biological efficiency is affected by an interaction between the sampler type and the microbial species.

- The gram-positive bacterium *E. faecalis* (43.3 min), and the bacterium without cell wall, *M. synoviae* (26.7 min), have longer half-lives in the air (at 20°C and RH 80%-85%) than the gram-negative bacteria *E. coli* (21.2 min) and *C. jejuni* (4.0 min). The half-life of Gumboro vaccine virus at 20°C and RH 70% is 11.9 min.

- The finding that a large proportion of Gumboro vaccine virus (95%) can be inactivated during aerosolization implies that the efficacy of current vaccination practice in the poultry industry is greatly reduced by wet-spraying the vaccine.

- In the experiment on airborne transmission of *Campylobacter* in broilers, the Andersen impactor, the AGI-30 and the OMNI-3000 could not detect culturable *Campylobacter* in the air, probably because their detection limits are higher than the bacterial concentrations.

- Although short distance (animal-to-animal) airborne transmission has been proven, the mechanisms of long distance (farm-to-farm) airborne transmission is still not well understood. The work reported in this thesis has made it clear that detection in the air is generally not sensitive enough for a good picture to be obtained of the possible role of airborne transmission.

- Although short distance (animal-to-animal) airborne transmission has been attributed as a possible explanation for infection, the mechanism of long distance (farm-to-farm) airborne transmission is still not very well understood.

RECOMMENDATIONS FOR FUTURE RESEARCH TOPICS

- Long distance airborne transmission: airborne transmission of pathogenic microorganisms between livestock farms requires further research. Studies should focus on elucidating the dispersion of microorganisms around the source farms.

- Combining mitigation techniques: these techniques (combining techniques of "control at source" and "air purification") have the potential to effectively reduce both the concentrations and the emissions of airborne microorganisms and dust in livestock production systems. However, the investment and operational costs and possible side effects of these combined techniques need to be adequately evaluated.

- Additives in acid scrubbers: in addition to sulfuric acid, alternative acid additives in the scrubbers should be further investigated. These additives should be used during outbreaks or threats of outbreaks of highly infectious diseases and should be able to effectively inactivate the highly pathogenic microorganisms concerned.

- Microbiological tracer: the PCR technology quantifies the genetic material from microorganisms themselves, which would extinguish the doubts about the distinctive size distributions between physical tracers and microorganisms in aerosols. The possible use of this microbiological tracer in aerosolization studies should be tested and validated; studies should be done on the decay of the gene, the variations in PCR tests and detection limits, for example.

- New bioaerosol samplers: microorganisms present in low concentrations in the air might still be able to spread diseases, which is why it is important to develop bioaerosol samplers with low detection limits. These samplers should have high physical and biological efficiencies, and also high sampling flow rates.

LITERATURE CITED

Aarnink, A. J. A., W. J. M. Landman, et al. (2005). Systems for eliminating pathogens from exhaust air of animal houses. Proc. ASAE Seventh International Symposium Livestock Environment VII, Beijing, China.

Aarnink, A. J. A., J. Mosquera, et al. (2009). Options for dust reduction from poultry houses. CIGR International Symposium on 'Agricultural Technologies in a Changing Climate', Brisbane, Australia.

Aarnink, A. J. A., R. F. M. M. Roelofs, et al. (1999). Dust sources in animal houses. Proceedings on dust control in animal production facilities, Horsens, Denmark, Danish institute of Agricultural Sciences.

Aarnink, A. J. A., T. van Hattum, et al. (2007). Reduction of fine dust emission by combi-scrubber of Big Dutchman. Lelystad, the Netherlands, Animal Sicences Group of Wageningen UR: 16.

Abadie, M., K. Limam, et al. (2001). Indoor particle pollution: effect of wall textures on particle deposition. Building and Environment 36: 821-827.

ACGIH (1989). Guidelines for assessment of bioaerosols in the indoor environment. Cincinnati, Ohio, American Conference of Governmental Industrial Hygienists, Cincinnati, Ohio.

Adams, D. J., J. C. Spendlove, et al. (1982). Aerosol stability of infectious and potentially infectious reovirus particles. Applied and Environmental Microbiology 44: 903-908.

Agranovski, V., Z. Ristovski, et al. (2004). Size-selective assessment of airborne particles in swine confinement building with the UVAPS. Atmospheric Environment 38: 3893-3901.

Al Homidan, A., J. F. Robertson, et al. (1997). Effect of temperature, litter and light intensity on ammonia and dust production and broiler performance. British Poultry Science 38: S5-S17.

Alexandersen, S. and A. I. Donaldson (2002). Further studies to quantify the dose of natural aerosols of foot-and-mouth disease virus for pigs. Epidemiology and Infection 128: 313-323.

Alexandersen, S., R. P. Kitching, et al. (2003). Clinical and laboratory investigations of five outbreaks of foot-and-mouth disease during the 2001 epidemic in the United Kingdom. The Veterinary Record 152: 489-496.

Alford, R. H., J. A. Kasel, et al. (1966). Human influenza resulting from aerosol inhalation. Proceedings of the Society for Experimental Biology and Medicine 122: 800-804.

Allos, B. M. (2001). Campylobacter jejuni infections: Update on emerging issues and trends. Clinical Infectious Diseases 32: 1201-1206.

Andersen, A. A. (1958). New sampler for the collection, sizing, and enumeration of viable airborne particles. Journal of Bacteriology 76: 471-484.

Andersson, A. M., N. Weiss, et al. (1999). Dust borne bacteria in animal sheds, schools and children's day care centres. Journal of Applied Microbiology 86: 622-634.

Appleby, M. C. and B. O. Hughes (1991). Welfare of laying hens in cages and alternative systems: Environmental, physical and behavioural aspects. World's Poultry Science Journal 47: 109-128.

Literature Cited

Attwood, P., R. Brouwer, et al. (1987). A study of the relationship between airborne contaminants and environmental factors in dutch swine confinement buildings. American Industrial Hygiene Association Journal 48: 745-751.

Auckenthaler, A., G. Raso, et al. (2002). Particle transport in a karst aquifer: natural and artificial tracer experiments with bacteria, bacteriophages and microspheres. Water Science and Technology 46: 131-138.

Baird-Parker, A. C. (1962). The occurrence and enumeration, according to a new classification, of micrococci and staphylococci in bacon and on human and pig skin. Journal of Applied Microbiology 25: 352-361.

Bakutis, B., E. Monstviliene, et al. (2004). Analyses of airborne contamination with bacteria, endotoxins and dust in livestock barns and poultry houses. Acta Veterinaria Brno 73: 283-289.

Baldocchi, D. D., B. B. Hincks, et al. (1988). Measuring biosphere-atmosphere exchanges of biologically related gases with micrometeorological methods. Ecology 69: 1331-1340.

Banhazi, T. M., J. Seedorf, et al. (2008). Identification of the risk factors for high airborne particle concentrations in broiler buildings using statistical modelling. Biosystems Engineering 101: 100-110.

Banhazi, T. M., J. Seedorf, et al. (2008). Identification of risk factors for sub-optimal housing conditions in Australian piggeries: Part 2. Airborne pollutants. Journal of Agricultural Safety and Health 14: 21-39.

Barlow, D. F. and A. I. Donaldson (1973). Comparison of the aerosol stabilities of foot-and-mouth disease virus suspended in cell culture fluid or natural fluids. Journal of General Virology 20: 311-318.

Barron, E. S. G. (1954). The role of free radicals and oxygen in reactions produced by ionizing radiations. Radiation Research 1: 109-124.

Baskerville, A. (1981). Mechanisms of infection in the respiratory tract. New Zealand Veterinary Journal 29: 235-238.

Baxby, D. and R. C. S. Woodroffe (1965). The location of bacteria in skin. Journal of Applied Microbiology 28: 316-321.

Beery, J. T., M. B. Hugdahl, et al. (1988). Colonization of gastrointestinal tracts of chicks by Campylobacter jejuni. Applied and Environmental Microbiology 54: 2365-2370.

Benbough, J. E. (1967). Death mechanisms in airborne Escherichia coli. Microbiology 47: 325-333.

Benbough, J. E. (1971). Some factors affecting the survival of airborne viruses. Journal of General Virology 10: 209-220.

Berendt, R. F. and E. L. Dorsey (1971). Effect of simulated solar radiation and sodium fluorescein on the recovery of Venezuelan equine encephalomyelitis virus from aerosols. Applied and Environmental Microbiology 21: 447-450.

Berrang, M. E., K. J. Northcutt, et al. (2004). The contribution of airborne contamination to Campylobacter counts on defeathered broiler carcasses. Journal of Applied Poultry Research 13: 1-4.

Berthelot-Herault, F., M. Gottschalk, et al. (2001). Experimental airborne transmission of Streptococcus suis capsular type 2 in pigs. Veterinary Microbiology 82: 69-80.

Besser, T. E., B. L. Richards, et al. (2001). Escherichia coli O157:H7 infection of calves: Infectious dose and direct contact transmission. Epidemiology and Infection 127: 555-560.

Brachman, P. S., R. Ehrlich, et al. (1964). Standard sampler for assay of airborne microorganisms. Science 144: 1295.

Brankston, G., L. Gitterman, et al. (2007). Transmission of influenza A in human beings. The Lancet Infectious Diseases 7: 257-265.

Brockmeier, S. L. and K. M. Lager (2002). Experimental airborne transmission of porcine reproductive and respiratory syndrome virus and Bordetella bronchiseptica. Veterinary Microbiology 89: 267-275.

Brown, J. L., J. W. Dominik, et al. (1982). Airborne Survival of Rift Valley Fever Virus. Maryland, USA, Army Medical Research Institute of Infectious Diseases.

Brown, J. S., K. L. Zeman, et al. (2002). Ultrafine particle deposition and clearance in the healthy and obstructed lung. American Journal of Respiratory and Critical Care Medicine 166: 1240-1247.

Brunekreef, B. and S. T. Holgate (2002). Air pollution and health. The Lancet 360: 1233-1242.

Bull, S. A., V. M. Allen, et al. (2006). Sources of Campylobacter spp. colonizing housed broiler flocks during rearing. Applied and Environmental Microbiology 72: 645-652.

Bundy, D. S. (1984). Rate of dust decay as affected by relative humidity, ionization and air movement. Transactions of the ASAE 27: 865-870.

Burton, N. C., S. A. Grinshpun, et al. (2007). Physical collection efficiency of filter materials for bacteria and viruses. Annals of Occupational Hygiene 51: 143-151.

Buswell, C. M., Y. M. Herlihy, et al. (1998). Extended survival and persistence of Campylobacter spp. in water and aquatic biofilms and their detection by immunofluorescent-antibody and -rRNA staining. Applied and Environmental Microbiology 64: 733-741.

Buzcu, B., M. P. Fraser, et al. (2003). Source identification and apportionment of fine particulate matter in Houston, TX, using positive matrix factorization. Environmental Engineering Science 20: 533-545.

Cafruny, W. A. and D. E. Hovinen (1988). The relationship between route of infection and minimum infectious dose: studies with lactate dehydrogenase-elevating virus. Journal of Virological Methods 20: 265-268.

Cambra-Lopez, M. (2010). Control of particulate matter emissions from poultry and pig houses. Valencia, Spain, Universidad Politécnica de Valencia. PhD dissertation.

Cambra-Lopez, M., A. J. A. Aarnink, et al. (2010). Airborne particulate matter from livestock production systems: A review of an air pollution problem. Environmental Pollution 158: 1-17.

Cambra-Lopez, M., A. Winkel, et al. (2009). Ionization for reducing particulate matter emissions from poultry houses. Transactions of the ASABE 52: 1757-1771.

Cappelier, J. M., C. Magras, et al. (1999). Recovery of viable but non-culturable Campylobacter jejuni cells in two animal models. Food Microbiology 16: 375-383.

CBS (2010). Statistical yearbook 2010, Statistics Netherlands. http://www.cbs.nl.

Chang, C. W., H. Chung, et al. (2001). Exposure of workers to airborne microorganisms in open-air swine houses. Applied and Environmental Microbiology 67: 155-161.

Chardon, W. J. and K. W. Van der Hoek (2002). Berekeningsmethode voor de emissie van fijn stof vanuit de landbouw [Calculation of particulate matter emissions from agriculture]. Bilthoven, the Netherlands, The National Institute for Public Health and the Environment (RIVM).

Chen, B. T., H. C. Yeh, et al. (1992). Evaluation of an environmental reaction chamber. Aerosol science and technology 17: 9-24.

Chen, M., J. Chen, et al. (2008). Agricultural phosphorus flow and its environmental impacts in China. Science of the Total Environment 405: 140-152.

Chiba, L. I., E. R. Peo, Jr., et al. (1985). Effect of dietary fat on pig performance and dust levels in modified open-front and environmentally regulated confinement buildings. Journal of Animal Science 61: 763-781.

Cho, J. G., S. A. Dee, et al. (2006). The impact of animal age, bacterial coinfection, and isolate pathogenicity on the shedding of Porcine reproductive and respiratory syndrome virus in aerosols from experimentally infected pigs. Canadian Journal of Veterinary Research 70: 297-301.

Clark, P. C. and J. B. McQuitty (1988). Air quality in farrowing barns. Canadian Agricultural Engineering 30: 173-178.

Clark, S., R. Rylander, et al. (1983). Airborne bacteria, endotoxin and fungi in dust in poultry and swine confinement buildings. American Industrial Hygiene Association Journal 44: 537-541.

Corbanie, E. A., M. G. Matthijs, et al. (2006). Deposition of differently sized airborne microspheres in the respiratory tract of chickens. Avian Pathology 35: 475-485.

Corbanie, E. A., J. P. Remon, et al. (2007). Spray drying of an attenuated live Newcastle disease vaccine virus intended for respiratory mass vaccination of poultry. Vaccine 25: 8306-8317.

Corbanie, E. A., C. Vervaet, et al. (2008). Vaccination of broiler chickens with dispersed dry powder vaccines as an alternative for liquid spray and aerosol vaccination. Vaccine 26: 4469-4476.

Cormier, Y., G. Tremblay, et al. (1990). Airborne microbial contents in two types of swine confinement buildings in Quebec. American Industrial Hygiene Association Journal 51: 304-309.

Cornick, N. A. and A. F. Helgerson (2004). Transmission and infectious dose of Escherichia coli O157:H7 in swine. Applied and Environmental Microbiology 70: 5331-5335.

Cox, C. S. (1966). The survival of Escherichia coli sprayed into air and into nitrogen from distilled water and from solutions of protecting agents, as a function of relative humidity. Journal of General Microbiology 43: 383-399.

Cox, C. S. (1968). The aerosol survival of Escherichia coli B in nitrogen, argon and helium atmospheres and the influence of relative humidity. Journal of General Microbiology 50: 139-147.

Cox, C. S. (1970). Aerosol survival of Escherichia coli B disseminated from the dry state. Applied and Environmental Microbiology 19: 604-607.

Cox, C. S. (1971). Aerosol survival of Pasteurella tularensis disseminated from the wet and dry states. Applied and Environmental Microbiology 21: 482-486.

Cox, C. S. (1989). Airborne bacteria and viruses. Science Progress 73: 469-499.

Cox, C. S. and L. J. Goldberg (1972). Aerosol survival of Pasteurella tularensis and the influence of relative humidity. Applied and Environmental Microbiology 23: 1-3.

Cray, W. C. and H. W. Moon (1995). Experimental infection of calves and adult cattle with Escherichia coli O157:H7. Applied and Environmental Microbiology 61: 1586-1590.

Crook, B., J. F. Robertson, et al. (1991). Airborne dust, ammonia, microorganisms, and antigens in pig confinement houses and the respiratory health of exposed farm workers. American Industrial Hygiene Association Journal 52: 271-279.

Curtis, S. E., J. G. Drummond, et al. (1975). Relative and qualitative aspects of aerial bacteria and dust in swine houses. Journal of Animal Science 41: 1512-1520.

Dark, F. A. and T. Nash (1970). Comparative toxicity of various ozonized olefins to bacteria suspended in air. Epidemiology and Infection 68: 245-252.

Dart, A. and J. Thornburg (2008). Collection efficiencies of bioaerosol impingers for virus-containing aerosols. Atmospheric Environment 42: 828-832.

