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LIVESTOCK RESEARCH
WAGENINGEN UR

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Samenvatting NL Met de huidige kennis zijn we ons bewust dat ziektekiemen vanuit stallen via de lucht een risico kunnen vormen voor de gezondheid van mensen en dieren in de omgeving van deze stallen. Welke processen en invloedsfactoren hierbij een rol spelen is nog niet duidelijk. Micro-organismen worden in de lucht blootgesteld aan meteorologische factoren zoals temperatuur, vochtigheid en straling. Deze factoren kunnen belangrijke effecten hebben op de overleving van micro-organismen. Deze effecten kunnen verschillend zijn voor verschillende typen micro-organismen. Het doel van deze studie is de effecten van temperatuur en luchtvochtigheid op de overlevingsduur van verschillende bacterietypen in de lucht vast te stellen.

In een laboratoriumstudie zijn de effecten van temperatuur (T) en relatieve luchtvochtigheid (RV) op de overleving van drie verschillende typen bacteriën onderzocht in een 3x3 experimentele set-up voor T = 10, 20 en 30^{o}C en RV = 40, 60 en 80%. De volgende bacteriën zijn onderzocht: *Escherichia coli* (*E.coli*; Gram -), *Enterococcus mundtii* (*E.mundtii;* Gram +) en *Mycoplasma synoviae* (*M.synoviae*; geen celwand). Natte en droge suspensies van deze drie typen bacteriën werden in de lucht gesprayd van een isolator. De bacteriën in de lucht werden periodiek bemonsterd met impingers tussen 0,5 en 30 min na het sprayen. Uit de kiemtellingen van de op kweek gezette monsters konden de halfwaardetijden van de verschillende bacteriën worden bepaald. Uranine werd gebruikt als tracer om te corrigeren voor de fysische verliezen van de bacteriën, als gevolg van bijvoorbeeld depositie van de aerosolen op vloer en wanden. Additionele verliezen worden veroorzaakt door biologische verliezen als gevolg van het afsterven van de bacteriën.
De bacteriën in de isolator lieten een sterke afname zien tijdens en in de eerste 30 sec na het vernevelen van de natte aerosolen en een veel geringere afname in de daarop volgende 30 min. Dit werd waarschijnlijk veroorzaakt door de plotselinge afkoeling van de bacteriën als gevolg van de verdamping van de kleine waterdruppels. Na het vernevelen van de droge aerosolen konden alleen detecteerbare niveaus van *E.mundtii* worden vastgesteld. De sterke initiële afname van bacteriën in de droge aerosolen werd waarschijnlijk veroorzaakt door de voor bacteriën stressvolle procedure voor het maken van het 'bacteriestof'. In de initiële fase van de natte aerosolen was de afname van *E.coli* en *M.synoviae* groter dan voor *E.mundtii*. Het temperatuurseffect was in deze fase niet consistent, aangezien het een interactie vertoonde met de relatieve luchtvochtigheid voor de verschillende typen bacteriën. Na de initiële afname hadden *E.mundtii* en *M.synoviae* de geringste afname bij een temperatuur van 20^{o}C, terwijl *E.coli* het langst overleefde bij 30^{o}C. *E.mundtii* bacteriën hadden een langere overlevingstijd in droge dan in natte aerosolen. Dit zou veroorzaakt kunnen zijn doordat ze in de natte aerosolen, als gevolg van verdamping, hun beschermende waterlaag verliezen, waardoor ze gevoeliger worden voor omgevingsinvloeden. Het zou ook veroorzaakt kunnen zijn door het feit dat de bacteriën in de droge aerosolen al een stressvolle bereidingsfase van het stof overleefd hebben, waardoor deze bacteriën misschien ook minder gevoelig zijn voor de stressvolle situatie in de lucht. De halfwaardetijden van de bacteriën in natte aerosolen varieerde van 2 tot 28 min. In droge aerosolen was dit nog langer voor *E.mundtii* (tot 90 min).
Uit dit onderzoek kan worden geconcludeerd dat de geteste bacteriën, in de onderzochte range van temperatuur (10-30^{o}C) en relatieve vochtigheid (40-80%), voldoende lang in de lucht overleven om een flinke afstand met de wind te worden meegevoerd.
E.coli en *M.synoviae* hebben de procedure voor het prepareren van droge aerosolen nauwelijks overleefd. De impact van de procedure voor het maken van droge aerosolen op de levensvatbaarheid van micro-organismen is niet duidelijk en zou (daarom) nader moeten worden onderzocht.

Summary UK It is generally agreed upon that pathogenic microorganisms emitted from livestock buildings in wet and dry aerosols may cause animal and human diseases by airborne transmission. The processes involved in the transmission of microorganisms via the airborne route are still not well revealed. Airborne microorganisms are exposed to meteorological factors, particularly temperature, humidity, wind velocity and solar radiation. These factors may have significant effects on the survival and spreading of these micro-organisms. Effects may be different for different species. The objective of this study is to investigate the effects of temperature and humidity on the survival of different bacteria types in the air.

In a laboratory study the effect of temperature (T) and relative humidity (RH) on the survival of three different types of airborne microorganisms was assessed in a 3x3 experimental set up for T = 10, 20 and 30°C and RH = 40, 60 and 80%. The microorganisms studied were *Escherichia coli* (*E.coli*; Gram -), *Enterococcus mundtii* (*E.mundtii;* Gram +), and *Mycoplasma synoviae* (*M.synoviae*; no cell wall). Wet and dry suspensions of these three microorganism species were aerosolized in an isolated air space. Airborne microorganisms were periodically sampled by impingement between 0.5 and 30 min after aerosolization. From the counted culturable microorganisms in the subsequent samples the half-life time of the three microorganisms was calculated. Uranine was used as a tracer to correct for the physical losses of bacteria (e.g. deposition of aerosols on walls and floor). Additional losses were contributed to biological losses, i.e. loss of viability.
The bacteria in the isolator showed a large biological decay during and in the first 30 seconds after aerosolization of wet aerosols and a much smaller decay in the following 30 minutes. This was probably caused by the suddenly cooling down effect caused by evaporation of water. In dry aerosols only detectable levels of *E.mundtii* were found after aerosolization. The large initial decay of bacteria in dry aerosols was probably caused by the rigid preparation procedure of the dry aerosols. In the initial phase of wet aerosolization, the decay of *E.coli* and *M.synoviae* was larger than of *E.mundtii*. The effect of temperature in this phase was not consistent while it interacted with relative humidity for the different types of bacteria. After the initial decay, *E.mundtii* and *M.synoviae*, in wet aerosols, survived longest at a moderate temperature (20°C), while *E.coli* survived longest at 30°C. *E.mundtii* bacteria survived longer in dry aerosols than in wet aerosols. This could be caused by the loss of their protective water film in wet aerosols, causing them to become more vulnerable for ambient influences or it could be caused by the survival of the fittest bacteria during the rigid preparation procedure of dry aerosols before they were spread in the isolator. The half-life time of bacteria in wet aerosols ranged from 2 min to 28 min and in dry aerosols this was even longer for *E.mundtii*.
It is concluded that the tested airborne bacteria, within the studied range of temperature (10-30°C) and relative humidity (40-80%), survive long enough to be transmitted over a long distance. *E.coli* and *M.synoviae* hardly survived the procedures for preparing dry aerosols. The impact of the procedures for preparing dry aerosols on the viability of microorganisms is not fully clear and should be further studied.

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Foreword

In een aantal gebieden in Nederland is sprake van publieke ongerustheid over de effecten van veehouderijbedrijven op de gezondheid van omwonenden. Deze ongerustheid betreft onder meer de risico's voor omwonenden als gevolg van de uitstoot van bio-aerosolen en mogelijk daaraan gebonden ziektekiemen. In diverse onderzoeksprojecten wordt daarom momenteel gewerkt aan het verkrijgen van verbeterd wetenschappelijk inzicht in de eventuele effecten van (grootschalige) intensieve veehouderij op de gezondheid van omwonenden. Daarnaast is er behoefte aan inzicht in de mogelijkheden die technische maatregelen bieden om de uitstoot door veehouderijbedrijven van bio-aerosolen en daaraan gebonden micro-organismen te verminderen. Welke processen en invloedsfactoren hierbij een rol spelen is nog onvoldoende duidelijk. Micro-organismen worden in de lucht blootgesteld aan meteorologische factoren zoals temperatuur, vochtigheid, windsnelheid en straling. Deze factoren kunnen belangrijke effecten hebben op de overleving en de verspreiding van de micro-organismen. In de voorliggende studie zijn in een laboratoriumopstelling de effecten van temperatuur en vochtigheid op overleving van drie verschillende bacterietypen onderzocht. De studie is uitgevoerd door medewerkers van Livestock Research. Bacteriële analyses zijn verzorgd door de Gezondheidsdienst voor Dieren te Deventer. Vinnie de Wilde en Rob Heinen hebben meegewerkt aan de uitvoering van de experimenten.

