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Emission of bioaerosols from livestock facilities -

Methods and results from available bioaerosol investigations in and around agricultural livestock farming

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Thünen Working Paper 138a

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Summary

The present report reviews literature from throughout the world on methods and results of bioaerosol investigations in and around agricultural livestock farming and summarises the most important points. The global trend towards intensification and industrialisation of animal production, with regional concentration of livestock facilities and increasing numbers of animals and greater stock densities, has led to an increase in bioaerosol emissions to the environment in certain areas and to increasing concern about health impairment of the population in the vicinity. The main sources of the bioaerosols are the animals and their faeces, the litter and feed. If the particles become airborne, they can be emitted from the stables into the environment. Hundreds of different viruses, bacteria and moulds have been detected in agricultural livestock farming worldwide. The bacterial group of the *Staphylococcaceae* appears to be most suitable for animal husbandry as a specific indicator or guiding parameter. Bioaerosols can be measured online with particle spectrometers and offline using classical methods, i.e. sampling on site with subsequent evaluation by means of culture-based or molecular biological methods in the laboratory. The classical detection methods are best suited to the complexity of bioaerosols in agricultural livestock farming. The sampling of bioaerosols should be carried out as far as possible using standardised systems that have high physical and biological collection efficiency, in order to ensure comparability of the data. The selection of a suitable collection system should primarily depend on the issue in question. After the bioaerosols have been collected in a sample, evaluation is usually carried out via cultivation and / or various biochemical and molecular biological methods. Especially the latter, in combination with the classical culture-based methods, enable a detailed insight into the composition of bioaerosols. However, further standardisation of the methods for bioaerosols is necessary here. Endotoxins, on the other hand, are predominantly detected using the LAL test, although this test remains relatively susceptible to disturbances.

Most data on bioaerosol measurements in agricultural livestock farming available for this review are from the USA and Germany. Here, the concentrations of bacteria, moulds and endotoxins were measured in the stalls of pigs, cattle and chickens. The highest concentrations of airborne bacteria were found in stalls for chickens, followed by turkeys, ducks, sheep, goats, pigs, cattle, horses and rabbits, with the different husbandry and production stages having a significant influence. Emission factors published for airborne microorganisms also differ in part considerably depending on the animal species and the type of keeping, also as a result of different sampling conditions, collection methods and different methods for determination of the concentrations. The concentrations of the airborne bacteria in livestock during the day and night can deviate by a factor of ten. The deviation may further increase by a factor of 1000 if emission factors are calculated on the basis of the specific volumetric flow rates. This must be taken into account in the calculation of annual average values of emission factors. During transportation, i.e. the transport of bioaerosols via the air, the microorganisms are largely exposed to wind and weather.

The extent to which they are carried is primarily dependent on two parameters: the tenacity, i.e. the ability to survive the airborne condition, and the size and composition of the bioaerosol particles, i.e. how quickly they sediment. How long microorganisms are viable in the air is dependent on very many factors and, due to the relatively unsuitable test systems used in the past, this aspect has not been studied sufficiently. Regarding particle size, most of the airborne microorganisms found in livestock farming have a significantly larger particle size or mass fraction than would be expected from the size of the individual cells of the organisms. 30% to 70% of the bacteria can be found in mass fractions larger than PM10, whereby the distribution of the different bioaerosol components can vary considerably and is not uniformly correlated with the distribution of the dust fractions. The immission concentrations of bioaerosols exponentially decrease with the distance from the emission source, mainly depending on the particle size and meteorological conditions. Instead of carrying out complex measurements, the spread of bioaerosols can also be simulated with computer models. Up to now, however, these models have often overestimated the emissions, since night reduction, particle size distributions and death rates of the microorganisms are still not taken into account.