Dawson, J. R. Minimizing dust in livestock buildings: Possible alternatives to mechanical separation. Journal of Agricultural Engineering Research 47: 235-248.

de Deus, N., C. Seminati, et al. (2007). Detection of hepatitis E virus in liver, mesenteric lymph node, serum, bile and faeces of naturally infected pigs affected by different pathological conditions. Veterinary Microbiology 119: 105-114.

de Jong, J. C., M. Harmsen, et al. (1975). Factors in the inactivation of Encephalomyocarditis virus in aerosols. Infection and Immunity 12: 29-35.

de Mik, G. and I. De Groot (1977). Mechanisms of inactivation of Bacteriophage phiX174 and its DNA in aerosols by ozone and ozonized cyclohexene. The Journal of Hygiene 78: 199-211.

de Mik, G., I. de Groot, et al. (1977). Survival of aerosolized bacteriophage X174 in air containing ozone–olefin mixtures. Epidemiology and Infection 78: 189-198.

de Reu, K., K. Grijspeerdt, et al. (2005). Bacterial eggshell contamination in conventional cages, furnished cages and aviary housing systems for laying hens. British Poultry Science 46: 149-155.

de Reu, K., W. Messens, et al. (2008). Bacterial contamination of table eggs and the influence of housing systems. World's Poultry Science Journal 64: 5-19.

de Rezende, C. L., E. T. Mallinson, et al. (2001). Effect of dry litter and airflow in reducing Salmonella and Escherichia coli populations in the broiler production environment. The Journal of Applied Poultry Research 10: 245-251.

Dennis, P. J. and J. V. Lee (1988). Differences in aerosol survival between pathogenic and non-pathogenic strains of Legionella pneumophila serogroup 1. Journal of Applied Microbiology 65: 135-141.

Derikx, P. J. L., H. C. Willers, et al. (1994). Effect of pH on the behaviour of volatile compounds in organic manures during dry-matter determination. Bioresource Technology 49: 41-45.

Deshpande, B. K., H. C. Frey, et al. (2009). Modeling of the Penetration of Ambient PM2. 5 to Indoor Residential Microenvironment. 102nd Annual Conference and Exhibition, Air & Waste Management Association, Detroit, USA.

Diekmann, O., J. A. P. Heesterbeek, et al. (1990). On the definition and the computation of the basic reproduction ratio R_0 in models for infectious diseases in heterogeneous populations. Journal of Mathematical Biology 28: 365-382.

Dinter, P. S. and W. Muller (1988). Viability of bacteria in the airborne state. VI. Viability of airborne Salmonella senftenberg. Zentralblatt fur Bakteriologie Mikrobiologie und Hygiene 186: 278-288.

Donaldson, A. I. (1972). The influence of relative humidity on the aerosol stability of different strains of foot-and-mouth disease virus suspended in saliva. Journal of General Virology 15: 25-33.

Donaldson, A. I., C. F. Gibson, et al. (1987). Infection of cattle by airborne foot-and-mouth disease virus: minimal doses with O1 and SAT 2 strains. Research in Veterinary Science 43: 339-346.

Donaldson, A. I., R. F. Sellers, et al. (1983). Quantitative data on airborne foot-and-mouth disease virus: Its production, carriage and deposition [and discussion]. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences (1934-1990) 302: 529-534.

Donham, K. J. and K. E. Gustafson (1982). Human occupational hazards from swine confinement. Annals of the American Conference of Governmental Industrial Hygienists 2: 137-142.

Donham, K. J., L. J. Scallon, et al. (1986). Characterization of dusts collected from swine confinement buildings. American Industrial Hygiene Association Journal 47: 404-410.

Donkoh, A. (1989). Ambient temperature: a factor affecting performance and physiological response of broiler chickens. International Journal of Biometeorology 33: 259-265.

Doyle, M. P. (1984). Association of Campylobacter jejuni with laying hens and eggs. Applied and Environmental Microbiology 47: 533-536.

Doyle, M. P. and D. J. Roman (1982). Prevalence and survival of Campylobacter jejuni in unpasteurized milk. Applied and Environmental Microbiology 44: 1154-1158.

Druett, H. A., D. W. Henderson, et al. (1953). Studies on respiratory infection. I. The influence of particle size on respiratory infection with anthrax spores. The Journal of Hygiene 51: 359-371.

Druett, H. A. and L. P. Packman (1972). The germicidal properties of ozone—olefin mixtures. Journal of Applied Microbiology 35: 323-329.

Druett, H. A., J. M. Robinson, et al. (1956). Studies on respiratory infection. II. The influence of aerosol particle size on infection of the guinea-pig with Pasteurella pestis. The Journal of Hygiene 54: 37-48.

Dubovi, E. J. and T. G. Akers (1970). Airborne stability of tailless bacterial viruses S-13 and MS-2. Applied and Environmental Microbiology 19: 624-628.

Duchaine, C., Y. Grimard, et al. (2000). Influence of building maintenance, environmental factors, and seasons on airborne contaminants of swine confinement buildings. American Industrial Hygiene Association Journal 61: 56-63.

Durand, K. T. H., M. L. Muilenberg, et al. (2002). Effect of sampling time on the culturability of airborne fungi and bacteria sampled by filtration. Annals of Occupational Hygiene 46: 113-118.

Eduard, W. and D. Heederik (1998). Methods for quantitative assessment of airborne levels of noninfectious microorganisms in highly contaminated work environments. American Industrial Hygiene Association Journal 59: 113-127.

Edwards, D. A., J. C. Man, et al. (2004). Inhaling to mitigate exhaled bioaerosols. Proceedings of the National Academy of Sciences of the United States of America 101: 17383-17388.

Ehrlich, R., S. Miller, et al. (1970). Effects of atmospheric humidity and temperature on the survival of airborne Flavobacterium. Applied Microbiology 20: 884-887.

Ehrlich, R., S. Miller, et al. (1970). Relationship between atmospheric temperature and survival of airborne bacteria. Applied and Environmental Microbiology 19: 245-249.

Elazhary, M. A. and J. B. Derbyshire (1979). Aerosol stability of bovine parainfluenza type 3 virus. Canadian Journal of Comparative Medicine 43: 295-304.

Elbers, A. R. W., J. A. Stegeman, et al. (2001). Factors associated with the introduction of classical swine fever virus into pig herds in the central area of the 1997/98 epidemic in the Netherlands. The Veterinary record 149: 377-382.

Elford, W. J. and J. van den Ende (1942). An investigation of the merits of ozone as an aerial disinfectant. Epidemiology and Infection 42: 240-265.

Engelhart, S., A. Glasmacher, et al. (2007). Air sampling of Aspergillus fumigatus and other thermotolerant fungi: Comparative performance of the Sartorius MD8 airport and the Merck MAS-100 portable bioaerosol sampler. International Journal of Hygiene and Environmental Health 210: 733-739.

Erisman, J. W., A. Bleeker, et al. (2008). Agricultural air quality in Europe and the future perspectives. Atmospheric Environment 42: 3209-3217.

European Comission (2008). Directive 2008/50/EC of the European Parliament and of the Council of 21 May 2008 on ambient air quality and cleaner air for Europe.

European Commission (1998). Air quality-Determination of the PM_{10} fraction of suspended particulate matter- Reference method and field test procedure to demonstrate reference equivalence of measurement methods, European Committee for Standardization, Brussels. EN 12341: 1-24.

European Commission (1999). Relating to limit values for sulphur dioxide, nitrogen dioxide and oxides of nitrogen, particulate matter and lead in ambient air (The first daughter directive), Council Directive 1999/30/EC, Official Journal of the European Communities, L163/41.

European Commission (2005). Ambient air quality-Standard gravimetric measurement method for the determination of the $PM_{2.5}$ mass fraction of suspended particulate matter, European Committee for Standardization, Brussels. EN 14907: 1-29.

European Commission (2005). Proposal for a directive of The European Parliament and of the Council on ambient air quality and cleaner air for Europe.

Literature Cited

European Union (2003). Directive 2003/99/EC of the European parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC: 31-40.

Fabian, P., J. J. McDevitt, et al. (2008). Influenza virus in human exhaled breath: an observational study. PLoS One 3: e2691.

Fan, L., J. Song, et al. (2002). Interaction of ozone and negative air ions to control micro organisms. Journal of Applied Microbiology 93: 144-148.

Fang, Z., Z. Ouyang, et al. (2008). Concentration and size distribution of culturable airborne microorganisms in outdoor environments in Beijing, China. Aerosol Science and Technology 42: 325-334.

Federighi, M., J. L. Tholozan, et al. (1998). Evidence of non-coccoid viable but non-culturableCampylobacter jejunicells in microcosm water by direct viable count, CTC-DAPI double staining, and scanning electron microscopy. Food Microbiology 15: 539-550.

Fogh, C. L., M. A. Byrne, et al. (1997). Size specific indoor aerosol deposition measurements and derived I/O concentrations ratios. Atmospheric Environment 31: 2193-2203.

Fouchier, R. A. M., A. D. M. E. Osterhaus, et al. (2003). Animal influenza virus surveillance. Vaccine 21: 1754-1757.

Fraser, M. P., Z. W. Yue, et al. (2003). Source apportionment of fine particulate matter in Houston, TX, using organic molecular markers. Atmospheric Environment 37: 2117-2123.

Gailiunas, P. and G. E. Cottral (1966). Presence and persistence of foot-and-mouth disease virus in bovine skin. Journal of Bacteriology 91: 2333-2338.

Gast, R. K., B. W. Mitchell, et al. (1999). Application of negative air ionization for reducing experimental airborne transmission of Salmonella enteritidis to chicks. Poultry Science 78: 57-61.

Gibson, C. F. and A. I. Donaldson (1986). Exposure of sheep to natural aerosols of foot-and-mouth disease virus. Research in Veterinary Science 41: 45-49.

Giese, A. C. and E. Christensen (1954). Effects of ozone on organisms. Physiological Zoology 27: 101-115.

Gloster, J. and S. Alexandersen (2004). New directions: Airborne transmission of Foot-and-mouth disease virus. Atmospheric Environment 38: 503-505.

Gloster, J., H. J. Champion, et al. (2003). Airborne transmission of foot-and-mouth disease virus from Burnside Farm, Heddonon-the-Wall, Northumberland, during the 2001 epidemic in the United Kingdom. Veterinary Record 152: 525-533.

Gloster, J., P. Williams, et al. (2007). Foot-and-mouth disease - Quantification and size distribution of airborne particles emitted by healthy and infected pigs. The Veterinary Journal 174: 42-53.

Goodhew, C. F., A. B. ElKurdi, et al. (1988). The microaerophilic respiration of Campylobacter mucosalis. Biochimica et Biophysica Acta 933: 114-123.

Gore, A. M., E. T. Kornegay, et al. (1986). The effects of soybean oil on nursery air quality and performance of weanling pigs. Journal of Animal Science 63: 1-7.

Graham, D. Y., G. R. Dufour, et al. (1987). Minimal infective dose of rotavirus. Archives of virology 92: 261-271.

Gray, J. T. and P. J. Fedorka-Cray (2001). Survival and Infectivity of Salmonella Choleraesuis in Swine Feces. Journal of Food Protection 64: 945-949.

Griffiths, W. D. and I. W. Stewart (1999). Performance of bioaerosol samplers used by the UK biotechnology industry. Journal of Aerosol Science 30: 1029-1040.

Griffiths, W. D., I. W. Stewart, et al. (1996). Effect of aerosolisation, growth phase and residence time in spray and collection fluids on the culturability of cells and spores. Journal of Aerosol Science 27: 803-820.

Grinshpun, S. A., C. W. Chang, et al. (1994). Inlet characteristics of bioaerosol samplers. Journal of Aerosol Science 25: 1503-1522.

Guarino, M., A. Caroli, et al. (1999). Dust concentration and mortality distribution in an enclosed laying house. Transactions of the ASAE 42: 1127-1133.

Gussman, R. A., L. C. Kenny, et al. (2002). Design, Calibration, and Field Test of a Cyclone for PM_1 Ambient Air Sampling. Aerosol Science and Technology 36: 361-365.

Gustafsson, G. (1999). Factors affecting the release and concentration of dust in pig houses. Journal of Agricultural Engineering Research 74: 379-390.

Haeussermann, A., M. Gotz, et al. (2007). Particulate emissions from deep-bedded growing-finishing pigs. Dustconf 2007 - How to improve air quality, Maastricht, the Netherlands.

Hambleton, P. (1970). The sensitivity of Gram-negative bacteria, recovered from aerosols, to lysozyme and other hydrolytic enzymes. Journal of General Microbiology 61: 197-204.

Handley, B. A. and A. J. F. Webster (1993). Some factors affecting airborne survival of Pseudomonas fluorescens indoors. Journal of Applied Microbiology 75: 35-42.

Harper, A. L., D. D. Ferguson, et al. (2010). An overview of livestock-associated MRSA in agriculture. Journal of Agromedicine 15: 101-104.

Harper, G. J. (1961). Airborne micro-organisms: Survival tests with four viruses. The Journal of Hygiene 59: 479-486.

Harper, G. J. and J. D. Morton (1953). The respiratory retention of bacterial aerosols: experiments with radioactive spores. The Journal of Hygiene 51: 372-385.

Harris, G. D., V. D. Adams, et al. (1987). Ultraviolet inactivation of selected bacteria and viruses with photoreactivation of the bacteria. Water Research 21: 687-692.

Hartung, J. (1992). Emissions of airborne substances from stalls of domestic animals. Pneumologie 46: 196-202.

Hatch, M. T. and J. C. Warren (1969). Enhanced recovery of airborne T3 coliphage and Pasteurella pestis bacteriophage by means of a presampling humidification technique. Applied and Environmental Microbiology 17: 685-689.

Hayden, F. G., J. J. Treanor, et al. (1996). Safety and efficacy of the neuraminidase inhibitor GG167 in experimental human influenza. Jama 275: 295-299.

Hayter, R. B. and E. L. Besch (1974). Airborne-particle deposition in the respiratory tract of chickens. Poultry Science 53: 1507-1511.

He, C., L. Morawska, et al. (2005). Particle deposition rates in residential houses. Atmospheric Environment 39: 3891-3899.

Heber, A. J., T. Lim, et al. (2006). Quality-assured measurements of animal building emissions : Particulate matter concentrations. Journal of the Air & Waste Management Association 56: 1642-1648.

Heber, A. J., M. Stroik, et al. (1988). Size distribution and identification of aerial dust particles in swine finishing buildings. Transactions of the ASAE 31: 882-887.

Heber, A. J., M. Stroik, et al. (1988). Influence of environmental factors on concentrations and inorganic content of aerial dust in swine finishing buildings. Transactions of the ASAE 31: 875-881.

Heidelberg, J. F., M. Shahamat, et al. (1997). Effect of aerosolization on culturability and viability of gram-negative bacteria. Applied and Environmental Microbiology 63: 3585-3588.

Heindel, T. H., R. Streib, et al. (1993). Effect of ozone on airborne microorganisms. Zentralblatt fuer Hygiene und Umweltmedizin 194: 464-480.

Henningson, E., R. Roffey, et al. (1982). A comparative study of apparatus for sampling airborne microorganisms. Grana 20: 155-159.

Hermann, J. R., S. L. Brockmeier, et al. (2008). Detection of respiratory pathogens in air samples from acutely infected pigs. Canadian Journal of Veterinary Research 72: 367-370.

Hermann, J. R., S. Hoff, et al. (2007). Effect of temperature and relative humidity on the stability of infectious porcine reproductive and respiratory syndrome virus in aerosols. Veterinary Research 38: 81-93.

Hess, G. E. (1965). Effects of oxygen on aerosolized Serratia marcescens. Applied and Environmental Microbiology 13: 781-787.

Heyder, J. (2004). Deposition of inhaled particles in the human respiratory tract and consequences for regional targeting in respiratory drug delivery. The Proceedings of the American Thoracic Society 1: 315-320.

Heyder, J., J. Gebhart, et al. (1986). Deposition of particles in the human respiratory tract in the size range 0, 005-15 μm. Journal of aerosol science 17: 811-825.

Higgins, R. D. and P. Dewell (1967). A gravimetric size-selecting personal dust sampler. Inhaled Particles and Vapours II. C. N. Davies. Oxford, Pergammon Press: 575-586.

Hijnen, W. A. M., E. F. Beerendonk, et al. (2006). Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo) cysts in water: A review. Water Research 40: 3-22.

Himathongkham, S., S. Bahari, et al. (1999). Survival of Escherichia coli O157:H7 and Salmonella typhimurium in cow manure and cow manure slurry. FEMS Microbiology Letters 178: 251-257.

Hinz, T. and S. Linke (1998). A comprehensive experimental study of aerial pollutants in and emissions from livestock buildings. part 2: Results. Journal of Agricultural Engineering Research 70: 119-129.

Hofacre, C. L., D. G. White, et al. (2001). Characterization of antibiotic-resistant bacteria in rendered animal products. Avian Diseases 45: 953-961.

Hofschreuder, P., A. J. A. Aarnink, et al. (2007). Measurement protocol for determining fine dust emission factors of animal housing systems. Proceedings of Dustconf 2007, How to Improve Air Quality, Maastricht, the Netherlands.