Dit onderzoek is uitgevoerd in opdracht van het ministerie van Economische Zaken.

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Summary

It is generally agreed upon that pathogenic microorganisms emitted from livestock buildings in wet and dry aerosols may cause animal and human diseases by airborne transmission. The processes involved in the transmission of microorganisms via the airborne route are still not well revealed. Airborne microorganisms are exposed to meteorological factors, particularly temperature, humidity, wind velocity and solar radiation. These factors may have significant effects on the survival and spreading of these micro-organisms. Effects may be different for different species. The objective of this study is to investigate the effects of temperature and humidity on the survival of different bacteria types in the air.

In a laboratory study the effect of temperature (T) and relative humidity (RH) on the survival of three different types of airborne microorganisms was assessed in a 3x3 experimental set up for T = 10, 20 and 30^{o}C and RH = 40, 60 and 80%. The microorganisms studied were *Escherichia coli* (*E.coli*; Gram -), *Enterococcus mundtii* (*E.mundtii;* Gram +), and *Mycoplasma synoviae* (*M.synoviae*; no cell wall). Wet and dry suspensions of these three microorganism species were aerosolized in an isolated air space. Airborne microorganisms were periodically sampled by impingement between 0.5 and 30 min after aerosolization. From the counted culturable microorganisms in the subsequent samples the half-life time of the three microorganisms was calculated. Uranine was used as a tracer to correct for the physical losses of bacteria (e.g. deposition of aerosols on walls and floor). Additional losses were contributed to biological losses, i.e. loss of viability.

The bacteria in the isolator showed a large biological decay during and in the first 30 seconds after aerosolization of wet aerosols and a much smaller decay in the following 30 minutes. This was probably caused by the suddenly cooling down effect caused by evaporation of water. In dry aerosols only detectable levels of *E.mundtii* were found after aerosolization. The large initial decay of bacteria in dry aerosols was probably caused by the rigid preparation procedure of the dry aerosols. In the initial phase of wet aerosolization, the decay of *E.coli* and *M.synoviae* was larger than of *E.mundtii*. The effect of temperature in this phase was not consistent while it interacted with relative humidity for the different types of bacteria. After the initial decay, *E.mundtii* and *M.synoviae*, in wet aerosols, survived longest at a moderate temperature (20^{o}C), while *E.coli* survived longest at 30^{o}C. *E.mundtii* bacteria survived longer in dry aerosols than in wet aerosols. This could be caused by the loss of their protective water film in wet aerosols, causing them to become more vulnerable for ambient influences or it could be caused by the survival of the fittest bacteria during the rigid preparation procedure of dry aerosols before they were spread in the isolator. The half-life time of bacteria in wet aerosols ranged from 2 min to 28 min and in dry aerosols this was even longer for *E.mundtii*.

It is concluded that the tested airborne bacteria, within the studied range of temperature (10-30^{o}C) and relative humidity (40-80%), survive long enough to be transmitted over a long distance. *E.coli* and *M.synoviae* hardly survived the procedures for preparing dry aerosols. The impact of the procedures for preparing dry aerosols on the viability of microorganisms is not fully clear and should be further studied.

1 Introduction

Since the outbreaks of infectious animal diseases like Avian Influenza and Q-fever in the Netherlands many residents in areas with intensive livestock farming are increasingly concerned about the negative effects of livestock production on human health. Opposition against intended expansion and relocation of livestock production facilities is growing. Pathogenic microorganisms emitted from livestock buildings may cause animal and human diseases by airborne transmission. However, many knowhow gaps in this field still exist; real health risks still have to be quantified and differentiated per infection and farm type. As a consequence, primarily basic risk analysis is necessary to assess the effect of pathogen reductions on health risks in the neighbourhood. A good starting point is offered by a multi-disciplinary research on possible health effects for people living in the neighbourhood of livestock production facilities (Heederik and IJzermans, 2011). This research included field measurements of fine dust and microbial components in fine dust, analysis of data registered by family doctors and additionally a comparative study with a group of asthmatic persons and a group of non-asthmatic persons. Increased concentrations of fine dust and endotoxins were measured in intensive pig and poultry production areas. Q-fever and MRSA bacteria were regularly found in the collected fine dust. From the results of the study the critical distance to the production facilities could not be determined nor at what concentrations health effects occur.

Microorganisms can emit in wet or in dry aerosols. Wet aerosols are mainly generated from the respiratory tract. Dry aerosols originate from dust sources, of which manure, skin particles, feed, and bedding material are reported to be the main sources (Aarnink et al., 1999; Heber et al., 1988); (Cambra-López et al., 2011). It is suggested that airborne dust particles may play an important role in the transport and survival of bacteria and viruses (Aarnink et al., 2011a). Zhao et al. (2011a) evaluated this relationship, but the processes involved in the transmission of microorganisms via the airborne route in association with dust are still not all revealed.

Airborne microorganisms are exposed to meteorological factors, particularly temperature, humidity and solar radiation (Dungan, 2010). These factors may have significant effects on the survival of these micro-organisms. Furthermore, airborne microorganisms might be protected from outside influences by (dust) particles coagulated with the viable particle. Proteins and membrane phospholipids appear to be the targets of relative humidity- and temperature-induced inactivity of bacteria (Zhao, 2011). Viruses with structural lipids are stable at low relative humidity, whereas viruses without lipids are more stable at high relative humidity. Zhao et al. (2012) investigated the response of the survival of airborne Gumboro vaccine virus to temperature and relative humidity. They found biphasic viral survival kinetics. The initial viral survival (within 2.3 min after aerosolization) was much worse than the secondary viral survival (2.3 to 20 min after aerosolization) at 10^{o}C and 20^{o}C and at 40% and 70% relative humidity. The best secondary viral survival was found at 20^{o}C. As relative humidity influences the water evaporation from airborne particles and thus their density and diameter, it also affects their settling velocity (Dungan 2010). Except differences between species of microorganisms, meteorological conditions presumably will determine to a large extent the survival time of microbial pathogens and the distance they can bridge to infect other farms and humans. Within this study we focused on effects of temperature and humidity. Although literature shows that radiation influences microbial survival (Beebe, 1959; King et al., 2011), this factor was not studied here. Of all radiation UV-C, with wavelengths between 100 – 280 nm, is considered to be germicidal (Zhao et al., 2013a). A lot of the UV-C radiation, however, is filtered out when passing through the atmosphere and solar radiation is absent during night time.

The objective of this study was to determine the survival rate of bacteria at different temperatures and relative humidity levels. This report describes the results of a laboratory study with three groups of bacteria, gram-positive, gram-negative and mycoplasma, represented by *Enterococcus mundtii, Escherichia coli* and *Mycoplasma synoviae* respectively. The chosen species are widely used as indicators for the groups of bacteria they are representing. Their suitability as study material for this type of experiments was recently extensively evaluated by Zhao (2011). He showed that the chosen species are little affected by procedures of preparation of bacterial suspensions or by sampling, nor by adding uranine to the suspensions.