From hundreds of publications, it has long been known that bioaerosols, probably interacting synergistically with other air pollutants, have a negative impact on the health of people who work in animal stalls and also on that of the animals. No dose / effect relationship has been established so far. To date there has been no clear statement as to the possible danger to residents living in the vicinity of livestock farms. Therefore, no general limit values have been formulated for bioaerosols, above which a detrimental effect on health is to be expected, except to a certain extent for endotoxins. Instead, an environmental assessment of individual cases usually takes place as a precautionary principle. A number of precautionary measures are available to reduce bioaerosol emissions. Thanks to good stall management and a hygiene concept supported by technical solutions, e.g. exhaust air cleaning, a significant reduction of bioaerosols originating from livestock husbandry of well over 90% can be achieved. It remains to be seen whether a dose/response relationship for bioaerosols or at least a valid environmental medical assessment of the emissions will be possible in the future. Until then, in the medium term, the indicator organisms and guiding parameters for bioaerosols from livestock husbandry should be (re)considered and viruses should be included. This comprises the validation and further development of high-volume collectors for bioaerosols. In the case of dispersion modelling, the particle size distributions of the microorganisms and the different levels of emissions between day and night must be considered for the short term. This also applies to tenacity, where new measurement systems are needed in order to obtain meaningful data. It should also be a medium-term goal to reduce bioaerosol concentrations already in the stalls. Concepts for adapted exhaust air cleaning systems are available for this purpose, which, together with further measures, can lead to a reduction of 90% to 99%. There is still a lot to do.

Keywords: *Bioaerosols, agriculture, animal husbandry, emissions, review, methods*

1 Introduction

The global trend towards industrialisation of animal production, with regional concentration of livestock facilities and increasing numbers of animals and greater stock densities, has led to an increase in bioaerosol emissions from the stalls into the environment and thus to increasing concern about health impairment of the population.

As a result of the global increase in demand for animal food products, animal production has changed dramatically in many industrialised and emerging countries over the past 50 years. The trend is moving away from small traditional farms typical of a particular country towards industrialised and specialised livestock facilities with increasing numbers of animals and high stock densities (Ko et al. 2010, Millner 2009, Thorne 2007, Thu 2002). The focus here is directed towards increasing efficiency and reducing costs. Gone are the days when production was solely for the domestic market, with export now becoming increasingly important (AVEC 2011, Deutscher Bauernverband 2016). In the meantime, intensive animal farming has become an important economic factor in many countries with regard to employment and export (Melse et al. 2009). Agriculture is the world's largest economic sector. In Germany, there are 650,000 full- and part-time farmers, who account for 1.5% of the German working population. Three million people are employed in the entire agricultural sector in this country, corresponding to 7% of those in gainful employment. Taken together, they account for as much as 13% of German gross domestic product. The number of animals produced is gigantic. In 2015, the amount of meat produced worldwide was 320 million tons, of which 47.1 million tons were produced in the EU (Deutscher Bauernverband 2016).

Throughout the world, chickens and pigs in particular are increasingly kept in closed stalls with forced ventilation, and, depending on the animal species, with different solid and liquid manure systems with or without litter, as e.g. in Canada (Letourneau et al. 2009), Ireland (Coggins et al. 2007), the Netherlands (Myrna et al. 2017), the USA (Greger & Koneswaran 2010, Hong et al. 2012), Korea (Jo & Kang 2005), China (Gao et al. 2017), Australia (Runge et al. 2007, Chinivasagam et al. 2009), South Africa (Venter et al. 2004) or Poland (Lonc & Plewa 2010). In contrast, turkeys, cattle, sheep and goats are still kept almost exclusively in freely ventilated open stalls, often also with exercise or grazing. Slurry or manure are generally stored on the farms, before spreading on the fields as agricultural fertiliser. The intensification of agricultural livestock farming is often accompanied by a concentration of increasing numbers of animals in confined spaces in the stalls. This poses special challenges for stall construction, especially with regard to the ventilation system and hygiene. An increase in the number of animals in the stalls inevitably leads to an increase in air pollutants there, such as various toxic gases (e.g. ammonia, methane), particulate matter and bioaerosols. The term bioaerosols refers to airborne organic particles of biological origin (based on DIN EN 13098). Specifically, these include airborne viruses, bacteria, mould spores, mould hyphae and pollen, and fragments and metabolic particles thereof, e.g. endotoxins