Hofschreuder, P., Y. Zhao, et al. (2008). Measurement protocol for emissions of fine dust from animal houses. Lelystad, the Netherlands, Animal Sciences Group, Wageningen UR Livestock Research.

Holt, P. S. (1993). Effect of induced molting on the susceptibility of white leghorn hens to a Salmonella enteritidis infection. Avian Diseases 37: 412-417.

Holt, P. S., B. W. Mitchell, et al. (1999). Use of negative air ionization for reducing airborne levels of Salmonella Enterica serovar enteritidis in a room containing infected caged layers. Journal of Applied Poultry Research 8: 440-446.

Hugh Jones, M., W. H. Allan, et al. (1973). The evidence for the airborne spread of Newcastle disease. Journal of Hygiene 71: 325-339.

Humphrey, T., S. O'Brien, et al. (2007). Campylobacters as zoonotic pathogens: A food production perspective. International Journal of Food Microbiology 117: 237-257.

Ijaz, M. K., A. H. Brunner, et al. (1985). Survival characteristics of airborne human Coronavirus 229E. Journal of General Virology 66: 2743-2748.

Ijaz, M. K., Y. G. Karim, et al. (1987). Development of methods to study the survival of airborne viruses. Journal of Virological Methods 18: 87-106.

Ijaz, M. K., S. A. Sattar, et al. (1994). Studies on the survival of aerosolized bovine rotavirus (UK) and a murine rotavirus. Comparative Immunology, Microbiology and Infectious Diseases 17: 91-98.

Ijaz, M. K., S. A. Sattar, et al. (1985). Comparison of the airborne survival of calf rotavirus and poliovirus type 1 (Sabin) aerosolized as a mixture. Applied and Environmental Microbiology 49: 289-293.

Ijaz, M. K., S. A. Sattar, et al. (1985). Effect of relative humidity, atmospheric temperature, and suspending medium on the airborne survival of human Rotavirus. Canadian Journal of Microbiology 31: 681-685.

Ingram, M. and R. B. Haines (1949). Inhibition of bacterial growth by pure ozone in the presence of nutrients. Journal of Hygiene 47: 146-158.

International Organization for Standardization (ISO) (2006). Microbiology of food and animal feeding stuffs - Horizontal method for detection and enumeration of Campylobacter spp. ISO10272:2006.

ISO 4833 (2003). Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of microorganisms -- Colony-count technique at 30 degrees C, International Standard Organization.

ISO 7402 (1985). Microbiology -- General guidance for the enumeration of enterobacteriaceae without resuscitation -- MPN technique and colony count technique, International Organization for Standardization.

Iversen, M., S. Kirychuk, et al. (2000). Human health effects of dust exposure in animal confinement buildings. Journal of Agricultural Safety and Health 6: 283-288.

Jacobs-Reitsma, W. F. (2002). Aanpassen van bemonsterings- en analysemethoden voor het aantonen van Campylobacter in omgevingsmonsters [Adaptation of sampling and analyzing methods to detect airborne Campylobacter]. Lelystad, the Netherlands, Institute of Animal Husbandry and Animal Health.

Jacobs-Reitsma, W. F., A. W. Van de Giessen, et al. (1995). Epidemiology of Campylobacter spp. at two dutch broiler farms. Epidemiology and Infection 114: 413-421.

James, A. C., W. Stahlhofen, et al. (1991). The respiratory tract deposition model proposed by the ICRP Task Group. Radiation Protection Dosimetry 38: 159-165.

Janni, K. A. and P. T. Redig (1986). Factors affecting the occurrence of aspergillosis in turkeys. ASAE Paper No. 86-4533, ASAE, St. Joseph, MI 49085.

Johnsen, G., H. Kruse, et al. (2007). Genotyping of thermotolerant Campylobacter from poultry slaughterhouse by amplified fragment length polymorphism. Journal of Applied Microbiology 103: 271-279.

Jones, C. D. and J. R. Bull (1987). Deposition and clearance of radiolabelled monodisperse polystyrene spheres in the calf lung. Research in Veterinary Science 42: 82-91.

Karim, Y. G., M. K. Ijaz, et al. (1985). Effect of relative humidity on the airborne survival of Rhinovirus-14. Canadian Journal of Microbiology 31: 1058-1061.

Katz, I. M., J. D. Schroeter, et al. (2001). Factors affecting the deposition of aerosolized insulin. Diabetes Technology & Therapeutics 3: 387-397.

Keener, K. M., M. P. Bashor, et al. (2004). Comprehensive review of Campylobacter and poultry processing. Comprehensive Reviews in Food Science and Food Safety 3: 105-116.

Kenny, L. C. (1998). Investigation in the effects of loading on $PM_{2.5}$ selectors. Sheffield, Health and Safety Laboratory report IR/L/A/98/13: 20.

Kenny, L. C., R. Gussman, et al. (2000). Development of a sharp-cut cyclone for ambient aerosol monitoring applications. Aerosol Science and Technology 32: 338-358.

Kesavan, J. S. and D. R. Schepers (2006). Characteristics and sampling efficiencies of OMNI 3000 aerosol samplers. Maryland, USA, Edgewood Chemical Biological Center.

Kim, J. G., A. E. Yousef, et al. (1999). Application of ozone for enhancing the microbiological safety and quality of foods: A review. Journal of Food Protection 62: 1071-1087.

Kim, K., H. J. Ko, et al. (2007). Farmer and Pig Exposure to Aerial Contaminants in a Pig Confinement Building. Transactions of the ASABE 50: 993-998.

Kim, K. Y., H. Ko, J.,, et al. (2008). Assessment of airborne bacteria and fungi in pig buildings in Korea. Biosystems Engineering 99: 565-572.

Kim, K. Y., H. J. Ko, et al. (2006). Effect of spraying biological additives for reduction of dust and bioaerosol in a confinement swine house. Annals of Agricultural and Environmental Medicine 13: 133-138.

Kim, K. Y., H. J. Ko, et al. (2007). Effect of ventilation rate on gradient of aerial contaminants in the confinement pig building. Environmental Research 103: 352-357.

Kim, K. Y., H. J. Ko, et al. (2005). Temporal and spatial distributions of aerial contaminants in an enclosed pig building in winter. Environmental Research 99: 150-157.

Kincaid, D. C. and T. S. Longley (1989). A water droplet evaporation and temperature model. Transaction of the ASAE 32: 457-463.

Kitching, R. P., M. V. Thrusfield, et al. (2006). Use and abuse of mathematical models: an illustration from the 2001 foot and mouth disease epidemic in the United Kingdom. Revue Scientifique et Technique-Office International des Epizooties 25: 293-311.

Kloos, W. E., R. J. Zimmerman, et al. (1976). Preliminary studies on the characterization and distribution of Staphylococcus and Micrococcus species on animal skin. Applied and Environmental Microbiology 31: 53-59.

Koop, T., B. Luo, et al. (2000). Water activity as the determinant for homogeneous ice nucleation in aqueous solutions. Nature 406: 611-614.

Kosch, R., V. Siemers, et al. (2005). Efficiency of a bioscrubber system for the reduction of ammonia and dust emissions in a broiler house ASAE Annual Meeting. Tampa, Florida.

Kournikakis, B., D. Netolitzky, et al. (1988). Effects of temperature and relative humidity on the survival of Newcastle disease virus aerosols in the rotating drum. Alberta, Canada, Defence Research and Development Canada.

Kowalski, W. J., W. P. Bahnfleth, et al. (1998). Bactericidal effects of high airborne ozone concentrations on Escherichia coli and Staphylococcus aureus. Ozone: Science & Engineering 20: 205-221.

Kristensen, C. S., A. Botner, et al. (2004). Experimental airborne transmission of PRRS virus. Veterinary Microbiology 99: 197-202.

Kuehn, T. H. (1988). Predicting air flow patterns and particle contamination in clean rooms. Journal of Aerosol Science 19: 1405-1408.

Lai, A. C. K. (2002). Particle deposition indoors: a review. Indoor Air 12: 211-214.

Lai, A. C. K. (2006). Particle Deposition and Decay in a Chamber and the Implications to Exposure Assessment. Water, Air, & Soil Pollution 175: 323-334.

Lai, H. T. L., A. J. A. Aarnink, et al. (2010). Size distribution of airborne particles in animal houses. Biosystems Engineering. (Submitted).

Landman, W. J. M., E. A. Corbanie, et al. (2004). Aerosolization of Mycoplasma synoviae compared with Mycoplasma gallisepticum and Enterococcus faecalis. Avian Pathology 33(2): 210-215.

Landman, W. J. M. and J. H. H. van Eck (2001). Aerosolization of Newcastle disease vaccine virus and Enterococcus faecalis. Avian Diseases 45: 684-687.

Langer, D. and J. A. Holcombe (1999). Thermophoretic collection and analysis of submicrometer Ag particles emitted from a graphite tube-type electrothermal vaporizer. Analytical Chemistry 71: 582-588.

Literature Cited

Larson, E. W., J. W. Dominik, et al. (1980). Aerosol stability and respiratory infectivity of japanese B encephalitis virus. Infection and Immunity 30: 397-401.

Larsson, K. A., A. G. Eklund, et al. (1994). Swine dust causes intense airways inflammation in healthy subjects. American Journal of Respiratory and Critical Care Medicine 150: 973-977.

Lee, S. A., A. Adhikari, et al. (2006). Personal exposure to airborne dust and microorganisms in agricultural environments. Journal of Occupational and Environmental Hygiene 3: 118-130.

Letellier, A., S. Messier, et al. (1999). Distribution of Salmonella in swine herds in Québec. Veterinary Microbiology 67: 299-306.

Li, C. S., M. L. Hao, et al. (1999). Evaluation of microbial samplers for bacterial microorganisms. Aerosol Science and Technology 30: 100-108.

Li, C. S. and Y. C. Lin (2001). Storage effects on bacterial concentration: determination of impinger and filter samples. Science of the Total Environment 278: 231-237.

Liang, Y., H. Xin, et al. (2005). Ammonia emissions from U. S. laying hen houses in Iowa and Pennsylvania. Transactions of the ASAE 48: 1927-1941.

Lighthart, B. (1973). Survival of airborne bacteria in a high urban concentration of carbon monoxide. Applied and Environmental Microbiology 25: 86-91.

Lighthart, B. and A. S. Frisch (1976). Estimation of viable airborne microbes downwind from a point source. Applied and Environmental Microbiology 31: 700-704.

Lin, W. H. and C. S. Li (1999). Collection efficiency and culturability of impingement into a liquid for bioaerosols of fungal spores and yeast cells. Aerosol Science and Technology 30: 109-118.

Lin, W. H. and C. S. Li (1999). Evaluation of impingement and filtration methods for yeast bioaerosol sampling. Aerosol Science and Technology 30: 119-126.

Lin, X. J., K. Willeke, et al. (1997). Effect of sampling time on the collection efficiency of all-glass impingers. American Industrial Hygiene Association Journal 58: 480-488.

Lippmann, M., D. B. Yeates, et al. (1980). Deposition, retention, and clearance of inhaled particles. British Medical Journal 37: 337-362.

Liu, B. Y. H. and D. Y. H. Pui (1981). Aerosol sampling inlets and inhalable particles. Atmospheric Environment 15: 589-600.

Loynachan, A. T. and D. L. Harris (2005). Dose determination for acute Salmonella infection in pigs. Applied and Environmental Microbiology 71: 2753-2755.

Lu, J., S. Sanchez, et al. (2003). Evaluation of broiler litter with reference to the microbial composition as assessed by using 16S rRNA and functional gene markers. Applied and Environmental Microbiology 69: 901-908.

Luechtefeld, N. W., W. L. Wang, et al. (1981). Evaluation of transport and storage techniques for isolation of Campylobacter fetus subsp. jejuni from turkey cecal specimens. Journal of Clinical Microbiology 13: 438-443.

Lundholm, I. M. (1982). Comparison of methods for quantitative determinations of airborne bacteria and evaluation of total viable counts. Applied and Environmental Microbiology 44: 179-183.

Lyngtveit, T. and W. Eduard (1997). Reduction of dust exposure by negative air ionisation. Annals of Agricultural and Environmental Medicine 4: 229-232.

Macher, J. M. and M. W. First (1984). Personal air samplers for measuring occupational exposures to biological hazards. American Industrial Hygiene Association Journal 45: 76-83.

Maciorowski, K. G., P. Herrera, et al. (2007). Effects on poultry and livestock of feed contamination with bacteria and fungi. Animal Feed Science and Technology 133: 109-136.

Madelin, T. M. and C. M. Wathes (1989). Air hygiene in a broiler house: comparison of deep litter with raised netting floors. British Poultry Science 30: 23-37.

Mainelis, G., A. Adhikari, et al. (2002). Collection of airborne microorganisms by a new electrostatic precipitator. Journal of Aerosol Science 33: 1417-1432.

Mainelis, G., D. Berry, et al. (2005). Design and performance of a single-pass bubbling bioaerosol generator. Atmospheric Environment 39: 3521-3533.

Mainelis, G. and M. Tabayoyong (2010). The effect of sampling time on the overall performance of portable microbial impactors. Aerosol Science and Technology 44: 75-82.

Manuzon, R. B., L. Y. Zhao, et al. (2007). A prototype acid spray scrubber for absorbing ammonia emissions from exhaust fans of animal buildings. Transactions of the ASABE 50: 1395-1407.

Marangon, S. and L. Busani (2007). The use of vaccination in poultry production. Revue Scientifique et Technique - Office International des Epizooties 26: 265-274.

Mars, M. H., C. J. Bruschke, et al. (1999). Airborne transmission of BHV 1, BRSV, and BVDV among cattle is possible under experimental conditions. Veterinary Microbiology 66: 197-207.

Marsh, L. S., S. W. Gay, et al. (2003). Performance evaluation of the SanScent® air scrubber for removal of dust, ammonia and hydrogen sulfide from the exhaust air of a swine nursery. 2003 ASAE Annual International Meeting. Las Vegas, Nevada, USA.

Marthi, B., V. P. Fieland, et al. (1990). Survival of bacteria during aerosolization. Applied and Environmental Microbiology 56: 3463-3467.

Martin, S. A., M. A. McCann, et al. (1998). Microbiological survey of Georgia poultry litter. The Journal of Applied Poultry Research 7: 90-98.

Martin, W. T., Y. H. Zhang, et al. (1996). Bacterial and fungal flora of dust deposits in a pig building. Occupational and Environmental Medicine 53: 484-487.

Matkovic, K., M. Vucemilo, et al. (2007). Qualitative structure of airborne bacteria and fungi in dairy barn and nearby environment. Czech Journal of Animal Science 52: 249-254.

Maus, R., A. Goppelsröder, et al. (2001). Survival of bacterial and mold spores in air filter media. Atmospheric Environment 35: 105-113.

Mayhew, C. J. and N. Hahon (1970). Assessment of aerosol mixtures of different viruses. Applied and Environmental Microbiology 20: 313-316.

McCambridge, J. and T. A. McMeekin (1981). Effect of solar radiation and predacious microorganisms on survival of fecal and other bacteria. Applied and Environmental Microbiology 41: 1083-1087.

McCullough, N. B. and C. W. Eisele (1951). Experimental human salmonellosis: III. Pathogenicity of strains of Salmonella newport, Salmonella derby, and Salmonella bareilly obtained from spray-dried whole egg. The Journal of Infectious Diseases 89: 209-213.

McGee, P., D. J. Bolton, et al. (2001). The survival of Escherichia coli O157:H7 in slurry from cattle fed different diets. Letters in Applied Microbiology 32: 152-155.

Melse, R. W. (2009). Air treatment techniques for abatement of emissions from intensive livestock production, PhD dissertation. Wageningen UR Livestock Research. Lelystad, the Netherlands., Wageningen University and Research Centre. PhD.

Melse, R. W. and N. W. M. Ogink (2005). Air scrubbing techniques for ammonia and odor reduction at livestock operations: review of on-farm research in the Netherlands. Transactions of the ASAE 48: 2303-2313.

Mikkelsen, T., S. Alexandersen, et al. (2003). Investigation of airborne foot-and-mouth disease virus transmission during low-wind conditions in the early phase of the UK 2001 epidemic. Atmospheric Chemistry and Physics Discussions 3: 2101-2110.

Milling, A., R. Kehr, et al. (2005). Survival of bacteria on wood and plastic particles: Dependence on wood species and environmental conditions. Holzforschung 59: 72-81.

Milne, A., M. K. Theodorou, et al. (1989). Survival of anaerobic fungi in feces, in saliva, and in pure culture. Experimental Mycology 13: 27-37.

Misra, C., M. D. Geller, et al. (2003). Development and evaluation of a PM_{10} impactor-inlet for a continuous coarse particle monitor. Aerosol Science and Technology 37: 271-281.