2 Materials and methods

2.1 Experimental set up

The effect of temperature (T) and relative humidity (RH) on the survival of three different types of airborne microorganisms was assessed in a 3x3 experimental set up for T = 10, 20 and 30°C and RH = 40, 60 and 80%. These temperatures and humidity levels were chosen to study a broad range of T and RH that can occur in the Netherlands. T < 10°C could not be studied, because of practical reasons. At these low temperatures the RH cannot be kept at the low levels when spraying the wet bacteria suspension (3 ml) in the air. Airborne microorganisms were sampled at 0.5, 10, 20 and 30 min after aerosolizing. From the counted culturable microorganisms in the subsequent samples the half-life time of the three microorganisms was calculated. Uranine was used as a tracer.
The microorganisms studied were the following bacteria species:

Escherichia coli (strain DSM-1936; Gram -)
Enterococcus mundtii (strain DSM-4838; Gram +)
Mycoplasma synoviae (originating from a spontaneous case of M.synoviae-associated amyloid arthropathy in brown layers (Landman & Feberwee, 2001), no cell wall)
All treatments (combinations of T and RH) were done in triplicate. The treatments were randomly scheduled over the different experimental days (Table 1). Triplicate measurements were done on the same day.

Table 1
Schedule of treatments

Date	Type aerosol	Bacteria	Treatment	
			T	RH
10-12-2012	Wet	E.coli, E.mundtii, M.synoviae	30	80
11-12-2012	Wet	E.coli, E.mundtii, M.synoviae	20	60
12-12-2012	Wet	E.coli, E.mundtii, M.synoviae	10	60
13-12-2012	Wet	E.coli, M.synoviae	20	40
14-12-2012	Wet	E.coli, E.mundtii, M.synoviae	10	80
17-12-2012	Wet	E.coli, E.mundtii, M.synoviae	10	40
18-12-2012	Wet	E.coli, E.mundtii, M.synoviae	30	40
19-12-2012	Wet	E.coli, E.mundtii, M.synoviae	20	80
20-12-2012	Wet	E.coli, E.mundtii, M.synoviae	30	60
21-12-2012	Wet	E.mundtii	20	40
28-01-2013	Dry	E.coli, E.mundtii	30	60
29-01-2013	Dry	E.coli, E.mundtii	20	40
30-01-2013	Dry	E.coli, E.mundtii	10	80
11-02-2013	Dry	E.coli, E.mundtii	10	40
12-02-2013	Dry	E.coli, E.mundtii	20	60
13-02-2013	Dry	E.coli, E.mundtii	10	60
25-02-2013	Dry	E.mundtii	30	40
26-02-2013	Dry	E.mundtii	30	80
27-02-2013	Dry	E.mundtii	20	80

2.2 Preparation of bacteria suspensions

Wet suspensions
Wet bacteria suspensions consisted of a bacteria culture in buffered peptone water. The preparation of wet suspensions followed the procedures as were described by (Zhao et al., 2011b).
E. coli was incubated at 37°C overnight on McConkey No. 3 agar (Oxford, Cambridge, UK) and then transferred into buffered peptone water (BPW, bioTRADING Benelux B.V., Mijdrecht, the Netherlands). *E. mundtii* suspensions were prepared similarly to *E. coli,* except that incubation was on sheep blood agar. The initial concentration of *E.coli* and *E.mundtii* was approximately 7 $\log_{10}$ CFU ml^{-1}.
M. synoviae was incubated in Mycoplasma experience (ME) broth (Mycoplasma Experience, Reigate, UK) at 37°C for 48 hours. The initial concentration of *M. synoviae* was approximately 8 $\log_{10}$ CFU ml^{-1}.
The necessary suspensions were prepared per test day (max. 9 per day) and stored in volumes of 3.0 ml at 4°C until the moment of aerosolization.

Dry suspensions
Dry bacteria suspensions consisted of a dried mixture of a suspension of *E.coli* or *E. mundtii* and dust. The procedure for preparing the bacteria suspensions used was similar to the procedure followed for the preparation of the wet suspensions. However, the bacteria concentration obtained this way (7 $\log_{10}$ CFU ml^{-1}) appeared to be too low to leave a detectable bacteria concentration in the aerosolized dry suspensions. In order to increase the bacteria concentration in the suspensions from 11-02-2013 *E.coli* and *E.mundtii* were incubated on broth, then the suspensions were centrifuged after which the pellets were resuspended in BPW. This procedure resulted into initial concentrations of 9 $\log_{10}$ CFU ml^{-1}.

Dust was prepared from pig faeces by the following procedure:

1. Pig faeces was sterilised (2.5 h at 134°C and +1 bar) in 1 l glass bottles, holding 500 g each.
2. Sterilised faeces was dried in a stove with forced air circulation at 110°C during 24 h.
3. Dried faeces was grinded in a cyclone mill over a 1 mm sieve (Cyclotec™ 1093 Sample Mill, Foss Tecator AB, Höganäs, Sweden).
4. Grinded faeces was subsequently sieved over 0.500, 0.250, 0.125, 0.063 and 0.036 mm sieves (Vibratory Sieve Shaker ANALYSETTE, Fritsch, Idar-Oberstein, Germany).

The dust fraction < 0.036 mm was used in the experiments. From 500 g fresh pig faeces approximately 4 g dried material < 0.036 mm was obtained. For each aerosolization event 150 mg of dust was used, as was weighed in a 2 ml Eppendorf tube. To this amount 1 ml of bacteria suspension and 150 µl 0.4% uranine was added. Dust, bacteria and uranine were mixed on a Fortex mixer (V-32), after which the tube was put in a freezer at -80°C. The next day (within 24 h) the tube was put in a vacuum freeze drier (Christ Epsilon 2-6d). The dried suspensions were stored at 4°C for maximum 72 h until the moment of aerosolization.

2.3 Isolators

Two identical stainless steel isolators (L = 1.6 m, W = 0.93 m, H = 1.1 m; V = 1.64 m^3) were used as aerosolization space. The isolators had a hinged plexy glass front window in each of which 4 gloves were fitted. The ventilation air was filtered with HEPA filters in the air inlet and air outlet. The isolators were equipped with a ventilator for mixing the inside air. The isolators were placed in a climatised room. The air conditioner (heater/cooler and drier) was positioned outside the room. Conditioned air was brought in through a perforated double ceiling. The relative humidity was regulated by two humidifiers inside the room. The set points of each treatment were set in the evening prior to the experiment, which ensured that there was enough time for reaching the desired climate conditions in the room. The set levels of the temperature and relative humidity were different from the levels of the different treatments (as indicated in Table 1). This was necessary because the effect of spreading the bacteria suspensions in the isolator on temperature and relative humidity had to be taken into account.
The climatic conditions were monitored with a temperature and humidity sensor installed at approximately 0.1 m beneath the ceiling; T and RH data were logged continuously. Necessaries could

be brought in and out via a water lock in the bottom of the isolator. Figure 1 shows a schematic diagram of an isolator.