and mycotoxins, as well as fragments of skin scales, hairs, feathers, faecal matter, litter material and remains of feed. In agricultural livestock farming, bioaerosols are produced along the entire chain of production, in the preceding production of animal feed (e.g. Abdel Hameed et al. 2003, Ghasemkhani et al. 2006, Kim et al. 2009, Straumfors et al. 2016), in the actual rearing and keeping of the animals (e.g. Clark et al. 1983, Whyte et al. 2001), in animal transportation (Rule et al. 2008), in slaughtering (e.g. Hagmar et al. 1999, Lutgring et al. 1997, Lues et al. 2007, Paba et al. 2014, Haas et al. 2005, Liang et al. 2013), in meat processing (Kotula & Kinner 1964, Lenhart et al. 1982, Lenhart & Olenchock 1984, Dobeic et al. 2011, Baikov & Petkov 1987), in the further processing of eggs (Boeniger et al. 2001, Smith et al. 1990), in the transportation (Dungan 2010) and spreading of manure or slurry on the fields as fertiliser (e.g. Jahne et al. 2015, Jahne et al. 2016, Boutin et al. 1988, Murayama et al. 2010) or in the treatment of waste water from livestock facilities (Kim et al. 2012). In the process, the bioaerosols also find their way out of the stalls into the environment and into residential areas (Fig. 1).

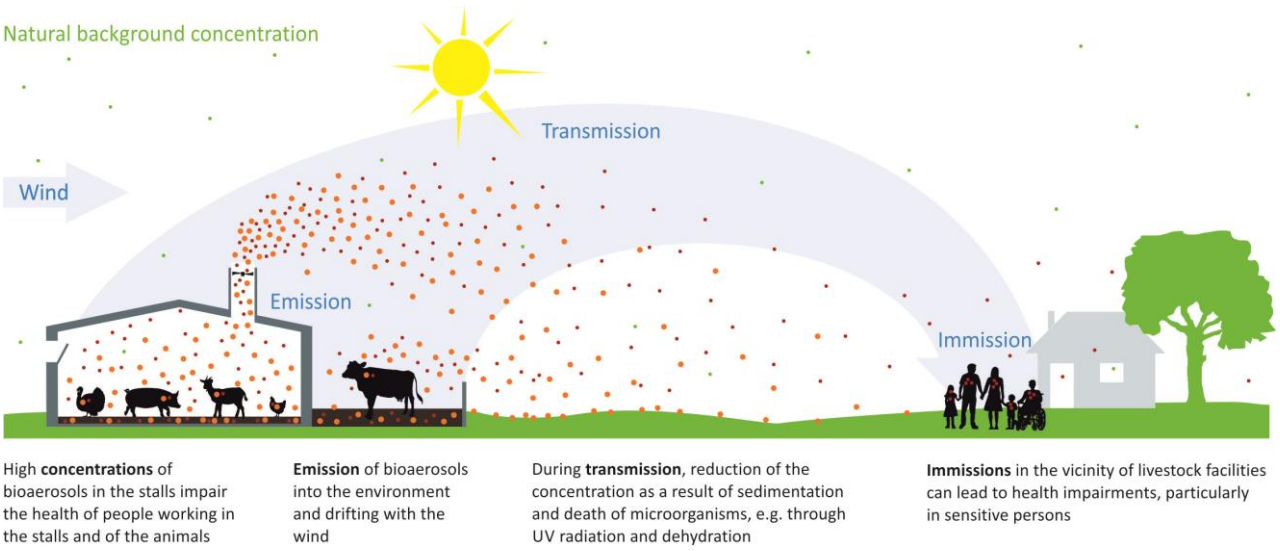


Fig. 1: Schematic illustration of the transportation of bioaerosols from livestock facilities into the environment