Mitchell, B. W., P. S. Holt, et al. (2000). Reducing dust in a caged layer room: an electrostatic space charge system. The Journal of Applied Poultry Research 9: 292-296.

Mitchell, B. W., L. J. Richardson, et al. (2004). Application of an electrostatic space charge system for dust, ammonia, and pathogen reduction in a broiler breeder house. Applied Engineering in Agriculture 20: 87-93.

Moe, K. and G. J. Harper (1983). The effect of relative humidity and temperature on the survival of bovine rotavirus in aerosol. Archives of Virology 76: 211-216.

Muller, W. and P. Wieser (1987). Dust and microbial emissions from animal production. Animal production and environmental health. D. Strauch, Elsevier: 47-89.

Nevalainen, A., J. Pastuzska, et al. (1992). Performance of bioaerosol samplers: collection characteristics and sampler design considerations. Atmospheric Environment 26A: 531-540.

Newell, D. G. and C. Fearnley (2003). Sources of Campylobacter colonization in broiler Chickens. Applied and Environmental Microbiology 69: 4343-4351.

Newman, M. E. J. (2002). Spread of epidemic disease on networks. Physical Review E 66: 16128.

Nicks, B., B. Canart, et al. (1993). Temperature, air humidity and air pollution levels in farrowing or weaner pig houses. Pig News and Information 14: 77-78.

Nishiguchi, A., S. Kobayashi, et al. (2007). Risk factors for the introduction of avian influenza virus into commercial layer chicken farms during the outbreaks caused by a low-pathogenic H5N2 virus in Japan in 2005. Zoonoses and Public Health 54: 337-343.

Literature Cited

NZFSA (2001). Campylobacter. Wellington, New Zealand. http://www.nzfsa.govt.nz/science/data-sheets/campylobacter.pdf (Accessed on June 19, 2010), New Zealand Food Safety Authority.

Ogink, N. W. M. and A. J. A. Aarnink (2003). Managing emissions from swine facilities: current situation in the Netherlands and Europe. Proceedings of the University of Illinois Pork Industry Conference, Champaign, Illinois.

Ogink, N. W. M. and B. J. J. Bosma (2007). Multi-phase air scrubbers for the combined abatement of ammonia, odor and particulate matter emissions. International Symposium on Air Quality and Waste Management for Agriculture. Broomfield, Colorado.

Ogink, N. W. M. and P. W. G. Groot Koerkamp (2001). Comparison of odour emissions from animal housing systems with low ammonia emission. Water Science and Technology 44: 245-252.

Ogink, N. W. M. and J. Hahne (2007). Removal of dust fractions by air scrubbers in livestock operations. Dustconf 2007: How to Improve Air Quality, Maastricht, the Netherlands.

Olsen, K. N., M. Lund, et al. (2009). Detection of Campylobacter bacteria in air samples for continuous real-time monitoring of Campylobacter colonization in broiler flocks. Applied and Environmental Microbiology 75: 2074-2078.

Omisakin, F., M. MacRae, et al. (2003). Concentration and prevalence of Escherichia coli O157 in cattle feces at slaughter. Applied and Environmental Microbiology 69: 2444-2447.

Otake, S., S. Dee, et al. (2010). Long distance airborne transport of infectious PRRSV and Mycoplasma hyopneumoniae from a swine population infected with multiple viral variants. Veterinary Microbiology 145: 198-208.

Ouellette, C. A., J. J. R. Feddes, et al. (1999). A portable environmental monitoring system to assess barn worker indoor air exposure. Journal of Agricultural Safety and Health 5: 383-394.

Pal, A., S. O. Pehkonen, et al. (2007). Photocatalytic inactivation of gram-positive and gram-negative bacteria using fluorescent light. Journal of Photochemistry and Photobiology A: Chemistry 186: 335-341.

Parks, S. R. (1996). An assessment of the sartorius MD8 microbiological air sampler. Journal of Applied Bacteriology 80: 529-534.

Pearson, C. C. and T. J. Sharples (1995). Airborne dust concentrations in livestock buildings and the effect of feed. Journal of Agricultural Engineering Research 60: 145-154.

Pedersen, S., V. Blanes-Vidal, et al. (2008). Carbon dioxide production in animal houses: A literature review. Agricultural Engineering International: The CIGR EJournal X: 1-19.

Pedersen, S. and C. B. Pedersen (1995). Animal activity measured by infrared detectors. Journal of Agricultural Engineering Research 61: 239-246.

Pell, A. N. (1997). Manure and Microbes: Public and Animal Health Problem? Journal of Dairy Science 80: 2673-2681.

Pérez, N., J. Pey, et al. (2008). Partitioning of major and trace components in PM_{10}-$PM_{2.5}$-PM_1 at an urban site in Southern Europe. Atmospheric Environment 42: 1677-1691.

Peters, T. M., R. W. Venderpool, et al. (1996). Development and evaluation of sampling components for measuring particulate matter under 2.5 micrometers, RTI Report No. 6360-001.

Phillips, G., G. J. Harris, et al. (1964). Effect of air ions on bacterial aerosols. International Journal of Biometeorology 8: 27-37.

Phillips, P. A. (1986). Dust levels in mechanically versus naturally ventilated hog barns. ASAE Paper No. 86-4041, ASAE, St. Joseph, MI 49085.

Power, C. A. (2005). An investigation into the potential role of aerosol dispersion of dust from poultry barns as a mode of disease transmission during an outbreak of avian influenza (H7:N3) in Abbotsford, BC in 2004. Bulletin of the Aquaculture Association of Canada 105: 7-14.

Predicala, B. Z., R. G. Maghirang, et al. (2001). Dust and bioaerosol concentrations in two swine-finishing buildings in Kansas. Transactions of the ASAE 44: 1291-1298.

Predicala, B. Z., J. E. Urban, et al. (2002). Assessment of bioaerosols in swine barns by filtration and impaction. Current Microbiology 44: 136-140.

Prescott, L. M., J. P. Harley, et al. (2005). Microbiology (Sixth ed.). New York, USA, McGraw-Hill.

Pui, D. Y. H. and B. Y. H. Liu (1988). Advances in instrumentation for atmospheric aerosol measurement. Physica Scripta 37: 252-269.

Quarles, C. L., R. F. Gentry, et al. (1970). Bacterial contamination in poultry houses and its relationship to egg hatchability. Poultry Science 49(1): 60-66.

Rabey, F., R. J. Janssen, et al. (1969). Stability of St. Louis encephalitis virus in the airborne state. Applied and Environmental Microbiology 18: 880-882.

Radon, K., B. Danuser, et al. (2002). Air contaminants in different European farming environments. Annals of Agricultural and Environmental Medicine 9: 41-48.

Rechsteiner, J. and K. C. Winkler (1969). Inactivation of respiratory syncytial virus in aerosol. Journal of General Virology 5: 405-410.

Reponen, T., K. Willeke, et al. (1997). Techniques for dispersion of microorganisms into air. Aerosol Science and Technology 27: 405 - 421.

Richardson, L. J., B. W. Mitchell, et al. (2003). Effect of an electrostatic space charge system on airborne dust and subsequent potential transmission of microorganisms to broiler breeder pullets by airborne dust. Avian Diseases 47: 128-133.

Rodenburg, T. B., M. C. Van der Hulst-van Arkel, et al. (2004). Campylobacter and Salmonella infections on organic broiler farms. Wageningen Journal of Life Sciences 52: 101-108.

Rodríguez, S., E. Cuevas, et al. (2008). Influence of sea breeze circulation and road traffic emissions on the relationship between particle number, black carbon, PM_1, $PM_{2.5}$ and $PM_{2.5-10}$ concentrations in a coastal city. Atmospheric Environment 42: 6523-6534.

Rosentrater, K. (2003). Performance of an electrostatic dust collection system in swine facilities [electronic resource]. CIGR ejournal.

Roy, D., P. K. Wong, et al. (1981). Mechanism of enteroviral inactivation by ozone. Applied and Environmental Microbiology 41: 718-723.

Roy, J. H. B. (1980). Factors affecting susceptibility of calves to disease. Journal of Dairy Science 63: 650-664.

Ruiz-Palacios, G. M., E. Escamilla, et al. (1981). Experimental Campylobacter diarrhea in chickens. Infection and Immunity 34: 250-255.

Saleh, M., J. Seedorf, et al. (2005). Influence of animal age and season on bio-aerosol concentrations in a broiler house. ISAH 2005, Warsaw, Poland.

Saltzman, B. E. (1984). Generalized performance characteristics of miniature cyclones for atmospheric particulate sampling. American Industrial Hygiene Association Journal 45: 671-680.

Sattar, S. A., M. K. Ijaz, et al. (1984). Effect of relative humidity on the airborne survival of Rotavirus SA11. Applied and Environmental Microbiology 47: 879-881.

Schaffer, F. L., M. E. Soergel, et al. (1976). Survival of airborne influenza virus: Effects of propagating host, relative humidity, and composition of spray fluids. Archives of Virology 51: 263-273.

Schauer, J. J., W. F. Rogge, et al. (1996). Source apportionment of airborne particulate matter using organic compounds as tracers. Atmospheric Environment 30: 3837-3855.

Schiffman, S. S. (1998). Livestock odors: implications for human health and well-being. Journal of Animal Science 76: 1343-1355.

Schlegelmilch, M., J. Streese, et al. (2005). Reducing odorous emissions from biowaste composting plants by means of biological waste gas treatment systems. Tenth International Waste Management and Landfill Symposium. Cagliari, Italy.

Schoenbaum, M. A., J. J. Zimmerman, et al. (1990). Survival of Pseudorabies virus in aerosol. American Journal of Veterinary Research 51: 331-333.

Schreider, J. P. and J. O. Hutchens (1979). Particle deposition in the guinea pig respiratory tract. Journal of Aerosol Science 10: 599-607.

Schuijffel, D. F., P. C. M. Van Empel, et al. (2005). Successful selection of cross-protective vaccine candidates for Ornithobacterium rhinotracheale infection. Infection and Immunity 73: 6812-6821.

Scott, D. B. M. and E. C. Lesher (1963). Effect of ozone on survival and permeability of Escherichia coli. Journal of Bacteriology 85: 567-576.

Seedorf, J., T. Banhazi, et al. (2005). Cleaning exhaust air by biological waste gas purification systems in livestock buildings-state of art and shortcomings. the XIIth ISAH Congress on Animal Hygiene, Warsaw, Poland.

Seedorf, J. and J. Hartung (1999). Reduction efficiencies of a biofilter and a bioscrubber for bioaerosols from two different piggeries. Berliner und Munchener Tierarztliche Wochenschrift 112: 444-447.

Seedorf, J., J. Hartung, et al. (1998). Concentrations and emissions of airborne endotoxins and microorganisms in livestock buildings in Northern Europe. Journal of Agricultural Engineering Research 70: 97-109.

Shaman, J. and M. Kohn (2009). Absolute humidity modulates influenza survival, transmission, and seasonality. Proceedings of the National Academy of Sciences 106: 3243-3248.

Shanker, S., A. Lee, et al. (1988). Experimental colonization of broiler chicks with Campylobacter jejuni. Epidemiology and Infection 100: 27-34.

Shen, T. T. and N. C. Pereira (1979). Electrostatic precipitation. Handbook of environmental engineering, volume 1:Air and noise pollution control. L. K. Wang and N. C. Pereira. Clifton, USA, Humana Press Inc.

Shipe, E. L., M. E. Tyler, et al. (1959). Bacterial aerosol samplers. II. Development and evaluation of the Shipe sampler. Applied Microbiology 7: 349-354.

Simmons, J. D. and Lott. B. D. (1996). Evaporative cooling performance resulting from changes in water temperature. Applied Engineering in Agriculture 12: 497-500.

Snell, H. G. J. and A. Schwarz (2003). Development of an efficient bioscrubber system for the reduction of emissions. ASAE Annual International Meeting. Las Vegas, Nevada.

Songer, J. R. (1967). Influence of relative humidity on the survival of some airborne viruses. Applied and Environmental Microbiology 15: 35-42.

Spearman, C. (1908). The method of right and wrong cases (constant stimuli) without Gauss's formulae. British Journal of Psychology 2: 227-242.

Spradbrow, P. B., J. L. Samuel, et al. (1988). Serological response of chickens to oral vaccination with newcastle disease virus. Veterinary Microbiology 16: 255-262.

Stark, K. D. C. (1999). The role of infectious aerosols in disease transmission pigs. Veterinary Journal 158(3): 164-181.

Steinfeld, H., P. Gerber, et al. (2006). Livestock's long shadow: Environmental issues and options, FAO.

Stephenson, E. H., E. W. Larson, et al. (1984). Effect of environmental factors on aerosol-induced Lassa virus infection. Journal of Medical Virology 14: 295-303.

Stewart, R. H. and D. N. Wright (1970). Recovery of airborne Streptococcal L-Forms at various relative humidities. Applied and Environmental Microbiology 19: 865-866.

Stewart, S. L., S. A. Grinshpun, et al. (1995). Effect of impact stress on microbial recovery on an agar surface. Applied and Environmental Microbiology 61: 1232-1239.

Strasters, K. C. and K. C. Winkler (1966). Viability of hospital staphylococci in air. Microbiology and Molecular Biology Reviews 30: 674-677.

Sun, W. and J. Ji (2007). Transport of droplet expelled by coughing in ventilated rooms. Indoor and Built Environment 16: 493-504.

Takai, H. (2007). Factors influencing dust reduction efficiency of spraying of oil-water mixtures in pig buildings. Dustconf 2007: How to Improve Air Quality, Maastricht, the Netherlands.

Takai, H. and S. Pedersen (2000). A comparison study of different dust control methods in pig buildings. Applied Engineering in Agriculture 16: 269-277.

Takai, H., S. Pedersen, et al. (1998). Concentrations and emissions of airborne dust in livestock buildings in Northern Europe. Journal of Agricultural Engineering Research 70: 59-77.

Talibart, R., M. Denis, et al. (2000). Survival and recovery of viable but noncultivable forms of Campylobacter in aqueous microcosm. International Journal of Microbiology 55: 263-267.

Tanaka, A. and Y. Zhang (1996). Dust settling efficiency and electrostatic effect of a negative ionization system. Journal of Agricultural Safety and Health 2: 39-47.

Tang, J. W. (2009). The effect of environmental parameters on the survival of airborne infectious agents. Journal of The Royal Society Interface 6: S737-S746.

Terzieva, S., J. Donnelly, et al. (1996). Comparison of methods for detection and enumeration of airborne microorganisms collected by liquid impingement. Applied and Environmental Microbiology 62: 2264-2272.

Theunissen, H. J., N. A. Lemmens-den Toom, et al. (1993). Influence of temperature and relative humidity on the survival of Chlamydia pneumoniae in aerosols. Applied and Environmental Microbiology 59: 2589-2593.

Thomas, C., D. Hill, et al. (2002). Culturability, injury and morphological dynamics of thermophilic Campylobacter spp. within a laboratory-based aquatic model system. Journal of Applied Microbiology 92: 433-442.

Thompson, M. W., J. Donnelly, et al. (1994). Method and test system for evaluation of bioaerosol samplers. Journal of Aerosol Science 25: 1579-1593.

Thomson, C. M., N. Chanter, et al. (1992). Survival of toxigenic Pasteurella multocida in aerosols and aqueous liquids. Applied and Environmental Microbiology 58: 932-936.

Thorne, P. S., M. S. Kiekhaefer, et al. (1992). Comparison of bioaerosol sampling methods in barns housing swine Applied and Environmental Microbiology 58: 2543-2551.

Topisirovic, G. (2003). Influence of different ventilation systems on inhalable and respirable dust particles concentrations distribution in weaning and finishing fattening pig houses. Journal of Agricultural Sciences, Belgrade 48: 187-204.

Torok, V. A., R. J. Hughes, et al. (2009). Influence of different litter materials on cecal microbiota colonization in broiler chickens. Poultry Science 88: 2474-2481.

Tseng, C. C. and C. S. Li (2005). Collection efficiencies of aerosol samplers for virus-containing aerosols. Journal of Aerosol Science 36: 593-607.

Tyler, M. E. and E. L. Shipe (1959). Bacterial aerosol samplers. I. Development and evaluation of the All-Glass Impinger. Applied Microbiology 7: 337-349.

Tyler, M. E., E. L. Shipe, et al. (1959). Bacterial aerosol samplers. III. Comparison of biological and physical effects in liquid impinger samplers. Applied Microbiology 7: 355-362.

US EPA (1997). Ambient air monitoring reference and equivalent methods, United States Environmental Protection Agency. Federal Register 40CFR Parts 50, 53 and 58.