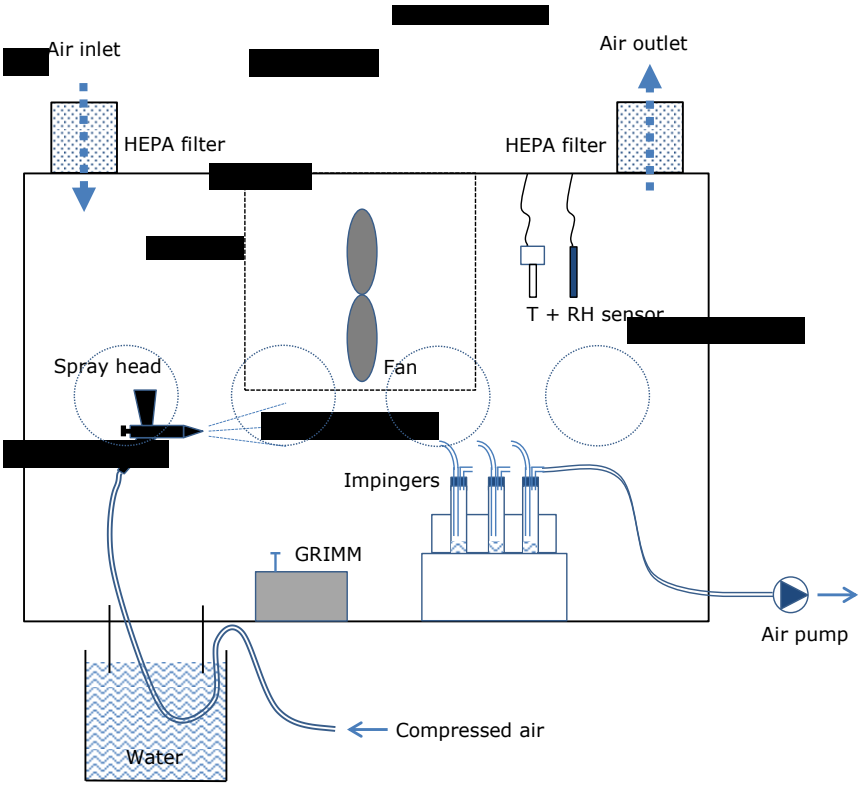

Figure 1 *Schematic diagram of the isolator used in the study*

2.4 Aerosolization

Wet suspensions
Wet bacteria suspensions containing uranine (tracer) were aerosolized by using a Walther Pilot spray-head with a 0.5 mm nozzle (Wather Spritz- und Lackiersysteme, Wuppertal, Germany). It was connected to a low pressure air system, which was set at 2.3 bar. The volume median diameter D (v, 0.5) of the prayed aerosol near the spray-head was approximately 10 µm (Zhao et al., 2011c). The bacteria suspensions were brought into the isolator before each aerosolization. The uranine stock solution was inside the isolator during the experiment. Uranine was added to and mixed with the bacteria suspension just before aerosolization. The suspensions were aerosolized by spraying from the left front corner diagonally in the isolator. The time of spraying was approximately 5 sec. During spraying the aerosols were mixed with the inside air by means of the ventilator. Directly after spraying the ventilator was put off.

Dry suspensions
Dry bacteria suspensions were aerosolized by using an air blow gun with a Ø 8 mm bent pipe (Air Boy, JWL, Hedensted, Denmark) onto which a dispersing nozzle (SAG 410+/L, Serial No. 107, Topas GmbH, Dresden, Germany) was mounted. The air blow gun was connected to a low pressure air system. The air pressure was set at 2.3 bar. The suspensions containing uranine, held in 2 ml plastic tubes (Eppendorf, HX07), were brought into the isolator just before the moment of aerosolization. The suspensions were sucked from the tubes into the nozzle via a 25 mm L pipe sticking in the tube. The aerosolization procedure was similar to the procedure used for wet suspensions.

To account for evaporation during sampling the actual size distributions of the aerosols were measured by a spectrometer (GRIMM 1.109, GRIMM Aerosol Technik GmbH & Co KG, Ainring, Germany) which was placed in one of the isolators.

2.5 Sampling

The airborne aerosols were sampled by using glass impingers (AGI-30) which were filled with 20 ml buffered peptone water. Sampling was done at 0.5, 10, 20 and 30 min after aerosolization. The dry *E.coli* aerosols were sampled at 0, 2, 5 and 10 min after aerosolization, from the moment it was noticed that after > 10 min no viable *E.coli's* in the samples could be detected. The sampling time was 2 min per sample at an air flow rate of 12.5 l/min. The impingers were placed inside the isolator.

After sampling a 2.0 ml subsample from each impinger tube for measuring the uranine concentration was taken by using a pipette. Subsampling was done before the impingers were taken out from the isolator.

2.6 Uranine

In this study uranine (CAS#518-47-8, Fischer Scientific, Landsmeer, the Netherlands) was used as a tracer. Uranine was chosen because it is an inert stable compound which can be detected in very low concentrations and which does not affect the culturability of bacteria in suspensions (Zhao *et al*. 2011a). Uranine was added to the wet suspensions in a concentration of approximately 0.02%. This was achieved by adding 0.3 ml uranine from a 0.2% (w/w) stock solution to 3.0 ml suspension, just before aerosolization. For the dry suspensions 150 µl 0.4% (w/w) uranine was added to a mixture of dust and bacteria. So, to wet and dry suspensions 600 µg uranine was added. The uranine was quantified by a fluorescent detector (HP 0146 A, HP, US) with a detection limit of 0.002 $\mu g.ml^{-1}$.

2.7 Bacteria quantification

E.coli and *E. mundtii* concentrations in the samples were determined by diluting the samples in 10-fold steps in physiological salt solution (bioTRADING Benelux B.V., Mijdrecht, the Netherlands), plating 100 µl of each dilution on sheep blood agar, counting the plates with 30 - 300 colonies and calculating the bacteria concentration in Colony Forming Units per ml (CFU/ml) in the original samples.
Decimal dilutions of *M. synoviae* samples were plated on ME agar, incubated at 37°C for approximately 7 days (until colonies were visible), after which the colonies were counted and the initial bacteria concentrations calculated, similarly to *E.coli* and *E.mundtii*.

All bacteria concentrations after aerosolization were corrected for the uranine decrease which is related to physical losses of bacteria, mainly caused by deposition of the aerosols on the floor and walls of the isolator. Additional losses were contributed to biological losses, i.e. loss of viability.

2.8 Data analysis

Calculation of initial uranine concentration
The initial uranine concentration of wet aerosols in the isolator ($C_{0,uranine,wet}$ in $\mu g/m^3$) was calculated from the amount of uranine aerosolized ($M_{aerosolized}$) and the volume of the isolator ($V_{isolator}$ in m^3) as:

$$C_{0,uranine,wet} = \frac{M_{aerosolized}}{V_{isolator}}$$

To calculate the initial uranine concentration of dry aerosols in the isolator ($C_{0,uranine,dry}$ in $\mu g/m^3$) the amount of uranine in the prepared suspensions ($M_{prepared}$ in µg) was corrected for the amount of suspension that was left in the tube after aerosolization (M_{left} in µg):

$$C_{0,uranine,dry} = \frac{(M_{prepared} - M_{left})}{V_{isolator}}$$

Calculation of uranine concentration after aerosolization
The uranine concentration in the isolator after aerosolization ($C_{t,uranine}$ in µg/m^3) was calculated from the uranine concentration in the samples ($C_{sample,uranine}$ in µg/ml) taking the liquid volume in the impingers after sampling ($V_{impinger}$) and the volume of sampled air into account (25 l sampled air):

$$C_{t,uranine} = C_{sample,uranine} * V_{impinger} * \frac{1000}{25}$$

Calculation of initial bacteria concentration
The initial bacteria concentration of wet aerosols ($C_{0,bact,wet}$) in the isolator (CFU/m^3) was calculated from the number of bacteria aerosolized ($N_{aerosolized}$) and the volume of the isolator ($V_{isolator}$ in m^3) as:

$$C_{0,bact,wet} = \frac{N_{aerosolized}}{V_{isolator}}$$

To calculate the initial bacteria concentration of dry aerosols ($C_{0,bact,dry}$) the number of bacteria in the prepared suspensions ($N_{prepared}$) was corrected for the amount of suspension that was left in the tube after aerosolization (M_{left}):

$$C_{0,bact,dry} = \frac{N_{prepared}}{V_{isolator}} * \frac{(M_{prepared} - M_{left})}{M_{prepared}}$$

Calculation of bacteria concentration after aerosolization
The bacteria concentration in the isolator after aerosolization ($C_{t,bact}$ in CFU/m^3) was calculated from the bacteria concentration in the samples ($C_{sample,bact}$ in CFU/ml) taken the liquid volume in the impingers after sampling ($V_{impinger}$) and the volume of sampled air (2*12.5 l = 25 l) into account:

$$C_{t,bact} = C_{sample,bact} * V_{impinger} * \frac{1000}{25}$$

Loss of viability of the bacteria was related to the imposed temperature (T, °C) and relative humidity (RH, %). First the loss of viability between the moment of aerosolization of the liquid or dust (C_0) and the moment of analysis of the first sample ($C_{0.5}$), taken at t = 0.5 min, was calculated by:

$$Log_{10\_loss\_initial} = Log_{10}\left(\frac{C_0}{C_{0.5}}\right)$$

The effects of bacteria, T, and RH on loss of viability were analysed applying the unbalanced ANOVA procedure of Genstat, with the following model:

$$Y_{ijkl} = \mu + B_i * T_i * RH_k + e_{ijkl}$$

Where: Y is loss of viability ($Log_{loss\_initial}$);
B is bacteria type (i = *E.coli, E.Mundtii, M.synoviae*);
T is temperature (j = 10, 20, 30 °C);
RH is relative humidity (k = 40, 60, 80 %);
e is error term.