It has long been known that the bioaerosol concentrations prevailing in the stalls are strongly increased compared with the natural background concentration (Kolk et al. 2009, Clauß 2013b) and that, together with other air pollutants, they can negatively impact the health of people working in agricultural operations (e.g. Cormier et al. 1991, Donham et al. 1977, 1982, 1984, 1986a, b, 1989, Iversen et al. 2000, Radon et al. 2000, Senthilselvan et al. 1997, Kirychuk et al. 1998, Preller et al. 1995, Rylander et al. 1989, Malmberg & Larsson 1993, Zejda et al. 1994, Duchaine et al. 2000, Cormier et al. 2000, Haglind et al. 1984, Reynolds 1988, Attwood et al. 1986). This also applies to animals kept in the stalls (e.g. Pauli et al. 1974, Wiseman et al. 1984, Huhn 1970, Jericho 1968, Kovacs et al. 1967, Bækbo 1998) and can lead to marked losses of performance (Kocaman et al. 2006, Curtis & Drumand 1982). The bioaerosols are emitted from the stalls via the ventilation into the environment, often in great numbers. They may also

accumulate in the vicinity of animal stalls in sedimentation dust or in the soil (Williams et al. 2016, Schulz et al. 2012). Throughout the world, there is growing evidence that these emissions are also having a negative impact on the general population, especially in areas with a high livestock density and in the vicinity of intensive livestock operations (Sneeringer 2009, Wouters et al. 2012, Radon 2004, Hoopmann 2004, O'Connor et al. 2010). Particularly problematical in this connection are the so-called "zoonoses". These are diseases that can be transmitted from animals to humans (Van der Giessen et al. 2010). Zoonoses account for an estimated 60% of all infectious diseases in humans (Taylor et al. 2001). This problem has been markedly exacerbated by the increasing numbers of animals being kept in close proximity to the human population (Klous et al. 2016, Beran 2008, Pearce-Duvet 2006). Wild animals in the vicinity of intensive livestock farms can also be problematical in this respect (Corn et al. 2009, Jonges et al. 2015). The transmission of zoonoses also takes place via emissions from the stalls (Hackert et al. 2012, Smit et al. 2012). Zoonoses have led to considerable economic losses in agricultural livestock farming in many countries over the past years (Verbeke 2003, Fitzgerald 2012, Chatard-Pannetier et al. 2004, Ogoshi et al. 2010, Bennett et al. 1999, Christou 2011, Leibler et al. 2008, Thuen & Ling 2017, Torgerson & Macpherson 2011, van Asseldonk et al. 2013). The question is how the subject of bioaerosols should be tackled in the future.

In Germany, the determination and assessment of emissions and immissions of particles is regulated by the Federal Pollution Control Act (Bundes-Immissionsschutzgesetz, BImSchG) and its provisions, as well as by the Technical Instructions on Air Quality Control (Technische Anleitung zur Reinhaltung der Luft, TA Luft). The aim of these regulations is to protect humans, animals and plants, the soil, the water, the atmosphere, as well as cultural and other material assets against harmful environmental influences and to prevent the development of harmful environmental influences. The TA Luft is currently under revision and it is being discussed how bioaerosols should be incorporated in the new version. As a result of different collection procedures and detection methods for bioaerosols and inconsistent results regarding the assessment of health impacts, this discussion has proved difficult up to now. The present report reviews literature from throughout the world on methods and results of bioaerosol investigations in and around agricultural livestock farming and summarises the most important points. Within the entire chain of production of agricultural livestock, the focus is directed here on the facilities for rearing and keeping the animals. This report is intended as a basis for the discussion concerning the incorporation of bioaerosols in the TA Luft and also as a basis for the development of a standardised test protocol for bioaerosols within the context of VERA (Verification of Environmental Technologies for Agricultural Production), a project being conducted jointly with Denmark and the Netherlands.

2 Source of bioaerosols in agricultural livestock farming

The major sources of bioaerosols are the animals and their faeces, the litter and the feed, from which bioaerosol particles find their way into the air and can be emitted from the stalls into the environment.