US EPA (2002). Ambient air monitoring reference and equivalent methods: designation of one new reference method for PM_{10}, four new equivalent methods for $PM_{2.5}$, and one new reference method for NO_2, United States Environmental Protection Agency. FRL–7166–8.

US EPA (2005). National emission inventory - Ammonia emissions from animal husbandry (Revised draft report), U.S. Environmental Protection Agency.

US EPA (2006). National Ambient Air Quality Standards for Particulate Matter; Final Rule. United States Environmental Protection Agency. 40 CFR Part 50.

US EPA (2008). List of designated reference and equivalent methods., United States Environmental Protection Agency. http://www.epa.gov/ttn/amtic/files/ambient/criteria/reference-equivalent-methods-list.pdf. (Accessed on Jan 12, 2009). U. S. E. P. Agency.

US EPA (2010). List of designated reference and equivalent methods. http://www.epa.gov/ttn/amtic/files/ambient/criteria/reference-equivalent-methods-list.pdf. (Accessed on June 15, 2010). U. S. E. P. Agency.

US EPA (2005). National emission inventory - Ammonia emissions from animal husbandry (Revised draft report), U.S. Environmental Protection Agency.

Vaithiyalingam, S., M. Nutan, et al. (2002). Preparation and characterization of a customized cellulose acetate butyrate dispersion for controlled drug delivery. Journal of Pharmaceutical Sciences 91: 1512-1522.

Valduga, G., G. Bertoloni, et al. (1993). Effect of extracellularly generated singlet oxygen on Gram-positive and Gram-negative bacteria. Journal of Photochemistry and Photobiology B: Biology 21: 81-86.

van Bunnik, B. A. D., T. J. Hagenaars, et al. (2009). Experimental study of a model system to gain insight in the mechanisms of indirect transmission. Proceedings of the VEEC Symposium on Epidemiology and Welfare, Deventer, the Netherlands.

van de Giessen, A. W., M. Bouwknegt, et al. (2006). Surveillance of Salmonella spp. and Campylobacter spp. in poultry production flocks in The Netherlands. Epidemiology and Infection 134: 1266-1275.

van der Hoek, K. W. (1998). Estimating ammonia emission factors in Europe: summary of the work of the UNECE ammonia expert panel. Atmospheric Environment 32: 315-316.

van Oirschot, J. T. (1979). Experimental production of congenital persistent swine fever infections: I. Clinical, pathological and virological observations. Veterinary Microbiology 4: 117-132.

Verreault, D., S. Moineau, et al. (2008). Methods for sampling of airborne viruses. Microbiology and Molecular Biology Reviews 72: 413-444.

Vittal, B. and S. Rasool (1995). Enumeration of airborne molds in some indoor environments of Madras city (India) by cultural and non-cultural volumetric samplers. Aerobiologia 11: 201-204.

von Essen, S. and D. Romberger (2003). The respiratory inflammatory response to the swine confinement building environment: the adaptation to respiratory exposures in the chronically exposed worker. Journal of Agricultural Safety and Health 9(3): 185-196.

von Essen, S. G. and B. W. Auvermann (2006). Health effects from breathing air near CAFOs for feeder cattle or hogs. Journal of Agromedicine 10: 55-64.

Vucemilo, M., K. Matkovic, et al. (2007). The effect of animal age on air pollutant concentration in a broiler house. Czech Journal of Animal Science 52: 170-174.

Wainwright, C. E., M. W. France, et al. (2009). Cough-generated aerosols of Pseudomonas aeruginosa and other gram-negative bacteria from patients with cystic fibrosis. Thorax 64: 926-931.

Literature Cited

Wang, C. C., G. Fang, C.,, et al. (2010). Bioaerosols as contributors to poor air quality in Taichung City, Taiwan. Environmental Monitoring and Assessment 166: 1-9.

Wang, Z., T. Reponen, et al. (2001). Effect of sampling time and air humidity on the bioefficiency of filter samplers for bioaerosol collection. Journal of Aerosol Science 32: 661-674.

Ward, R. L., D. I. Bernstein, et al. (1986). Human rotavirus studies in volunteers: determination of infectious dose and serological response to infection. The Journal of Infectious Diseases 154: 871-880.

Wathes, C. M., K. Howard, et al. (1986). The survival of Escherichia coli in an aerosol at air temperatures of 15 and 30 C and a range of humidities. The Journal of Hygiene 97): 489-496.

Webb, S. J. (1959). Factors affecting the viability of air-borne bacteria. I. Bacteria aerosolized from distilled water. Canadian Journal of Microbiology 5: 649-669.

Weber, T. P. and N. I. Stilianakis (2008). Inactivation of influenza A viruses in the environment and modes of transmission: A critical review. Journal of Infection 57: 361-373.

Webster, R. G., M. Yakhno, et al. (1978). Intestinal influenza: Replication and characterization of influenza viruses in ducks. Virology 84: 268-278.

Wedding, J. B., M. A. Welgand, et al. (1982). A 10-mm cutpoint inlet for the dichotomous sampler. Environmental Science and Technology 16: 602-606.

Weesendorp, E., W. J. M. Landman, et al. (2008). Detection and quantification of classical swine fever virus in air samples originating from infected pigs and experimentally produced aerosols. Veterinary Microbiology 127: 50-62.

Welford, R. A., J. J. R. Feddes, et al. (1992). Pig building dustiness as affected by canola oil in the feed. Canadian Agricultural Engineering 34: 365-365.

Wells, W. F. (1934). On air-borne infection: study II. droplets and droplet nuclei. American Journal of Epidemiology 20: 611-618.

Welsh, H. H., E. H. Lennette, et al. (1958). Air-borne transmission of Q fever: the role of parturition in the generation of infective aerosols. Annals of the New York Academy of Sciences 70(3): 528-540.

WHO (1990). Indoor air quality: Biological contaminants. Copenhagen, World Health Organization Regional Publications, European Series No. 31, Copenhagen.

Wilson, I. G. (2004). Airborne Campylobacter infection in a poultry worker: case report and review of the literature. Communicable Disease and Public Health 7: 349-353.

Wilson, S. C., J. Morrow-Tesch, et al. (2002). Airborne Microbial Flora in a Cattle Feedlot. Applied and Environmental Microbiology 68: 3238-3242.

Wing, S. and S. Wolf (2000). Intensive livestock operations, health, and quality of life among eastern North Carolina residents. Environmental Health Perspectives 108: 233-238.

Won, W. D. and H. Ross (1969). Reaction of airborne Rhizobium meliloti to some environmental factors. Applied and Environmental Microbiology 18: 555-557.

Wright, D. N., G. D. Bailey, et al. (1968). Role of relative humidity in the survival of airborne Mycoplasma pneumoniae. Journal of Bacteriology 96: 970-974.

Literature Cited

Wright, D. N., G. D. Bailey, et al. (1968). Survival of airborne Mycoplasma as affected by relative humidity. Journal of Bacteriology 95: 251-252.

Wu, C. S. (2009). Antibacterial and static dissipating composites of poly (butylene adipate-co-terephthalate) and multi-walled carbon nanotubes. Carbon 47: 3091-3098.

Wunsch, O. (1994). Oscillating sedimentation of spheres in viscoplastic fluids. Rheologica Acta 33: 292-302.

Wylie, L. M., G. W. Robertson, et al. (2001). Effects of ambient temperature and restricted feeding on the growth of feathers in growing turkeys. British Poultry Science 42: 449-455.

Yao, M. H. and G. Mainelis (2006). Effect of physical and biological parameters on enumeration of bioaerosols by portable microbial impactors. Journal of Aerosol Science 37: 1467-1483.

Yoder, M. F. and G. L. Van Wicklen (1988). Respirable aerosol generation by broiler chicken. Transactions of the ASAE 31: 1510-1517.

Yoon, I. J., H. Joo, et al. (1993). Persistent and contact infection in nursery pigs experimentally infected with porcine reproductive and respiratory syndrome (PRRS) virus. Journal of Swine Health and Production 1: 5-8.

Yoon, K. J., J. J. Zimmerman, et al. (1999). Effect of challenge dose and route on porcine reproductive and respiratory syndrome virus (PRRSV) infection in young swine. Veterinary Research 30: 629-638.

Yu, I. T. S., Y. Li, et al. (2004). Evidence of airborne transmission of the severe acute respiratory syndrome virus. The New England Journal of Medicine 350: 1731-1739.

Zeitler, M. H., M. Konig, et al. (1987). Effect of the type of feed (meal, pellets or fluid) and season on the concentration and particle size of dust particles in pig houses. Deutsche Tierarztliche Wochenschrift 94: 420-424.

Zhang, Y., J. A. Polakow, et al. (2001). An aerodynamic deduster to reduce dust and gas emissions from ventilated livestock facilities. Livestock Environment VI: Proceedings of the 6th International Symposium, Kentucky, USA.

Zhang, Y. H. (2004). Indoor air quality engineering Boca Raton, Florida, CRC Press.

Zhao, Y., A. J. A. Aarnink, et al. (2011). Evaluation of efficiencies of four bioaerosol samplers for collecting aerosolized Gumboro vaccine virus. Applied and Environmental Microbiology. Submitted.

Zhao, Y., A. J. A. Aarnink, et al. (2011). Effectiveness of multi-stage scrubbers on reducing emissions of airborne bacteria and dust from pig houses. Transactions of the ASABE 54: 285-293.

Zhao, Y., A. J. A. Aarnink, et al. (2011). Investigation of the efficiencies of bioaerosol samplers for collecting aerosolized bacteria using a fluorescent tracer. I: Effects of non-sampling processes on bacterial culturability. Aerosol Science and Technology 45: 423-431.

Zhao, Y., A. J. A. Aarnink, et al. (2011). Investigation of the efficiencies of bioaerosol samplers for collecting aerosolized bacteria using a fluorescent tracer. II: Sampling efficiency, and half-life time. Aerosol Science and Technology 45: 432-442.

Zhao, Y., A. J. A. Aarnink, et al. (2008). Removal efficiency of dust and bacteria by multi-stageair scrubbers in a pig house. The Eighth International Symposium of Livestock Environment, Iguassu Falls, Brasil.

Zhao, Y., A. J. A. Aarnink, et al. (2009). Evaluation of an impaction and a cyclone pre-separator for sampling high PM_{10} and $PM_{2.5}$ concentrations in livestock houses. Journal of Aerosol Science 40: 868-878.

Zhen, S., K. Li, et al. (2009). A comparison of the efficiencies of a portable BioStage impactor and a Reuter centrifugal sampler (RCS) High Flow for measuring airborne bacteria and fungi concentrations. Journal of Aerosol Science 40: 503-513.

Zimmerman, J. J., K. Schwartz, et al. (1993). Influence of dose and route on transmission of encephalomyocarditis virus to swine. Journal of Veterinary Diagnostic Investigation 5: 317-321.

Ziprin, R. L., R. E. Droleskey, et al. (2003). Failure of viable nonculturable Campylobacter jejuni to colonize the cecum of newly hatched leghorn chicks. Avian Diseases 47: 753-758.

Zucker, B. A., S. Trojan, et al. (2000). Airborne gram-negative bacterial flora in animal houses. Journal of Veterinary Medicine, Series B 47: 37-46.

SUMMARY

Summary

Intensified livestock production enhances agricultural yields (e.g. milk, meat and eggs) per unit of labor, feed input and land but is associated with high concentrations and emissions of aerial pollutive agents which create serious local, regional and global air pollution. Two of the most important of these pollutive agents are airborne microorganisms and dust (together these are also referred to as particulate matter). Concentrations of airborne microorganisms and dust in livestock production systems are much higher than those in urban ambient air, and often exceed the thresholds legislated by some occupational health organizations for human environments.. The concentrations of microorganisms in urban areas are around 3 $\log_{10}$ colony forming units (CFU) m^{-3}. The microbial concentrations measured in livestock houses can be as high as 6 $\log_{10}$ CFU m^{-3} and even up to 9 $\log_{10}$ CFU m^{-3}. Research on airborne dust concentration is increasingly focusing on the fractions with small particle sizes, e.g. PM_{10} (particulate matter smaller than 10 µm) and $PM_{2.5}$ (particulate matter smaller than 2.5 µm). These fractions are more harmful to human health because they can be deposited deeper in the respiratory tract than the larger particle fractions. The European Commission has stipulated that in ambient air the annual average concentrations of PM_{10} and $PM_{2.5}$ should not exceed 40 µg m^{-3} and 25 µg m^{-3} respectively. However, the PM concentrations in livestock production systems, especially in pig and poultry houses, never achieve this. For environmental protection there is therefore a strong need to mitigate the concentration and emission of airborne microorganism and dust from livestock production systems.

Besides their detrimental effect on the environment, emissions of the pollutant agents may also pose a health hazard to animals in nearby livestock units and to humans living in the vicinity. One outcome of the several epidemics of highly infectious diseases in recent decades is that it is now suspected that disease may be spread between farms via airborne transmission of pathogenic microorganisms, possibly on dust particles. The suspicion is based on the fact that no route other than airborne transmission can explain the infection of animals on farms with no history of movement of objects (e.g. vehicles) or living beings (animals, or people). However, whether and to what extent the emitted pathogenic microorganisms are aerially transmitted from the infected livestock unit to nearby recipient units, and how dust plays a role in the transmission, remain unclear. To enhance our understanding of airborne transmission, knowledge of its processes needs to be expanded, and sampling techniques should be validated for accurate measurement of airborne microorganisms and dust from livestock production systems.

The objective of this thesis was to gain knowledge about airborne microorganisms from livestock production systems and their relation to dust by 1) reviewing previous studies about the fate of microorganisms and dust in the processes of airborne transmission, 2) investigating the performance of mitigation techniques for reducing emissions of airborne microorganisms and dust from livestock production systems, 3) evaluating the efficiencies and suitability of samplers for microorganisms and dust which could be used in practical livestock production systems, and 4) validating these samplers

for microorganisms in an experiment on the airborne transmission of *Campylobacter* in broilers. The detailed objectives were:

- to review the sources, concentrations, physical and biological decay in the transmission, deposition in the respiratory tracts of human and animal, and sampling and mitigation techniques of airborne microorganisms and dust. (Chapter 2).

- to evaluate the performance of multi-stage air scrubbers on reducing emissions of airborne microorganisms, dust, ammonia and CO_2 from pig farms. (Chapter 3).

- to investigate the overloading problem of the EU reference PM_{10} and $PM_{2.5}$ samplers (with impaction pre-separators) when used in livestock houses, and to validate alternative PM_{10} and $PM_{2.5}$ samplers (with cyclone pre-separators) for their suitability for use in livestock houses. (Chapter 4).

- to evaluate the physical and biological efficiencies of the Andersen six-stage impactor, the AGI-30, the OMNI-3000 and the MD8 for collecting four aerosolized bacterial species (*Enterococcus faecalis*, *Escherichia coli*, *Campylobacter jejuni* and *Mycoplasma synoviae*) and one viral species (Gumboro vaccine virus). (Chapters 5, 6 and 7).

- to detect airborne *Campylobacter* with three bioaerosol samplers (Andersen six-stage impactor, AGI-30 and OMNI-3000) in a transmission experiment with broilers. (Chapter 8).

AIRBORNE MICROORGANISMS AND DUST IN LIVESTOCK PRODUCTION SYSTEMS (CHAPTER 2)

This desk study presented a state-of-art review of airborne microorganisms and dust from livestock production systems with respect to their sources, concentrations, physical and biological decay in airborne transmission, deposition in human and animal respiratory tracts, infection in animals, and sampling and mitigation techniques. It is concluded that the sources of airborne microorganisms and dust are similar in livestock houses and include animal excretion, secretion, hair and skin, feed and bedding materials, which, however, contribute differently to the concentrations in the air. The concentrations of microorganisms and dust in livestock houses, especially in pig and poultry houses, are generally very high. They may be transmitted to the outside, and during this process may undergo physical decay. In addition, microorganisms may undergo biological decay. Microorganisms can be inhaled and deposited in the respiratory tract; the deposition pattern depends on their size. Sampling techniques of dust for ambient air may not be applicable for sampling in livestock houses, where dust concentrations are high. The efficiencies of bioaerosol samplers need to be assessed. The mitigation techniques for airborne microorganisms and dust include control at source and air purification. Combined techniques may be more effective in reducing their concentrations and emissions in livestock production systems.