Secondly, the loss of viability between the moment of analysis of the first sample ($C_{0.5}$) and the moment of the other samplings (C_t) was calculated by:

$$Log_{10\_loss\_isolator} = Log_{10}\left(\frac{C_{0.5}}{C_t}\right)$$

The effects of T and RH were analysed for each bacteria with the REML procedure of Genstat, with the following model:

$$Y_{ijk} = \mu + T_i + RH_j + T_i * RH_j + e_{ijk}$$

Where: T = 10, 20, 30 ^{o}C;
RH = 40, 60, 80 %

Within this analysis the spraying moment within a sampling day was taken as the random component.

Calculation of half-life time

Half-life time is the amount of time in which the concentration of viable bacteria decreases by half. The half-life time of the bacteria ($t_{1/2}$, min) was calculated by first determining the regression coefficient of log_{10} reduction in bacteria concentration in the isolator on the sampling time:

$$log_{10}\left(\frac{C_0}{C_t}\right) = \beta \,.\, t$$

The half-life time of the bacteria can then be calculated as follows:

$$t_{1/2} = \frac{Log_{10}(2)}{\beta}$$

The regression coefficient β was calculated in a simple linear regression with groups (Genstat Committee, 2010), in which groups were the factor levels of temperature or relative humidity. Analysis with the REML procedure (Genstat Committee, 2010) showed that the interaction between the factors temperature and relative humidity was not significant, therefore temperature and humidity effects could be determined in separate regression analyses.

3 Results

3.1 Set and measured T and RH

The real temperatures (T) and relative humidity (RH) levels during the experiments were slightly different from the desired values of the different treatments . The measured T and RH showed some variation during the measurements due to limited controllability. Table 2 shows the set T and RH and the measured values per treatment (= day of experiment).

Table 2
Set points and mean measured values of T and RH for the different treatment combinations. Standard deviations are given between brackets.

Date	Type aerosol	Set points treatment		Measured values	
		T	RH	T	RH
10-12-2012	Wet	30	80	30.0 (0.51)	83.1 (4.48)
11-12-2012	Wet	20	60	20.4 (0.48)	58.1 (4.12)
12-12-2012	Wet	10	60	12.7 (0.37)	59.0 (6.72)
13-12-2012	Wet	20	40	19.8 (0.40)	36.9 (3.38)
14-12-2012	Wet	10	80	12.0 (0.88)	71.0 (6.25)
17-12-2012	Wet	10	40	14.6 (0.63)	42.3 (2.36)
18-12-2012	Wet	30	40	30.2 (0.52)	38.9 (4.09)
19-12-2012	Wet	20	80	20.8 (0.57)	80.6 (2.67)
20-12-2012	Wet	30	60	29.6 (0.25)	58.4 (2.54)
21-12-2012	Wet	20	40	20.5 (0.26)	45.3 (0.94)
28-01-2013	Dry	30	60	29.8 (0.16)	62.7 (2.81)
29-01-2013	Dry	20	40	20.4 (0.32)	42.0 (2.08)
30-01-2013	Dry	10	80	13.1 (0.98)	80.2 (2.27)
11-02-2013	Dry	10	40	10.4 (0.51)	40.2 (4.49)
12-02-2013	Dry	20	60	20.0 (0.36)	60.8 (2.67)
13-02-2013	Dry	10	60	9.5 (0.21)	62.7 (0.47)
25-02-2013	Dry	30	40	29.1 (0.99)	41.0 (2.16)
26-02-2013	Dry	30	80	30.2 (0.40)	78.0 (1.41)
27-02-2013	Dry	20	80	20.8 (0.62)	80.3 (0.47)

3.2 Particle size distribution

Figure 2 shows the typical course of the PM10, PM2.5 and PM1particle concentrations in the isolator after wet and dry aerosolization during the experiments.

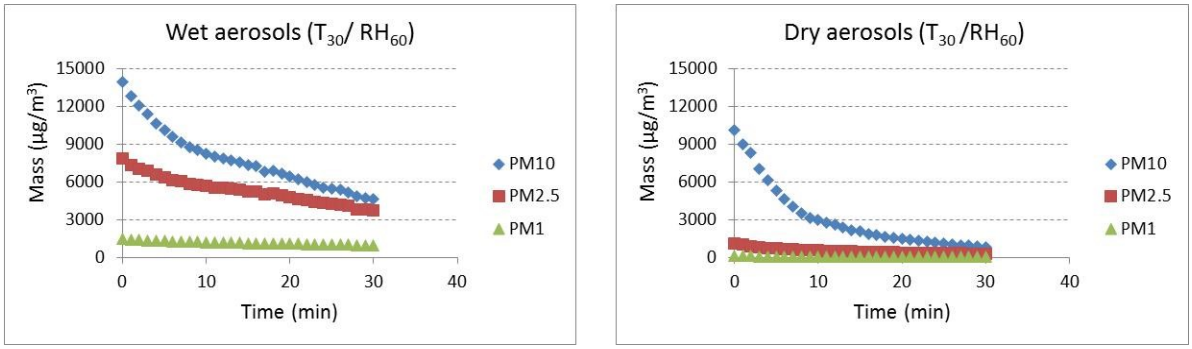

Figure 2 *Typical course of PM10, PM2.5 and PM1 mass concentrations in the isolator after wet and dry aerosolization during the experiments.*

Figure 2 shows the course of the particle concentrations in the isolator at 30°C and 60% relative humidity. Different aerosolizations show different concentrations but the course of the concentrations

over time are rather similar. The initial concentrations of wet PM10, PM2.5 and PM1 aerosols are higher than of dry aerosols. The share of PM10 in dry aerosols is higher than in wet aerosols, so on average the size of dry aerosol particles is bigger than of wet aerosols. Bigger particles show a faster decrease than smaller particles, with regression coefficients of -0.0216 (s.e. 0.0017), -0.0154 (s.e. 0.0015) and -0.0067 (s.e. 0.0010) for PM10, PM2.5 and PM1 respectively in wet aerosols and regression coefficients of -0.05432 (s.e. 0.00187), -0.03983 (s.e. 0.00198) and -0.03413 (s.e. 0.00195) respectively in dry aerosols. So, dry aerosols showed a faster decrease than wet aerosols.

3.3 Uranine

Figure 3 shows the average uranine concentration (on log-scale) in the isolator during wet and dry aerosol sampling as measured in the subsamples from the AGI-30 impingers.

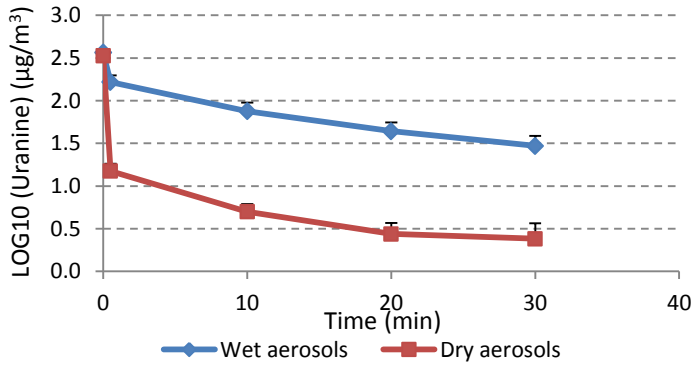

Figure 3 *Uranine concentrations and standard deviations (┬) in the isolator versus time.*

The decrease of the uranine concentration in time is used as a measure of the physical decrease of the bacteria concentration in the isolator, so not attributed to biological decay. Figure 3 shows that the concentration of dry aerosolized uranine decreases much stronger than wet aerosolized uranine in the first 0.5 min after aerosolization. After the initial decrease, the trend of wet and dry aerosolized uranine in time is pretty similar. The concentration levels strongly varied from day to day, but followed a more or less similar course (on log scale). Significant effects ($P<0.001$) of the isolator, day and serial number of aerosolization on the level of uranine concentration were observed, however, interactions with sample time were not significant. This means that the course of the uranine concentration was not influenced by above mentioned factors. Therefore corrections of the measured bacteria concentrations were based on average uranine concentrations per sample time, relative to the original concentrations (at t=0), for wet and dry aerosols, as shown in Figure 3.