Over the land surface in natural surroundings, only approx. 25% of airborne dust is made up of biological particles (Matthias-Maser & Jaenicke 1994, Matthias-Maser & Jaenicke 2000, Jones & Harrison 2004). In municipal and predominantly agricultural regions, this percentage is generally higher (Matthias-Maser & Jaenicke 1995). In agricultural livestock farming operations, bioaerosols can even account for well over 90% of airborne dusts (Aengst 1984). The main sources of bioaerosols in the stalls are the animals, feed, litter and faeces (Kotimaa et al. 1991, Hartung & Whyte 1994, Heber et al. 1988, Chien et al. 2011), but also manipulable material (Wagner et al. 2016), people in the stalls, e.g. staff and visitors (Lewis et al. 1969, Nishiguchi et al. 2007, Clauß et al. 2013a), or the drinking water. Natural outdoor air also contains bioaerosols (Kolk et al. 2009), which find their way into the stalls with the unfiltered incoming air. The primary sources of bioaerosols can vary, depending on the animal species. In poultry, feather fragments and faecal particles are predominantly found, in pigs it is mostly dried slurry residues (Cambra-Lopez 2010). The biological particles find their way into the air more or less by chance, e.g. through material being swirled up by turbulence or skin scales or feather fragments being stripped off.

Beside undigested feed residues and fragments of intestinal mucosa, the animals' faeces naturally contain a huge number of microorganisms (Pell 1997). Many of them are harmless, but pathogenic organisms are also found, such as salmonellae (Gray & Fedorka-Cray 2001, Himathongkham et al. 1999), or various different viruses (Van Oirschot 1979, Spradbrow et al. 1988, Yoon et al. 1993, Pell 1997, Fouchier et al. 2003, Webster et al. 1978, De Deus et al. 2007). The skin of animals is also colonised by numerous microorganisms, on the surface and especially in the pores. These are predominantly staphylococci, a group of Gram-positive bacteria (Baird-Parker 1962, Gailiunas & Cottral 1966, Kloos et al. 1976). From investigations in humans, it is known that large amounts of these bacteria seated on scales of skin are continuously released into the air (Lewis et al. 1969, Clauß et al. 2013a). Various types of litter are provided in the stalls, usually for reasons of animal welfare, in order to support natural behaviours (Gunnarsson et al. 2000, Appleby & Hughes 1991). Various different materials are used as litter, e.g. sand, straw, sawdust or wood chippings. These materials can already contain large amounts of microorganisms by nature. And this is constantly added to by the animals' faeces, drinking water, sloughed skin or feathers, as well as remains of feed, for as long as the litter remains in the stalls (Torok et al. 2009). Together, they form an ideal culture medium for microorganisms (Lu et al. 2003, Martin et al. 1998). Therefore, the concentrations of airborne microorganisms in housing with litter are usually higher than in housing without litter (Madelin & Wathes 1989, Vucemilo et

al. 2007, Letourneau et al. 2009). The climatic conditions in the stalls, with relatively high temperatures and relative air humidity, additionally promote their growth. A small proportion of the bioaerosols in the stalls comes from the outside air, but this amount is generally negligible compared with the high concentrations found within the stalls. However, this becomes relevant in the case of highly infectious pathogens, e.g. in the case of certain viruses (Donaldson et al. 1977, Gibson & Donaldson 1986, Gloster et al. 2010) or the Q-fever pathogen (Hackert et al. 2012, Smit et al. 2012). Here, transmission via the supposedly uncontaminated outdoor air can certainly occur if there are contaminated farms in the vicinity. As a result of activity of the animals, during feeding (Chang et al. 2001a, Pearson & Sharples 1995, Maciorowski et al. 2007), or also due to work being performed in the stalls (Wijnand 1997), particles of faeces, litter or feed find their way into the air and, with them, also the microorganisms they contain. For example, Rautilia et al. (2003) found that the concentrations of Gram-negative bacteria in the air increased in part 10-fold when spreading litter for pigs. Bioaerosols can also come directly from the animals' respiratory tract and be released by coughing, snorting or breathing. This process has been thoroughly investigated in humans (Duguid 1946, Loudon & Roberts 1967, Papineni & Rosenthal 1997, Nicas et al. 2005, Yang et al. 2007, Gralton et al. 2011). In animals, few studies are available on this subject (Cho et al. 2006). Once the bioaerosol particles are in the air, depending on their size and their aerodynamic diameter (AD) they are carried further via the thermal uplift from the animals, turbulence and the ventilation system, and can ultimately be emitted via the exhaust airflow out of the stalls into the environment. Dust that has already sedimented in the stalls or in the exhaust air ducts also contains many bacteria (Skora et al. 2016), which may remain viable for a very long time (Schulz et al. 2016). If this is swirled up by turbulence, these bacteria similarly find their way into the environment with the airflow.