PERFORMANCE OF MULTI-STAGE SCRUBBERS IN REDUCING AIR POLLUTANTS (CHAPTER 3)

The concentrations of airborne bacteria, dust, ammonia and carbon dioxide (CO_2) were measured before and after three multi-stage air scrubbers (two double-stage scrubbers: acid stage + bio-filter/bio-scrubber, and a triple-stage scrubber: water stage + acid stage + bio-filter) installed in three different pig houses. The differences in concentrations in incoming and outgoing air were used to calculate the removal efficiencies of these air pollutants. The removal efficiencies of these multi-stage scrubbers were 46%-85% for total bacteria, 61%-93% for PM_{10}, 47%-90% for $PM_{2.5}$, and 70%-100% for ammonia. The emissions of CO_2 were not reduced. The multistage scrubbers were more effective at removing larger bacteria in the size range > 3.3 µm (53%-92%) than smaller bacteria in the range of 0.65-3.3 µm (-42%-20%). The most efficient in removing dust and ammonia was the triple-stage scrubber, probably due to its longer residence time. Compared to the single-stage scrubbers, it seems that multi-stage scrubbers perform more consistently in reducing total bacteria, PM_{10}, $PM_{2.5}$, and ammonia emissions from livestock houses. It should be noted that all the measurements were done in winter when the ventilation rates were relatively low, thus at low loadings of the multi-stage scrubbers. To give a full view of the performance of these scrubbers, they must be evaluated at high ventilation rates.

CYCLONE PRE-SEPARATORS FOR PM SAMPLING IN LIVESTOCK HOUSES (CHAPTER 4)

It was suspected that the EU reference impaction pre-separator (IPS), in which the large particles are impacted on a greased plate, easily becomes overloaded when sampling PM in environments with high dust concentrations. The overloading problem was tested by operating IPSs in layer houses with intermittent cleaning of the greased impaction plates used to retain the particles larger than PM_{10} or $PM_{2.5}$. The results showed that the PM_{10} IPS did not become overloaded in 24 h measurements in layer houses, whereas $PM_{2.5}$ IPS became overloaded within 1 h. Therefore, a $PM_{2.5}$ cyclone pre-separator (CPS) was tested for its vulnerability to become overloaded in dusty environments. It was found that this CPS did not become overloaded during 48 h sampling in a layer house. When the EU standard protocol for candidate PM sampler validation was followed, both the PM_{10} and $PM_{2.5}$ CPSs showed good precision and comparability with the impaction pre-separator in ambient air with low dust concentrations. In pig and broiler houses with high dust loads, PM_{10} CPS could give comparable results with the IPS when a correction factor was introduced. This standard protocol, however, was not applicable for validating $PM_{2.5}$ CPS in livestock houses due to the severe overloading problem of the $PM_{2.5}$ reference sampler (for outside air) with an IPS. It is concluded that $PM_{2.5}$ IPS is not suitable for sampling in livestock houses, and that $PM_{2.5}$ CPS is very tolerant of high dust loads. For PM_{10} sampling, both IPS and CPS can be used.

Summary

LOSSES OF BACTERIAL CULTURABILITY IN NON-SAMPLING PROCESSES (CHAPTER 5)

When assessing bioaerosol deposition and the physical efficiencies of samplers for collecting aerosolized bacteria using a tracer, the bacteria may lose culturability 1) due to the tracer, 2) in the pre-sampling process (nebulization/aerosolization of bacterial suspensions), 3) in the sampling process (collecting bioaerosols with samplers), and 4) in post-sampling processes (air sample handling procedures). Only the losses in the sampling process are associated with the efficiencies of samplers. Before assessing the efficiency of four bioaerosol samplers for four aerosolized bacteria, the losses of bacterial culturability in the other three processes, i.e. non-sampling processes, were investigated. The results showed that uranine, a fluorescent tracer, had no significant effect on the culturability of *Enterococcus faecalis*, *Escherichia coli*, and *Mycoplasma synoviae* in suspensions, whereas the culturability of *Campylobacter jejuni* was reduced (P = 0.01). *E. faecalis*, *E. coli*, and *M. synoviae* retained their culturability during aerosolization, and only 29% of *C. jejuni* were still culturable. In the air sample handling procedures, the four bacteria were recovered without significant losses from the samples of Andersen impactor, but only 33%-60% of uranine was recovered. *E. faecalis*, *E. coli* and *M. synoviae* were recovered without significant losses from the samples of the Airport MD8. More *C. jejuni* was recovered (172%), probably due to multiplication. It is concluded that the tracer and bacteria should be aerosolized separately when the tracer reduces the bacterial culturability. Losses of bacterial culturability (or multiplication) may occur in pre- and post-sampling processes and should be excluded when calculating the sampling efficiencies.

EFFICIENCIES AND HALF-LIFE (BACTERIA, CHAPTER 6)

The physical and biological efficiencies of Andersen 6-stage impactor, all-glass impinger (AGI-30), high air flow rate sampler OMNI-3000, and Airport MD8 with gelatin filter for collecting aerosolized *E. faecalis*, *E. coli*, *C. jejuni* and *M. synoviae* were investigated, correcting for the losses of bacterial culturability and uranine in the non-sampling processes. The physical efficiency of the AGI-30 (74%) did not significantly differ from that of the Airport MD8, which was selected as the reference sampler due to its high physical efficiency. The OMNI-3000 (49%) had lower physical efficiency than the Airport MD8. The low physical efficiency of the Andersen impactor (18%) was mainly caused by the incomplete recovery of uranine when handling the air samples, so could not be ascribed to the sampler efficiency. All the bioaerosol samplers had high biological efficiencies for all four bacterial species, except for *C. jejuni* (1%) when measured by the OMNI-3000 and for *E. coli* (38%) and *C. jejuni* (2%) when measured by the Airport MD8. The low biological efficiencies indicated that the sampling stresses inactivated the bacterial culturability. The mean half-life in the air was 43.3 min for *E. faecalis,* 26.7 min for *M. synoviae*, 21.2 min for *E. coli,* and 4.0 min for *C. jejuni* at 21-23°C temperature and 80%-85% relative humidity (RH). It is concluded that bioaerosol samplers vary in their physical and biological efficiencies for collecting different microbial species. Efficiency

assessment is essential so that suitable samplers for airborne microorganisms can be selected and in order to assure that the measurements are accurate.

EFFICIENCIES AND HALF-LIFE (GUMBORO VACCINE VIRUS, CHAPTER 7)

Losses of Gumboro vaccine virus in non-sampling processes and the efficiencies of four bioaerosols samplers were investigated in a similar way as described in Chapters 5 and 6. The results showed that an addition of 0.1% uranine (the tracer) did not reduce the infectivity of the virus. The loss of viral infectivity during aerosolization was remarkable: after this process, only 5% of virus remained infective. In the sample-handling procedures, the loss of infective virus increased further when the agar plates of the Andersen impactor, with virus, were stored for a long period (= 1 h) before further treatment. However, no loss of virus was noticed in the sample-handling procedure for the MD8, as long as its gelatin filters were dissolved within 60 min after being loaded with virus. The physical and biological efficiencies of the Andersen impactor, the AGI-30 and the MD8 were not significantly different from 100% for collecting the aerosolized virus. However, both the physical efficiency (62%) and biological efficiency (23%) of the OMNI-3000 were significantly lower than 100%.

DETECTING *CAMPYLOBACTER* IN EXPERIMENTAL BROILER ROOMS (CHAPTER 8)

Using the Andersen impactor, AGI-30 and OMNI-3000, the air in four broiler rooms was sampled to detect *Campylobacter jejuni* (*C. jejuni*). In each room there were five *C. jejuni*-infected broilers (infected when 14 days old) kept in a cage in the middle of the room, and ten healthy broilers kept individually 75 cm away from the infected ones. Air samples were taken on eight days, i.e. the day before inoculation (BI) as a negative control, and 1, 3, 6, 9, 14, 21, 29 days post-inoculation (PI). The presence of culturable *C. jejuni* was tested using a culture-based method, and the presence of *C. jejuni* DNA was tested with the PCR test. Results showed healthy broilers in all four rooms developed *C. jejuni* infections. PCR tests showed there was DNA of *C. jejuni* in the air on day 1 PI, but no bacterial DNA on the following days. No culturable *C. jejuni* was detected in any of the air samples. The failure to collect culturable *C. jejuni* was probably due to the low number of infected broilers in the rooms and the unfavorable survival conditions for these bacteria, leading to *C. jejuni* concentrations below the detection limits of the bioaerosol samplers. It is concluded that the three samplers used in this study are incapable of detecting *C. jejuni* outbreaks via aerial transmission when bacterial concentrations are low. New sampling techniques with low detection limits need to be developed for assessing bio-security.

GENERAL CONCLUSION

Outbreaks of infectious diseases in livestock production which cannot be explained by commonly known transmission mechanisms have led to the spread of the disease being ascribed to airborne transmission. However, the status of this form of transmission between livestock farms remains concessive. In this thesis, we conclude that the doubt surrounding airborne transmission comes from the knowledge gaps in every process, from microorganism suspension, transportation, deposition in respiratory tract and finally to infection. There is great need for more knowledge. The knowledge should be gained by collaborative interdisciplinary research (by scientists from veterinary, animal, microbiologic, and epidemiological sciences). The end-of-pipe technology, i.e. multi-stage air scrubbers, is promising for reducing the emissions of airborne microorganisms and dust from livestock houses. The EU impaction pre-separator for $PM_{2.5}$ sampling for ambient air cannot be directly transferred for use in livestock production systems, where dust concentrations are much higher. A cyclone pre-separator is very tolerant of high dust concentrations, and is recommended as PM sampler in livestock production systems. This thesis also shows that bioaerosol samplers for sampling airborne microorganisms vary in their efficiencies, and these must be investigated so that suitable samplers can be selected for measurements in practice. One problem identified in this thesis is that the tested bioaerosol samplers (the Andersen impactor, the AGI-30 and the OMNI-3000) were not capable of detecting a specific bacterial species (*Camplyobacter*) in the air, probably because their detection limits are higher than the bacterial concentration. More research should be done on improving the sampling protocol for airborne microorganisms (e.g. a proper sampling strategy and samplers with low detection limits).

SAMENVATTING

Intensivering van de veehouderij geeft hogere producties (b.v. van melk, vlees en eieren) per eenheid arbeid, voer en grond, maar wordt tevens geassocieerd met hogere concentraties en emissies van vervuilende componenten in de lucht, die lokaal, regionaal en wereldwijd serieuze problemen kunnen geven. Twee van de meest belangrijke vervuilende componenten zijn micro-organismen en stof. Concentraties micro-organismen en stof in stallucht zijn vele malen hoger dan bijvoorbeeld de luchtconcentraties in een stedelijke omgeving en ze overschrijden vaak advieswaarden of wettelijke grenswaarden. Concentraties micro-organismen in de lucht van een stedelijk omgeving zijn ongeveer 3 $\log_{10}$ kolonievormende eenheden (kve) m^{-3}. De microbiële concentraties die in stallen gemeten worden kunnen variëren van 6 $\log_{10}$ kve m^{-3} tot 9 $\log_{10}$ kve m^{-3}. Onderzoek naar concentraties stof in de lucht focussen steeds meer op de fracties kleine stofdeeltjes, zoals PM_{10} (Particulate Matter 10 = stofdeeltjes < 10 µm) en $PM_{2.5}$ (stofdeeltjes < 2,5 µm). Deze kleine deeltjes zijn schadelijker voor de gezondheid dan de grotere deeltjes, omdat ze dieper doordringen in de longen. De Europese Commissie heeft grenswaarden vastgesteld voor de gemiddelde jaarlijkse concentratie van PM_{10} en $PM_{2.5}$; deze mogen niet hoger zijn dan respectievelijk 40 en 25 µg m^{-3}. PM concentraties in stallen, vooral in varkens- en pluimveestallen, kunnen hier echter bij lange na niet aan voldoen. Er is daarom een sterke noodzaak om concentraties en emissies van micro-organismen en stof in en uit stallen te reduceren.

Buiten het schadelijke effect voor het milieu, kunnen de emissies van stof en micro-organismen ook gezondheidsproblemen veroorzaken bij dieren in nabijgelegen stallen of bij mensen die in de buurt wonen. Eén van de uitkomsten van de vele ziekte-uitbraken in de afgelopen tientallen jaren is dat aerogene transmissie (transmissie via de lucht) mogelijk een rol speelt bij de verspreiding van de ziekte. Hierbij zou stof tevens een rol kunnen spelen als drager van de ziektekiem. Deze gedachte is opgekomen als gevolg van het feit dat sommige bedrijven besmet werden zonder dat dit toegewezen kon worden aan bijvoorbeeld transport van objecten (auto's, vrachtauto's) of van mensen of dieren. Echter, het blijft nog steeds onduidelijk wat de precieze bijdrage is van de aerogene transmissieroute aan de verspreiding van ziektekiemen van bedrijf naar bedrijf en wat de rol is van stof in dit proces. Om deze kennislacune te vullen moet onze kennis op dit gebied worden verbreed. Hiervoor zijn gevalideerde bemonsteringstechnieken nodig die nauwkeurig concentraties micro-organismen en stof in stallucht kunnen bepalen.

De doelstelling van dit onderzoek was om meer kennis te verkrijgen rond micro-organismen in stallucht en de relatie met stof door: 1) een literatuuronderzoek naar de processen en factoren die de verspreiding van micro-organismen en stof via de stallucht beïnvloeden; 2) onderzoek naar de efficiëntie van systemen om de emissie van micro-organismen en stof via de stallucht te reduceren; 3) evaluatie van de efficiëntie en geschiktheid van monsternameapparatuur (samplers) voor bepaling van concentraties micro-organismen en stof in stallen; 4) valideren van deze samplers in een experiment naar aerogene transmissie van *Campylobacter* bij vleeskuikens. De gedetailleerde doelstellingen waren:

- literatuuronderzoek naar de bronnen, concentraties, fysische en biologische afbraak tijdens transmissie, depositie in de longen van mens en dier, en bemonsterings- en reductietechnieken voor micro-organismen en stof. (Hoofdstuk 2).

- bepalen van de efficiëntie van gecombineerde luchtwassers om emissies van micro-organismen, stof, ammoniak en CO_2 uit varkensstallen te reduceren. (Hoofdstuk 3).

- onderzoek naar het probleem van overlading van de EU referentie sampler voor PM_{10} en $PM_{2.5}$ wanneer ze worden gebruikt in stallen, en het testen van alternatieve PM_{10} en $PM_{2.5}$ apparatuur (met cycloon voorafscheiders). (Hoofdstuk 4).

- bepalen van de fysische en biologische efficiëntie van de 'Andersen six-stage impactor', de AGI-30, de OMNI-3000 en de MD8 voor het bemonsteren van 4 in de lucht vernevelde bacteriesoorten (*Enterococcus faecalis*, *Escherichia coli*, *Campylobacter jejuni* en *Mycoplasma synoviae*) en één virussoort (Gumboro vaccin virus). (Hoofdstukken 5, 6 en 7).

- detectie van *Campylobacter jejuni* met drie typen samplers ('Andersen six-stage impactor', AGI-30 en OMNI-3000) in een transmissie-experiment bij vleeskuikens. (Hoofdstuk 8).

MICRO-ORGANISMEN EN STOF IN STALLUCHT (HOOFDSTUK 2)

In deze literatuurstudie is onderzoek gedaan naar de achtergronden van micro-organismen en stof in stallen: de bronnen, de concentraties, de fysische en biologische afbraak tijdens transmissie, de depositie in de longen van mens en dier, de besmetting van dieren, en de bemonsterings- en reductietechnieken. Uit dit onderzoek kan geconcludeerd worden dat de bronnen van micro-organismen en stof in stallucht vergelijkbaar zijn. De belangrijkste bronnen zijn: excrementen (feces en urine), secreties, haar en huid, voer en strooisel materiaal. De bijdrage van deze bronnen aan de concentraties in de stallucht is verschillend. De concentraties micro-organismen en stof in stallucht, vooral in varkens- en pluimveestallen, zijn in het algemeen zeer hoog. Deze kunnen naar buiten emitteren en kunnen tijdens dit proces fysische veranderingen ondergaan. Micro-organismen kunnen ook biologische veranderingen ondergaan. Micro-organismen kunnen ingeademd worden en neerslaan in de luchtwegen. Waar ze neerslaan hangt af van de grootte van het deeltje. Monsternametechnieken voor stof in de omgevingslucht zijn misschien niet geschikt om toe te passen in stallen, waar de concentraties vele malen hoger zijn. De efficiëntie van bioaerosol samplers zullen tevens vastgesteld moeten worden. Reductietechnieken voor micro-organismen en stof in de lucht bestaan uit oplossingen bij de bron en luchtzuivering. Gecombineerde technieken bieden misschien betere mogelijkheden om deze concentraties en emissies in stallen vergaand te reduceren.