3.4 Wet aerosols

3.4.1 Initial decay

Figure 4 shows the concentration on log-scale of viable *E.Coli, E.mundtii* and *M.synoviae* in the isolator at 0 and at 0.5 minutes after aerosolization and the biological decay at the combinations of temperature and relative humidity studied.

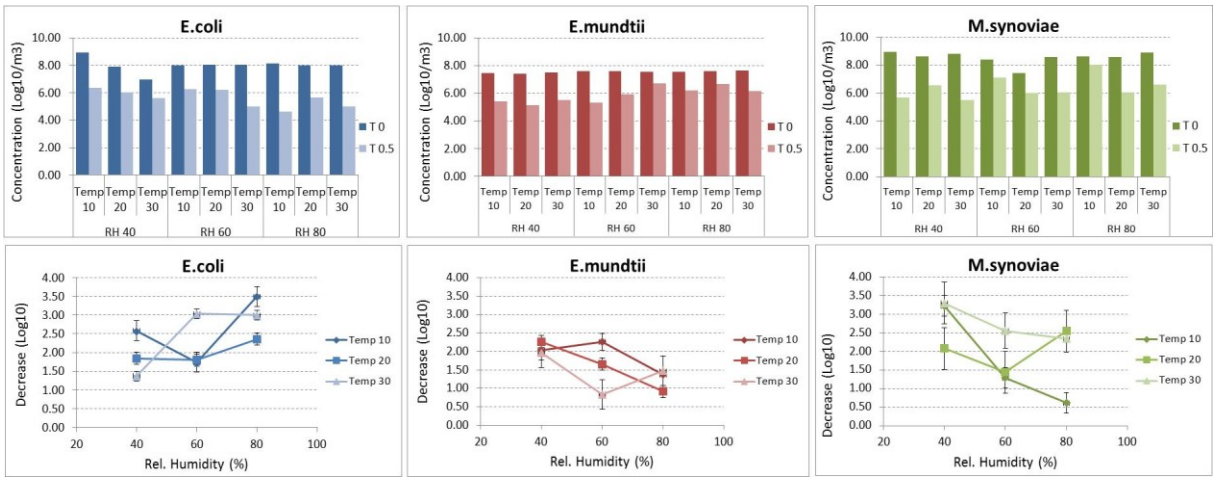

Figure 4 *Concentrations of viable E.coli, E.Mundtii and M.synoviae in the isolator at 0 and at 0.5 minutes after wet aerosolization (above) and the biological decay (below; in Log10 decrease) with standard deviations ($_{T}$) at different climatic conditions.*

Figure 4 shows that the biological decay of the bacteria concentration in the first half minute after wet aerosolization is variable with clear differences between bacteria types and climatic conditions. The decay of *E.coli* and *M.synoviae* is larger than of *E.mundtii*. The differences between bacteria types are significant (P<0.01). The decay is influenced by temperature and relative humidity in a variable way. *E.coli* showed, on average, a larger decay at higher relative humidity, especially at 80% (P<0.01). The effect of temperature was not consistent, while it interacted with relative humidity. *E.mundtii* showed a smaller decay at higher temperatures (not significant) and higher levels of relative humidity (P=0.01). *M.synoviae* showed a tendency towards a larger decay at higher temperatures (P=0.06) and had a relatively large decay at 40% humidity, however, the effect of humidity was not significant (P=0.13).

3.4.2 Decay in time

Figure 5 shows the concentration on log-scale of viable *E.Coli, E.mundtii* and *M.synoviae* in the isolator versus time (0.5 to 30 min) after wet aerosolization at the combinations of temperature and relative humidity studied.

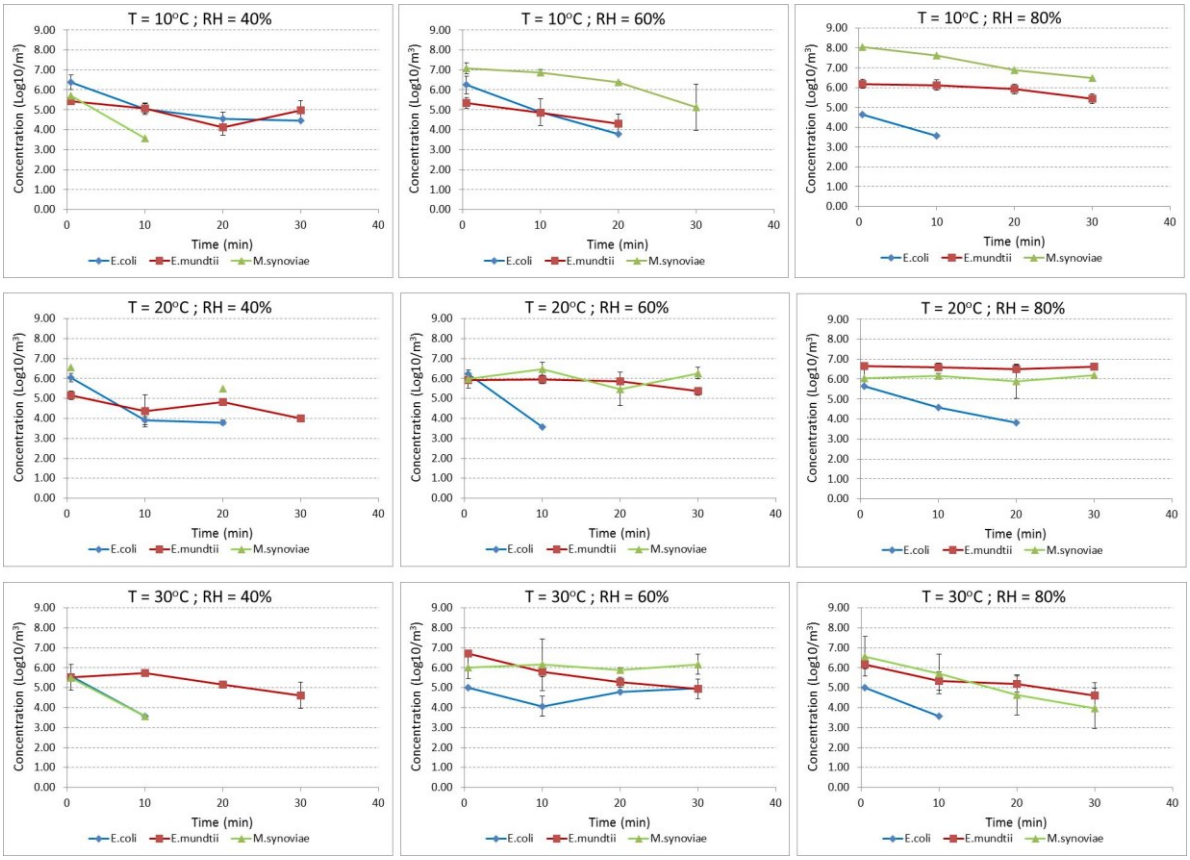

Figure 5 Concentrations and standard deviations ($\top$) of viable *E.coli, E.Mundtii* and *M.synoviae* versus time after wet aerosolization at different climatic conditions.

The concentrations of the three bacteria in the isolator showed different changes in the course of time from 0.5 min after aerosolization onwards, with different effects of temperature and relative humidity. For *E.coli* and *E.mundtii* a significant effect of temperature level on the decay was found ($P<0.05$) and there was a tendency towards a temperature effect on the decay of *M.synoviae*. On average, *E.coli* showed larger decay than *E.mundtii* and *M.synoviae*. At medium temperature (20°C) the decay of *E.coli* was significantly ($P<0.05$) larger than at 10 and 30°C. Contradicting to this, *E.mundtii* and *M.synoviae* showed lowest decay at 20°C. *E.mundtii* showed highest decay at 30°C and intermediate at 10°C. *M.synoviae* showed similar decay at 10 and 30°C. No significant effects of relative humidity on the decay of the three tested bacteria were found.

3.4.3 Half-life time

In Table 3 half-life time values of *E.coli, E.mundtii* and *M.synoviae* after wet aerosolization, at the temperatures studied, are given.

Table 3
Half-life times of E.coli, E.mundtii and M.synoviae after wet aerosolization at different temperatures. Superscripts represent significance of differences; if letters in a row do not correspond means that the difference between temperatures is significant (P<0.05). Relative standard deviations (%) are put between brackets.