EFFICIËNTIE VAN GECOMBINEERDE LUCHTWASSERS OM EMISSIES UIT STALLEN TE BEPERKEN (HOOFDSTUK 3)

De concentraties bacteriën, stof, ammoniak en kooldioxide (CO_2) in de lucht zijn gemeten voor en na drie gecombineerde luchtwassers (twee wassers met twee reinigingsstappen: een wasfase met zuur gecombineerd met een bio-filter / bio-wasser, en één wasser met drie reinigingsstappen: een waterfase, een wasfase met zuur, vervolgens een bio-filter) die geïnstalleerd waren op drie verschillende varkensstallen. Het verschil tussen ingaande en uitgaande concentraties werd gebruikt om de verwijderingsrendementen van de verschillende componenten te berekenen. De verwijderingsrendementen waren 46%-85% voor totaal aantal bacteriën, 61%-93% voor PM_{10}, 47%-90% voor $PM_{2.5}$ en 70%-100% voor ammoniak; emissies van CO_2 werden niet beïnvloed. De luchtwassers waren effectiever in het verwijderen van grotere bacteriën in de range > 3.3 µm (53%-92%) dan kleinere bacteriën in the range van 0.65-3.3 µm (-42%-20%). De driefasen wasser was het meest efficiënt in het verwijderen van stof en ammoniak, waarschijnlijk als gevolg van een langere verblijftijd van de lucht in deze wasser. Vergeleken met enkelvoudige wassers, lijken gecombineerde luchtwassers constanter te presteren ten aanzien van de reductie van bacteriën, PM_{10}, $PM_{2.5}$ en ammoniakemissie uit stallen. Hierbij moet opgemerkt worden dat alle metingen in de winter zijn gedaan bij relatief lage ventilatiehoeveelheden, dus bij relatief lage belasting van de luchtwassers. Om een volledig beeld te krijgen van de werking van deze luchtwassers zullen ze ook bij hoge ventilatiehoeveelheden moeten worden getest.

CYCLOON VOORAFSCHEIDERS VOOR PM MONSTERNAME IN STALLEN (HOOFDSTUK 4)

De EU referentie sampler (monsternameapparaat) met een impactie voorafscheider (IPS), waarbij de grotere deeltjes neerslaan op een ingevette plaat, zou weleens snel overbeladen kunnen raken in stoffige omgevingen, zoals in stallen. Dit overladingsprobleem is onderzocht in een leghennenstal, waarbij de ingevette impactieplaat regelmatig werd schoongemaakt. Op deze plaat impacteren de deeltjes die groter zijn dan PM_{10} of $PM_{2.5}$. De resultaten lieten zien dat PM_{10} IPS niet werd overbeladen gedurende een 24-uurs meting in leghennenstallen, terwijl $PM_{2.5}$ IPS al binnen 1 uur werd overbeladen. Daarom is een $PM_{2.5}$ cycloon voorafscheider (CPS) getest op gevoeligheid voor overbelading in stoffige omgevingen. Deze test liet zien dat CPS niet werd overbeladen gedurende 48 uren monstername in een leghennenstal. Bij het volgen van het EU standaard protocol voor validatie van kandidaat PM samplers, werd voor zowel PM_{10} als voor $PM_{2.5}$ CPSs in omgevingslucht bij lage stofconcentraties een goede nauwkeurigheid en een goede vergelijkbaarheid gevonden met de impactie voorafscheider. In varkens- en pluimveestallen met hoge stofconcentraties, gaf PM_{10} CPS vergelijkbare resultaten als de IPS wanneer een correctiefactor werd toegepast. Dit standaard protocol kon echter niet worden toegepast voor het valideren van $PM_{2.5}$ CPS in stallen, aangezien de $PM_{2.5}$ referentie IPS sampler (voor buitenlucht) in stallen zwaar wordt overbeladen. Uit dit onderzoek kon geconcludeerd worden dat $PM_{2.5}$ IPS niet geschikt is voor toepassing in stallen, maar $PM_{2.5}$ CPS wel, aangezien deze zeer tolerant is voor hoge stofconcentraties. Voor PM_{10} monstername kunnen zowel de IPS als de CPS worden gebruikt.

VERLIES VAN LEVENSVATBAARHEID VAN MICRO-ORGANISMEN IN 'NON-SAMPLING' PROCESSEN (HOOFDSTUK 5)

Bij verneveling van een vloeistof met bacteriën waaraan een tracer is toegevoegd, voor het bepalen van de depositie van bioaerosolen en de efficiëntie van de samplers, kunnen bacteriën hun levensvatbaarheid (in feite het vermogen om zich te vermenigvuldigen tijdens de kweek) verliezen als gevolg van: 1) de tracer; 2) het vernevelen; 3) de monstername; 4) de nabehandeling van de monsters. Alleen de verliezen tijdens de monstername zijn direct geassocieerd met de efficiëntie van de samplers. Voordat we de efficiëntie van vier bioaerosol samplers voor vier verschillende bacteriën hebben bepaald, hebben we de verliezen als gevolg van de 'non-sampling' processen bepaald. De resultaten lieten zien dat uranine, een fluorescerend tracer, geen effect had op de levensvatbaarheid van *Enterococcus faecalis*, *Escherichia coli* en *Mycoplasma synoviae* in de vloeistof, terwijl de levensvatbaarheid van *Campylobacter jejuni* wel werd gereduceerd (P = 0.01). *E. faecalis*, *E. coli* en *M. synoviae* behielden hun levensvatbaarheid tijdens vernevelen, terwijl slechts 29% van *C. jejuni* levensvatbaar bleef. Tijdens de monsternamebehandeling werden geen significante verliezen waargenomen voor de Andersen impactor, echter deze sampler had slechts een uranine recovery van 33%-60%. Monsters genomen met de Airport MD8 van *E. faecalis*, *E. coli* en *M. synoviae* konden zonder verliezen worden nabehandeld. Voor *C. jejuni* werden na behandeling meer bacteriën teruggevonden (172%), waarschijnlijk als gevolg van multiplicatie. De conclusie van dit onderzoek is dat tracer en bacteriën apart moeten worden verneveld als de tracer de levensvatbaarheid van de bacterie beïnvloedt. Verlies aan levensvatbaarheid van bacteriën (of multiplicatie) kan optreden in processen voor en na monstername. Hiervoor moet worden gecorrigeerd wanneer de monstername efficiëntie wordt berekend.

EFFICIËNTIE EN HALFWAARDETIJD (BACTERIËN, HOOFDSTUK 6)

De fysische en biologische efficiëntie van de Andersen 6-stage impactor, all-glass impinger (AGI-30), hoog volume sampler (OMNI-3000) en Airport MD8 met gelatine filter voor het bemonsteren van vernevelde *E. faecalis*, *E. coli*, *C. jejuni* en *M. synoviae* is bepaald, waarbij is gecorrigeerd voor verlies van levensvatbaarheid van bacteriën en verlies van uranine als gevolg van de 'non-sampling' processen. De fysische efficiëntie van de AGI-30 (74%) verschilde niet significant van die van de Airport MD8, die als referentie diende vanwege zijn hoge fysische efficiëntie. De OMNI-3000 (49%) had een lagere fysische efficiëntie dan de Airport MD8. De lage fysische efficiëntie van de Andersen impactor (18%) werd vooral veroorzaakt door de onvolledige recovery van uranine tijdens de nabehandeling van de monsters, dus kon dit niet toegeschreven worden aan de sampler. Alle bioaerosol samplers hadden een hoge biologische efficiëntie voor alle vier bacteriesoorten, behalve voor *C. jejuni* (1%) wanneer gemeten met de OMNI-3000 en voor *E. coli* (38%) en *C. jejuni* (2%) wanneer gemeten met de Airport MD8. Lage biologische efficiëntie wordt veroorzaakt door inactivering van bacteriën als gevolg van sampling stress. De halfwaardetijd in de lucht was 43,3 min voor *E. faecalis,* 26,7 min voor *M. synoviae*, 21,2 min voor *E. coli* en 4,0 min voor *C. jejuni* bij een temperatuur van 21-23°C en een luchtvochtigheid van 80%-85%. De conclusie is dat bioaerosol samplers variëren in hun fysische en biologische efficiëntie afhankelijk van de bacterie die wordt

bemonsterd. Daarom is het essentieel dat de efficiëntie van samplers wordt bepaald, zodat de juiste sampler kan worden geselecteerd voor het nauwkeurig meten van een bepaalde bacterie.

EFFICIËNTIE EN HALFWAARDETIJD (GUMBORO VACCIN VIRUS, HOOFDSTUK 7)

Verliezen van Gumboro vaccin virus in 'non-sampling' processen en de efficiëntie van vier bioaerosol samplers zijn onderzocht in een vergelijkbare opzet als beschreven in hoofdstukken 5 en 6. De resultaten lieten zien dat toevoeging van 0,1% uranine (als tracer) geen invloed had op de levensvatbaarheid (= vermogen om een embryo in een ei te infecteren) van het virus. De levensvatbaarheid van het virus nam opvallend sterk af tijdens het vernevelen. Na het vernevelen bleef slechts 5% van het virus levensvatbaar. Wanneer de agarplaten van de Andersen impactor, met het virus, lang (= 1 uur) werden opgeslagen voordat ze verder werden behandeld nam het aandeel levensvatbare virussen verder af. Bij de MD8 werd geen verlies aan levensvatbaarheid gevonden wanneer de gelatine filters binnen 60 min na monstername werden opgelost in een vloeistof. De fysische en biologische efficiëntie van de Andersen impactor, de AGI-30 en de MD8 waren niet significant verschillende van 100% voor het bemonsteren van de vernevelde virusdeeltjes. Echter, zowel de fysische (62%) als de biologische (23%) efficiëntie van de OMNI-3000 was significant lager dan 100%.

DETECTIE VAN *CAMPYLOBACTER* IN PROEFAFDELINGEN VOOR VLEESKUIKENS (HOOFDSTUK 8)

De lucht in vier kleine vleeskuikenafdelingen werd bemonsterd op aanwezigheid van *Campylobacter jejuni* (*C. jejuni*) met een Andersen impactor, AGI-30 en OMNI-3000. In elke afdeling werden vijf met *C. jejuni* geïnfecteerde vleeskuikens geplaatst (geïnfecteerd op 14 dagen leeftijd) in een kooi in het midden van de afdeling, en tien gezonde vleeskuikens die individueel waren gehuisvest op 75 cm afstand van de geïnfecteerde dieren. Luchtmonsters werden genomen op de volgende 8 dagen: de dag voor inoculatie met *C. jejuni* (BI) als de negatieve controle en 1, 3, 6, 9, 14, 21 en 29 dagen na inoculatie (PI). De aanwezigheid van levensvatbare (= kweekbare) *C. jejuni* werd getest met behulp van een kweekmethode en de aanwezigheid van *C. jejuni* DNA werd bepaald met de PCR test. De resultaten lieten zien dat in alle afdelingen een aantal niet geïnoculeerde vleeskuikens een *C. jejuni* infectie ontwikkelde. PCR testen toonden DNA aan van *C. jejuni* in de lucht op dag 1 PI, maar geen DNA op de volgende dagen. In geen van de luchtmonsters werd levensvatbare *C. jejuni* aangetoond. Het niet kunnen aantonen van levensvatbare *C. jejuni* werd waarschijnlijk veroorzaakt door het geringe aantal geïnfecteerde vleeskuikens in de afdelingen en de ongunstige omgevingscondities voor deze bacterie, met als gevolg dat *C. jejuni* concentraties in de lucht onder de detectielimiet lag van de bioaerosol samplers. Hieruit kan worden geconcludeerd dat de drie samplers die zijn gebruikt in deze studie niet geschikt zijn om *C. jejuni* uitbraken via aerogene transmissie aan te tonen bij lage concentraties. Nieuwe bemonsteringstechnieken zijn nodig met een lage detectielimiet om deze transmissieroute te kunnen monitoren voor bio-security.

ALGEMENE CONCLUSIE

Uitbraken van infectieuze ziekten in de veehouderij die niet toegeschreven konden worden aan de normale transmissiemechanismen hebben geleid tot het toeschrijven van de besmetting aan de aerogene transmissieroute. Echter, in welke mate deze transmissieroute bijdraagt aan de verspreiding van ziekten tussen bedrijven blijft onduidelijk. Uit dit proefschrift kan geconcludeerd worden dat de twijfels die er zijn rond aerogene transmissie voortkomt uit gebrekkige kennis van de verschillende processtappen, van opname van de micro-organismen in de lucht, het transport via de lucht, de depositie in de luchtwegen, tot de infectie. Daarom is er sterke behoefte aan meer kennis op dit gebied. Deze kennis moet ontwikkeld worden in een samenwerking tussen onderzoekers uit verschillende disciplines (veterinairen, zoötechnici, microbiologen en epidemiologen). Gecombineerde luchtwassers, een end-of-pipe technologie, zijn veelbelovend om emissies van micro-organismen en stof via de stallucht te reduceren. De EU impactie voorafscheider voor bemonstering van $PM_{2.5}$ in de omgevingslucht kan niet worden gebruikt in de stoffige omgeving van stallen. Voor deze omgevingen is een cycloon voorafscheider beter geschikt, aangezien deze niet gevoelig is voor overbelading bij hoge stofconcentraties. De cycloon voorafscheider wordt daarom aanbevolen voor PM monstername in stallen. Dit proefschrift laat ook zien dat de efficiëntie van bioaerosol samplers voor het bemonsteren van verschillende micro-organismen sterk kan variëren. Daarom zal deze efficiëntie moeten worden vastgesteld om geschikte samplers te selecteren voor metingen in de praktijk. Een probleem dat in dit proefschrift naar voren kwam is dat de geteste bioaerosol samplers (de Andersen impactor, de AGI-30 en de OMNI-3000) niet in staat waren om een bepaalde bacterie (*Camplyobacter*) in de lucht aan te tonen, waarschijnlijk als gevolg van een hogere detectielimiet dan de concentraties in de lucht. Meer onderzoek is daarom nodig naar betere monsternameprotocollen voor aerogene micro-organismen (o.a. een goede bemonsteringsstrategie en samplers met een lage detectielimiet).

LIST OF PUBLICATIONS

PEER REVIEWED PAPER

Zhao, Y., Zhao, Y.J., Li, B.M., Shi, Z.X., 2006. The influence of density and a partially shadowed layer on the behaviour and welfare of broiler breeders in battery cages during laying. Zoological Research 27, 433-440.

Zhao, Y.J., **Zhao, Y.**, Shi, Z.X., Li, B.M., 2006. Feather pecking of broiler laying hens in light versus dark regions in shade battery cage. Journal of China Agricultural University 11, 13-17.

Zhao, Y., Aarnink, A.J.A., Hofschreuder, P., Groot Koerkamp, P.W.G., 2009. Evaluation of an impaction and a cyclone pre-separator for sampling high PM_{10} and $PM_{2.5}$ concentrations in livestock houses. Journal of Aerosol Science 40, 868-878.

Cambra-López, M., Aarnink, A.J.A., **Zhao, Y.**, Calvet, S., Torres, A.G., 2010. Airborne particulate matter from livestock production systems: A review of an air pollution problem. Environmental Pollution 158, 1-17.

Zhao, Y., Aarnink, A.J.A., Doornenbal, P., Huynh, T.T.T., Groot Koerkamp, P.W.G., de Jong, M.C.M., Landman, W.J.M., 2011. Investigation of the efficiencies of bioaerosol samplers for collecting aerosolized bacteria using a fluorescent tracer. I: Effects of non-sampling processes on bacterial culturability. Aerosol Science and Technology 45, 423-431.

Zhao, Y., Aarnink, A.J.A., Doornenbal, P., Huynh, T.T.T., Groot Koerkamp, P.W.G., Landman, W.J.M., de Jong, M.C.M., 2011. Investigation of the efficiencies of bioaerosol samplers for collecting aerosolized bacteria using a fluorescent tracer. II: Sampling efficiency, and half-life time. Aerosol Science and Technology 45, 432-442.

Zhao, Y., Aarnink, A.J.A., de Jong, M.C.M., Ogink, N.W.M., Groot Koerkamp, P.W.G., 2011. Effectiveness of multi-stage scrubbers on reducing emissions of airborne bacteria and dust from pig houses. Transactions of the ASABE 54, 285-293.

Zhao, Y., Aarnink, A.J.A., Wang, W., Fabri, T., Groot Koerkamp, P.W.G., de Jong, M.C.M., 2011. Evaluation of efficiencies of four bioaerosol samplers for collecting aerosolized Gumboro vaccine virus. Applied and Environmental Microbiology. (Submitted).

Zhao, Y., Aarnink, A.J.A., Groot Koerkamp, P.W.G., Hagenaars, T.J., Katsma, W.E.A., de Jong, M.C.M., 2011. Detection of airborne *Campylobacter* with three bioaerosol samplers for alarming bacteria transmission in broilers. Biological Engineering. (Submitted).

Zhao, Y., Aarnink, A.J.A., Groot Koerkamp, P.W.G., de Jong, M.C.M., 2011. Airborne microorganisms and their relation to dust from livestock production systems. Critical Reviews in Environmental Science and Technology. (Submitted).