	Half-life time (min)					
	10°C		20°C		30°C	
E.coli	3.17[a]	(11.4)	1.97[b]	(10.5)	6.04[a]	(33.9)
E.mundtii	9.08[a]	(15.6)	20.3[a]	(28.3)	5.65[b]	(7.80)
M.synoviae	5.37[a]	(19.6)	28.1[b]	(95.3)	4.93[a]	(18.4)

Table 3 shows that the half-life times of wet aerosols vary considerably between bacteria and temperatures. The half-life time at 10°C and 20°C of *E.coli* is short compared with *E.mundtii* and *M.synoviae*. *E.mundtii* and *M.synoviae* show much longer half-life times at 20°C than at 10°C and 30°C.

3.5 Dry aerosols

3.5.1 Initial decay

Figure 6 shows the concentration of viable *E.mundtii* in the isolator at 0 and at 0.5 minutes after aerosolization and the biological decay at the combinations of temperature and relative humidity studied.

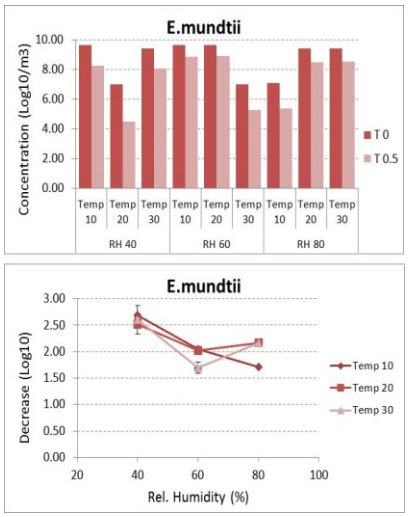

Figure 6 *Concentrations of viable E.Mundtii in the isolator at 0 and at 0.5 minutes after dry aerosolization (above) and the biological decay (below; in Log10 decrease) at different climatic conditions.*

Figure 6 shows that the decay of *E.mundtii* in the first half minute after dry aerosolization was rather unambiguous with small differences between temperatures. The effect of relative humidity, however, was significant ($P<0.01$). The largest decay was found at low relative humidity.

3.5.2 Decay in time

Figure 7 shows the concentration of viable *E.mundtii* in the isolator versus time after dry aerosolization at the combinations of temperature and relative humidity studied.

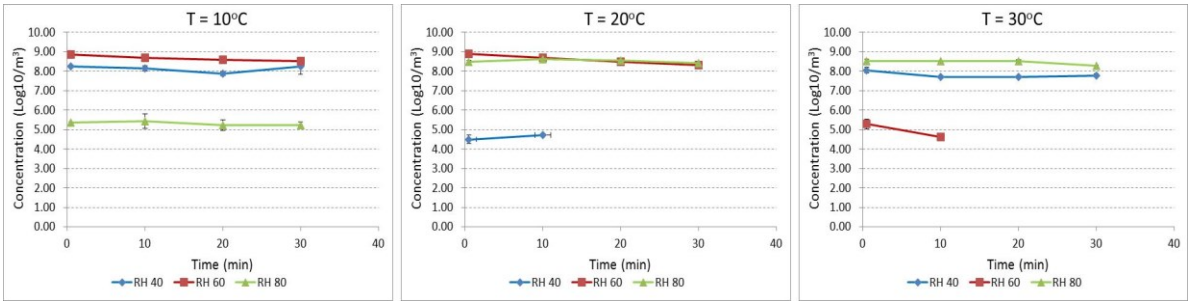

Figure 7 *Concentrations of viable E.mundtii versus time after dry aerosolization at different climatic conditions.*

The concentration of *E.mundtii* in the isolator shows only small changes in the course of time between 0.5 and 30 min after aerosolization. The largest changes are visible between 0.5 and 10 min.

However, the fact that for the treatments T20/RH40 and T30/RH60 only one measurement is available means that the predicted decay for these treatments is less reliable than for the other treatments. The effect of relative humidity on the biological decay of *E.mundtii* was significant (P<0.05); the effect of temperature was not significant.

3.5.3 Half-life time

In Table 4 half-life time values of *E.mundtii* after dry aerosolization, at the temperatures studied, are given.

Table 4
Half-life times of E.mundtii after dry aerosolization at different temperatures and relative humidities. Superscripts represent significance of differences; if letters in a column do not correspond means that the difference between temperatures or relative humidities is significant (P<0.05). Between brackets the relative standard deviations (%) are given.

	Half-life time (min)			Half-life time (min)	
T 10°C	36.6[a]	(32.0)	RH 40%	32.9[ab]	(30.2)
T 20°C	30.5[a]	(30.4)	RH 60%	16.6[a]	(15.1)
T 30°C	27.2[a]	(26.9)	RH 80%	90.9[b]	(73.0)

The half-life times of dry aerosols of *E.mundtii* were not influenced by temperature but they were substantially affected by relative humidity. The half-life time value as presented for RH 40% and 60% may be less reliable than for RH 80% because they are based on less measurements. It is clear that the half-life times of dry aerosols of *E.mundtii* are much longer than of wet aerosols.

4 Discussion

Material and methods

The chosen experimental setup has proven to be very suitable for testing airborne microorganisms. Isolators were used before in this type of experiments (Landman et al., 2004; Zhao 2011). However, for future experiments control of the climate inside the isolator should be improved. It is advisable to install accurate climate control equipment with sufficient capacity inside the isolator instead of controlling the climate in the surrounding room.

For practical reasons repeated tests were done at the same day which introduced a risk of confounding effects between day and treatment. To minimize these confounding effects the test procedure was standardized as good as possible. Despite standardisation of the procedure the uranine concentration showed considerable variation between days, which could not be clarified. As uranine is an attractive tracer due to its chemo-physical properties it is worthwhile to further look into the cause of the observed variations; starting-points could be the strategy of mixing and sampling and the accuracy of quantification. Standardization and validation of sampling and analysis protocols would be welcome as bioaerosol concentration studies often show large variation in samples (Millner, 2009). Also in our study we found large variations in survival rate of bacteria. These variations can probably be reduced by further standardization of the experimental procedure, especially concerning the behaviour of urea during sampling and analysis.

The experiments were done with wet as well as dry bioaerosols because livestock production is an important source of both. Wet aerosols originate mainly from sneezing, coughing and urine splashing (Wainwright et al., 2009), dry aerosols mainly from manure, skin particles, feed, and bedding (Aarnink et al., 1999; Heber et al., 1988); (Cambra-López et al., 2011). Both wet and dry aerosols emitted from livestock production facilities may contain pathogenic microorganisms. It should be noted that small wet aerosols evaporate free water very quickly, within (tenths of) seconds (Kincaid and Longley, 1989). Therefore wet aerosols become dry aerosols very quickly when relative humidity is not close to saturation. Studies on effects of wet aerosols, however, remain important as our study showed that bacteria are very vulnerable for decay during the evaporation phase after aerosolization of wet aerosols. Reductions up to 3.5 magnitude in the first 30 seconds after aerosolization of *E.coli* (at high relative humidity) and *M.synoviae* (at low relative humidity) were found. Comparable differences in vulnurability between bacteria types were reported by Marthi et al., 1990).

Physical bacterial decay

The average particle size of wet aerosols is smaller than of dry aerosols. The particle size of wet aerosols is greatly determined by water evaporation from the particle directly after aerosolization. The difference in particle size between wet aerosols and dry aerosols explains why the decay of dry aerosols in the isolator is faster than the decay of wet aerosols. The difference in decay velocity between wet and dry aerosols is consistent with the fact that the uranine concentration after dry aerosolization decreases faster than after wet aerosolization. So the physical decay of bacteria in dry aerosols is faster than in wet aerosols due to a higher settling velocity of the heavier dry aerosols. From figures 2 and 3 it seems that uranine concentrations decrease faster (figure 3) than PM10 mass concentrations (figure 2). This was probably caused by the fact that the impingers also sample larger particles than 10 µm. These particles deposit very fast on floor and walls of the isolator.

Biological bacterial decay

Airborne *E.coli, E.mundtii* and *M.synoviae* show different viability under comparable climatic conditions. The bacteria show biphasic inactivation in wet aerosols as well as in dry aerosols as was also observed in several previous studies; the initial inactivation (first 0.5 min after aerosolization) is much stronger than the second inactivation (0.5 – 30 min after aerosolization). The high initial inactivation of bacteria in wet aerosols is probably caused by the evaporation stress, as mentioned before. Because of the fast evaporation of free water from the droplets there is a fast cool-down of the

bacteria inside the droplets. This probably affects their viability, as was also shown in the study of Zhao et al. (2012) for virus. The high initial inactivation of bacteria in dry aerosols is probably caused by the rigid preparation procedure of the bacteria in the dry aerosols before they were spread in the isolator. It is advised to investigate the losses in this preparation procedure (mainly during freeze drying).

In wet aerosols the initial inactivation was significantly influenced by temperature and relative humidity. The second inactivation was significantly affected by temperature but not by relative humidity. *E.coli* showed the strongest initial inactivation at high RH, while the effect of temperature interacted with humidity level. *E.mundtii* showed the strongest inactivation at low RH. In wet aerosols *E.mundtii* survived longer than *E.coli* and *M.synoviae* at low temperatures.

In dry aerosols RH had a significant effect on the initial inactivation of *E.mundtii;* the inactivation decreased with increasing RH. In dry aerosols the second inactivation was strongly influenced by RH; the survival time was longer at high RH, but the effect was not unambiguous. This raises doubts about RH as a meaningful parameter in relation to biological decay of microorganisms. Some authors propose absolute humidity or evaporation potential, the difference between actual water content in the air and the water content in saturated air at the same temperature, to be of greater biological significance (Shaman and Kohn, 2009; Zhao et al., 2013b) . However, calculations in this study showed that evaporation potential did not explain more variation in decay than temperature and relative humidity. Therefore this variable was not included in the analysis.

Half-life

Maximum half-life times of *E.mundtii* and *M.synoviae* in wet aerosols, found at a temperature of 20^{o}C, were much longer than of *E.coli* (Table 3) which is consistent with the results of Zhao et al. 2011. The observed longer half-life time of *E.mundtii* (Gram +) compared with *E.coli* (Gram -) was expected, while gram positive bacteria are better protected against environmental influences than gram negative bacteria because of a thicker cell wall. It is remarkable however that *M.synoviae* showed such a long half-life time, while *M.synoviae* has no cell wall at all. A possible explanation is lying in the size of the microorganisms. As *M.synoviae* is the smallest bacteria (0.5 micro in diameter), after aerosolization they could attach to and be protected by wet aerosols better than larger bacteria. In other words, the exposure surface of a *M.synoviae* cell (when it attached to the droplet) to the ambient is less, thus the microbe is less affected by ambient changes. Another possible explanation is that microorganisms with a less complicated structure are more resistant than their counterparts. E.g. viruses have a simpler structure as compared to bacteria, but they are very robust in air (Zhao et al. 2013). Gumboro virus has two major components, i.e. RNA and protein coating. The inactivation of a Gumboro virus is mainly achieved at the point of the more vulnerable or less tolerant component which is the protein coating (Zhao et al. 2012). For bacteria, the degradation of the cell wall is possibly the main reason for bacterial inactivation in the air. If that is the case, perhaps lack of a cell wall is an advantageous characteristic for M.synoviae survival in the air. On this topic there is still some work to be done.

The results show that *E.mundtii* survives longer in dry aerosols than in wet aerosols. Two possible explanations for this finding are proposed: (1) evaporation and (2) survival of the fittest. Due to evaporation after aerosolization microorganisms in wet aerosols lose their protective water film and become more vulnerable for ambient influences. Culturable *E.mundtii* found in dry aerosols survived a rigid preparation procedure before they were spread in the isolator. The bacteria were mixed with dust and uranine, freezed at -80^{o}C and put in a vacuum freeze drier for a couple of days before they were aerosolized. From the initial count of ca. 9.7 $\log_{10}$ CFU/m^3 culturable *E.mundtii* a detectable count of ca. 7.1 $\log_{10}$ CFU/m^3 survived this procedure (approx. 2.5 promille). *E.coli* and *M.synoviae* were not detected directly after aerosolization, which indicates that these species did not survive the preparation procedure. However, for *E.coli* and *M.synoviae* the detection limit was higher than for *E.mundtii*; the latter was detected after centrifugation of the impinged samples. This procedure was not applied for *E.coli* and *M.synoviae.* This leaves uncertainty about the effect of the preparation procedure on these bacteria and about their quality as suitable indicator organism in studies into the effect of reduction measures of airborne pathogens from livestock buildings. Therefore, the impact of the preparation procedure of dry aerosols on the viability of these types of bacteria should be further studied.

The half-life time of bacteria in wet aerosols ranged from 2 min (*E.coli*, at 20°C) to 28 min (M.synoviae, at 20°C). *E.mundtii* in dry aerosols survived even longer. From these data the transmission distance of bacteria in wet aerosols can be estimated. At a wind speed of 5 m/s (average in the Netherlands) and no other influencing factors the transmission distance of wet bioaerosols would range from 600 – 8400 m, far enough to be a potential health risk for surrounding urban areas and animal production facilities.

Mitigation measures on the emission of airborne pathogens from livestock buildings should be taken to reduce the risk of spreading animal diseases and minimize the health risk of humans. The effect of air scrubbing, which was introduced in Dutch livestock production to reduce gaseous emissions, on the removal of microorganisms from the exhaust air from animal buildings was studied by Aarnink et al. (2011b). They found a reduction of total bacterial count of 70% when using an acid scrubber. While in certain situations large numbers of microorganisms can be emitted, they concluded that the present available commercial scrubbers are unable to substantially reduce pathogen emissions from pig houses.

5 Conclusions

The following conclusions can be drawn from our experiments.

1 The studied bacteria, *E.coli, E.mundtii* and *M.synoviae*, representing Gram-, Gram+, and bacteria without a cell wall, respectively, stay airborne and alive from several minutes to more than one hour after aerosolization within the range of temperature (10-30^{o}C) and relative humidity (40-80%) studied.
2 Two phases of decay are observed: 1) a fast initial decay during and directly after spraying of wet aerosols and during the preparation phase and aerosolization of the dry aerosols; 2) a slow decay in the air after the initial phase.
3 In the initial phase, the decay of *E.coli* and *M.synoviae* is larger than of *E.mundtii*. The effect of temperature in this phase is not consistent while it interacts with relative humidity for the different types of bacteria.
4 In the second phase, *E-coli* shows a larger decay than *E.mundtii* and *M.synoviae.*
5 In the second phase, *E.mundtii* and *M.synoviae* survive longest at moderate temperature (20^{o}C), while *E.coli* survived longest at 30^{o}C.
6 *E.mundtii* bacteria survive longer in dry aerosols than in wet aerosols. This could be caused by the loss of their protective water film in wet aerosols, causing them to become more vulnerable for ambient influences or by the survival of the fittest bacteria during the rigid preparation procedure of dry aerosols before they were spread in the isolator.
7 *E.coli* and *M.synoviae* hardly survived the preparation procedures for dry aerosols. The impact of these procedures on the viability of microorganisms isn't fully clear and should be further studied.
8 The experimental setup, using an isolator and uranine as a tracer for deposition and impaction of aerosols, is suitable for testing the effect of ambient influences on the survival of airborne microorganisms.

6 References

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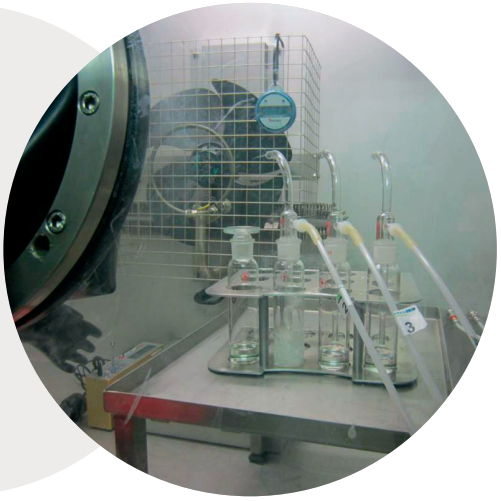

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