Zhao, Y., Aarnink, A.J.A., de Jong, M.C.M., Groot Koerkamp, P.W.G., 2011. Effects of temperature, relative humidity, absolute humidity and evaporation potential on survival of airborne Gumboro vaccine virus. Applied and Environmental Microbiology. (Submitted).

Aarnink, A.J.A., van Harn, van Hattum, T.G., **Zhao, Y.**, Ogink, N.W.M., 2011. Dust reduction in broiler houses by spraying rapeseed oil. Transactions of the ASABE. (Submitted).

Adell, E., Moset, V., **Zhao, Y.**, Cerisuelo, A., Cambra-López, M., 2011. Concentration and distribution of airborne aerobic mesophilic bacteria in broiler farms. Información Técnica Económica Agraria. (Submitted).

MANUSCRIPT IN PREPARATION

Zhao, Y., Aarnink, A.J.A., Cambra-López, M., de Jong, M.C.M., Groot Koerkamp, P.W.G., 2011. Sources and emission of airborne Gumboro vaccine virus from an experimental broiler room.

Zhao, Y., Aarnink, A.J.A., Groot Koerkamp, P.W.G., 2011. Clearance of airborne *Enterococcus faecalis* and Gumboro vaccine virus by a UV-TiO$_2$ scrubber.

CONFERENCE PROCEEDING, PAPER AND ABSTRACT

Hofschreuder, P., Aarnink, A.J.A., **Zhao, Y.**, Ogink, N.W.M., 2007. Measurement protocol for determining fine dust emission factors of animal housing systems. In: Proceedings of Dustconf 2007, How to Improve Air Quality, Maastricht, the Netherlands.

Zhao, Y., Aarnink, A.J.A., Groot Koerkamp, P.W.G., Vroegindeweij, B.A., Hagenaars, T.J., Katsma, W.E.A., de Jong, M.C.M., 2008. Air sampling for detecting airborne transmission of *Campylobacter* in broilers. In: The Eighth International Symposium of Livestock Environment, Iguassu Falls, Brazil.

Zhao, Y., Aarnink, A.J.A., Groot Koerkamp, P.W.G., Ogink, N.W.M., De Jong, M.C.M., 2008. Removal efficiency of dust and bacteria by multi-stage air scrubbers in a pig house. In: The Eighth International Symposium of Livestock Environment, Iguassu Falls, Brasil, 139-145.

Aarnink, A., van Harn, J., van Hattum, T., **Zhao, Y.**, Ellen, H., 2008. Dust reduction in broiler houses by spraying rapeseed oil. In: The Eighth International Symposium of Livestock Environment, Iguassu Falls, Brazil, 133-138.

Aarnink, A.J.A., **Zhao, Y.**, Hofschreuder, P., Ogink, N.W.M., 2008. Validation of the cyclone as a pre-separator for airborne fine dust sampling in animal houses. In: The Eighth International Symposium of Livestock Environment, Iguassu Falls, Brasil, 79-84.

Zhao, Y., Aarnink, A.J.A., Groot Koerkamp, P.W.G., de Jong, M.C.M., Doornenbal, P., Huynh, T.T.T., Landman, W.J.M., 2009. Assessment of the efficiency of four air bioaerosol samplers

to detect aerosolized *Enterococcus faecalis*. In: 16th Congress of World Veterinary Poultry Association, Marrakesh, Morocco.

Zhao, Y., Aarnink, A.J.A., Groot Koerkamp, P.W.G., de Jong, M.C.M., Doornenbal, P., Huynh, T.T.T., Landman, W.J.M., 2009. Assessment of the efficiency of four air bioaerosol samplers after aerosolization of *Enterococcus faecalis* suspensions: A preliminary study. In: The 2009 CIGR International Symposium of the Australian Society for Engineering in Agriculture (SEAG2009), Brisbane, Australia.

Zhao, Y., Aarnink, A.J.A., Groot Koerkamp, P.W.G., de Jong, M.C.M., Doornenbal, P., Huynh, T.T.T., Landman, W.J.M., 2010. A Method for evaluating sampling efficiency of bioaerosol samplers for airborne bacteria and virus. In: ASHRAE IAQ 2010 Conference, Kuala Lumpur, Malaysia.

Zhao, Y., Aarnink, A.J.A., de Jong, M.C.M., Groot Koerkamp, P.W.G., 2011. Measuring airborne microorganisms and dust from livestock production systems. In: XV ISAH Congress 2011 "Animal Hygiene and Sustainable Livestock Production", Vienna, Austria.

INTERNAL REPORT

Aarnink, A.J.A., van Hattum, T., Hol, A., **Zhao, Y.**, 2007. Reduction of fine dust emission by combi-scrubber of Big Dutchman. Wageninge UR Livestock Research, Lelystad, The Netherlands, pp. 16.

Hofschreuder, P., **Zhao, Y.**, Aarnink, A.J.A., Ogink, N.W.M., 2008. Measurement protocol for emissions of fine dust from animal houses. Wageningen UR Livestock Research, Lelystad, The Netherlands, pp. 38.

Zhao, Y., Landman, W.J.M., Doornenbal, P., Aarnink, A., 2009. Clearance of airborne *Enterococcus faecalis* and Gumboro vaccine virus by a Philips air scrubber. Wageningen UR Livestock Research, Lelystad, The Netherlands, pp. 18.

Aarnink, A.J.A., **Zhao, Y.**, van Hattum, T., Hol, A., 2009. Reduction of emission of fine dust by a Inno+ multi-stage scrubber. Wageningen UR Livestock Research, Lelystad, The Netherlands, pp. 18.

Aarnink, A.J.A., **Zhao, Y.**, van Hattum, T., Hol, A., 2009. Reduction of emission of fine dust by a Uniqfill multi-stage scrubber. Wageningen UR Livestock Research, Lelystad, The Netherlands, pp. 19.

ACKNOWLEDGEMENTS

First of all, I would express my most sincere THANKS to my daily supervisor, Dr. André Aarnink. It's you, who brought me into the world of science. You act as a trustful supporter in my work, and your inspired ideas always help me out of the problems. Your charisma is not limited in science, but also in life. You act as a reliable friend for sharing feelings and experiences. I will never forget our beer meetings in De Vlaamsche Reus, the dinners in your house, the lovely trip to China with you and your family, and the warm welcome to the Dutch trips of my family. André, you are far beyond a supervisor! I am looking forward your book: "Philosophy in being an admired supervisor".

I would like to express my deep gratefulness to my two promoters, Professor Peter Groot Koerkamp and Professor Mart de Jong, for their scientific supervision. This thesis would have not been completed without your efforts. Peter, I am very lucky to be guided by you. Thank you also for sharing party with me and for helping my Chinese guests. Mart, your critical comments are of valuable to improve my work. Thanks are expressed to Dr. Wil Landman for revision of the journal papers. You can always organize the sentences and paper structure in a clear way, which I learnt and am still learning. Prof. Klaas Frankena, I appreciate your assistances in data analysis.

Great appreciations are expressed to my wonderful colleagues in Wageningen UR Livestock research for creating such a harmonious atmosphere to work in. You will not be forgotten: Mr. Sjoerd Bokma, Mr. Fridtjof de Buisonje, Mr. Hendrik Jan van Dooren, Mr. Hilco Ellen, Mr. Alfred Gerrits, Ms. Karin Groenestein, Mr. Theo van Hattum, Mr. Jan van Harn, Mr. Paul Hoeksma, Mrs. Annemieke Hol (and her family), Mr. Wim Houwers, Mr. Jos Huis In T Veld, Mr. Gerrit Kasper, Mr. Geert Kupers, Mr. Eef Lovink (and his family), Mr. Roland Melse (and his family), Mr. Julio Mosquera Losada, Mr. Guus Nijeboer (and his family), Mr. Nico Ogink (and his family), Mr. Johan Ploegaert, Mr. Michel Smits, Mr. Dick Starmans, Mr. Nico Verdoes, Mr. Jerke de Vries.

I thank my co-authors and co-workers: Dr. Nico Bolder, Mr. Frans Putirulan, Ms. Ria van der Hulst, Dr. Thomas Hagenaars, Ms. Elly Katsma, Dr. Thuy Huynh, Mr. Jean Slangen, Mr. Bastiaan Vroegindeweij, Mr. Dennis Snoek, Mr. Xu Lu, and Mr. Frans van Korlaar. My thanks also go to the Groups in Spelderholt, in Zodiac farm, and in Runderweg farm.

Parts of the thesis were done in Animal Health Service (GD) in Deventer. I would like to express my thanks to the co-workers over there. Thanks go to Mrs. Thea von Bannisseht, Mr. Piet Doornenbal, Mr. Machiel Esmann, Mr. Ad Klein Koerkamp, Ms. Constance Reugebrink, Mr. Wilco Vije, Mr. Johan Welner, and Mr. Dave Ufee for assisting in the experiment. Acknowledgements are given to Mr. Teun Fabri, Mr. Floris Ruiterkamp, Mr. Gejan Bolink, Ms. Annelies Hoogkamer and Mr. Huub van de Sande as well.

My thanks go to Professor Martin Verstegen, Mrs. Mariet Verstegen, Mrs. Joke Aarnink, Ms. Mien Lieferink, Ms. Els Lieferink, Ms. Garla Lieferink, and Ms. Désirée Meijer for helping me to emerge

into Dutch society. Martin, you are the best professor in life. Mariet, you and your positive attitude encourage me to finish my PhD. Joke, I like your delicious dishes in the dinners, and your special "stamppot" service to me. You appear cool from outside, but are warm in heart. Mien, Els and Garla, thank you for your hospitality, your lovely laughing, and your handmade card. Désirée, thank you for warm welcome to me and your nice food. Thank Dr. Phung Le Dinh, who helped me when I began my life in Wageningen.

Dr. Huong Lai in the soon future and Dr. Maria Cambra-Lopez, I appreciate your friendship which I believe that will last for the rest of my life. I want to say "good friends are stars, you don't always see them, but you know they are there." (a sentence in the birthday card that I received from Huong).

Dr. Joy Burrough, I appreciate your great contribution in English correction of my scientific papers and PhD thesis.

I would like to express my special gratitude to my study (co-)supervisors in China Agricultural University, Professor Baoming Li, Professor Zhengxiang Shi, Professor Shiwei Huang, and Dr. Yajun Zhao. Your guidance will never be forgotten.

My friends in Wageningen, I appreciate that you have shared a wonderful time with me in past and are creating a sweet memory for future. Our friendship is the treasure of my life.

At last, I would give my big hugs to my mother, my father, and my wife. I succeed today because you always stand beside me. Mom, you are definitely the most important person in my life. Thank you very much for endless love and support. Dad, I learn from you how to behave correctly in some cases. Wei, my wife, I appreciate you so much for your understanding and encouragement in these years when I am abroad. Thank you for all your efforts in establishing our own house in China. Thanks also go to the members of my parents' families.

爸妈，感谢你们对我无尽的爱，支持和培养。王伟，感谢你这些年来对我的理解与鼓励。我要感谢我大家庭中的长辈及兄弟姐妹，感谢并缅怀在我求学期间去世的爷爷奶奶。谨以此文献给你们！
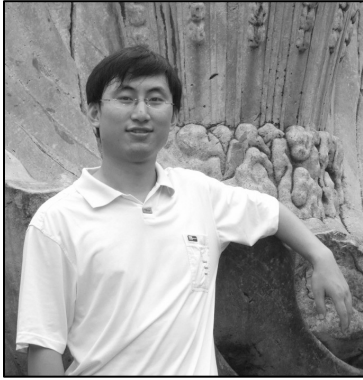
感谢所有在我人生道路上帮助过我的人。

Zhao Yang

April 2011

Wageningen, the Netherlands.

ABOUT THE AUTHOR

Zhao Yang（赵阳）was born on September 19, 1981 in Qingdao, Shandong province, China. He spent 12 years on his junior and senior education in his hometown. In 2000, he went to Beijing to study Agricultural Structure Environment and Energy Engineering at China Agricultural University (CAU). After graduating with a BSc in 2004, he continued to pursue his Master degree in Agri-biological Environmental and Energy Engineering at CAU. He received his MSc diploma in 2006. In the same year, he was given the opportunity to work as a PhD student in the Animal Sciences Group of Wageningen University and Research Centre (WUR) in the Netherlands. The topic of his research was "Measuring airborne microorganisms and dust from livestock houses". In June 2011 he will go to Iowa State University (ISU) in the United States to do postdoc research. His dream is to work for his city, and his country.

TRAINING AND SUPERVISION PLAN

Training and Supervision Plan			Graduate School WIAS	
Name PhD student	Zhao Yang			
Project title	Measuring airborne microorganisms and dust from livestock houses			
Group	Animal Sciences Group			
Daily supervisor(s)	Dr. Ir. André Aarnink			
Supervisor(s)	Prof. Peter Groot Koerkamp & Prof. Mart de Jong			
Project term	From Aug. 2006	Until Apr. 2011		
Submitted	Nov. 2010	Certificate		

EDUCATION AND TRAINING		
The Basic Package	year	credits *
WIAS Introduction Course	2008	
Course on philosophy of science and/or ethics "Biology underpinning animal sciences: Broaden your Horizon" <Oct 25; Nov 1,9,15,22>	2007	
Introduction interview with WIAS scientific director and secretary: <Nov 9>	2006	
Introduction interview with WIAS education coordinator: <Oct 2>	2006	
Introduction interview with WIAS PhD students confidant: <Nov 9>	2006	
Subtotal Basic Package		**3**
Scientific Exposure (conferences, seminars and presentations)		
International conferences		
< full name of conference, place, start and end dates >		
International Dust Conference, Maastricht, The Netherlands, Apr 23-24	2007	
International Livestock Symposium (ILES VIII) <1 oral, 1 poster>, Iguassu, Brazil, Sep 1-5	2008	
SEAG2009 <1 oral>, Brisbane, Australia, Sep 13-16	2009	
IAQ 2010 <1 oral>, Kuala Lumpur, Malaysia, Nov 10-12	2010	
Seminars and workshops		
< full name of seminar / workshop, place, date >		
WIAS science day, Wageingen, The Netherlands, Mar 8	2007	
Dynamics of knowledge management in agricultural R&D, Wageningen, Nov 13	2006	
Immune response to viruses: a comparable approach, Wageningen, Nov 17	2006	
Publish or perish, wageningen, Sep 17	2008	
WIAS science day, Wageingen, The Netherlands, Mar 12	2009	
WIAS science day, Wageingen, The Netherlands, Jan 28	2010	
Presentations		
< title of presentation, place, date, oral / poster >		
WIAS science day, Wageingen, The Netherlands, Mar 13 <1 oral>	2008	
International Livestock Symposium (ILES VIII) <oral & poster presentation>, Iguassu, Brazil, Sep 1-5	2008	
KB 8 Projectendag, <oral presentation> Lelystad, The Netherlands, Nov 6	2008	
SEAG2009, <oral presentation>, Brisbane, Australia, Sep 13-16	2009	
WIAS science day <poster presentation>, Wageingen, The Netherlands, Jan 28	2010	
IAQ 2010, <oral presentation>, Kuala Lumpur, Malaysia, Nov 10-12	2010	
Subtotal Scientific Exposure		**12**
In-Depth Studies		
Management of Microbiological Hazards in Foods, Wageningen, Mar 28-Apr 1	2011	
Advanced statistics courses		
Orientation on mathematical modelling in biology	2010	
WIAS Advanced Statistics Course: Design of Animal Experiments, Wageningen, Feb 13-15	2008	

Statistics for the Life Sciences, Wageningen, May 30, 31 and Jun 1, 4, 5	2007	
MSc level courses		
Quantitative Veterinary Epidemiology, Wageningen (QVE-30306)	2007	
Subtotal In-Depth Studies		**10**
Professional Skills Support Courses		
Writing for academic publication, Lelystad	2006	
Working with Endnote 9.0, Wageningen	2006	
Information Literacy Course, Wageningen	2006	
Effective behaviour in your professional surroundings, Wageningen , Feb 23 and Mar 23	2009	
Subtotal Professional Skills Support Courses		**3**
Research Skills Training		
Preparing own PhD research proposal	2006	
Subtotal Research Skills Training		**6**
Didactic Skills Training		
Supervising practicals and excursions		
MSc Excursion in Animal Health Service (GD)	2010	
MSc Excursion in Animal Health Service (GD)	2011	
Supervising theses		
Bastiaan Vroeginderij (BSc)	2007	
Xu Lu (MSc major thesis)	2010	
Subtotal Didactic Skills Training		**3**
Education and Training Total		**37**

* one ECTS credit equals a study load of approximately 28 hours