3 Mirobial indicator organisms for bioaerosols from livestock farming

Hundreds of different viruses, bacteria and moulds have been detected in agricultural livestock farming throughout the world, whereby the bacterial group of the *Staphylococcaceae* appears to be most suitable as a specific indicator parameter for animal husbandry.

Hundreds of different species of bacteria and moulds have been detected in the air of animal stalls, both with the classical culture-based procedures (e.g. Wilson et al. 2002, Brodka et al. 2012, Pavan & Manjunath 2014, Sowiak et al. 2012, Vela et al. 2012, Fritz 2017) and with the more modern molecular biological methods (e.g. Hong et al. 2012, Nonnenmann et al. 2010, Nehme et al. 2009, Martin et al. 2010b, Fallschissel 2011, Wang et al. 2011, Kristiansen et al. 2012, Gao et al. 2017, Schaeffer et al. 2017, O'Brien & Nonnemann 2016). In addition, there is a variety of different viruses (e.g. Hugh-Jones et al. 1973, Christensen et al. 1993), endotoxins (e.g. Zucker & Müller 2000, Myrna et al. 2017), mycotoxins (Wang et al. 2008, Wang et al. 2011) and allergens (Radon et al. 2000, Hinze et al. 1996, Virtanen et al. 1988, Kullmann et al. 1998, Rimac et al. 2010). Certain groups of microorganisms are repeatedly found in large numbers in animal stalls throughout the world. These groups appear to be specific for animal housing and are so-called "indicator parameters" for animal husbandry. The term indicator parameter is defined by the Association of German Engineers (Verein Deutscher Ingenieure, VDI) in VDI 4250 Sheet 3: "Components of bioaerosols that are characteristic for the emission from an installation and are detectable with currently available sampling and analytical methods". The latter is particularly important, as, e.g. in the case of bacteria, it is generally estimated that only around 0.01% – 1% are detectable at all with the commonly used standard methods via cultivation (Amann et al. 1995, Oliver 2005, Chi & Li 2006, Xu et al. 1982).

In the standards VDI 4250 Sheet 3 and VDI 4253 Sheet 3, general sum parameters ("Total bacteria" and "Total moulds"), indicator parameters specifically occurring in animal stalls, as well as "special measurement parameters" relevant to particular questions are stated, beside cultivation methods and confirmation reactions. Here, the selection of indicator organisms appears to be strongly related to medical aspects and does not necessarily reflect the actual occurrence of the selected microorganisms in native bioaerosol samples. Table 1 provides an overview of the sum, indicator, and special measurement parameters from the VDI standards for Germany and, beyond this, from the worldwide literature the most important microorganism groups and viruses that have been detected in bioaerosol samples from agricultural livestock farming and thus are transmissible aerogenically, as well as their incidence and their medical relevance.

Table 1: Microorganism groups and viruses that have been detected in bioaerosol samples from agricultural livestock farming throughout the world, their incidence and their medical relevance

Measurement parameters	Animal species	Medical relevance	Detectable in bioaerosol samples	Notes	Literature reference
Bacteria					
Total cell count	All	Potentially also containing pathogens	Microscopy	Important sum parameter for plausibility testing of measurements	
Total bacteria (22 °C and 36 °C)	All	Potentially also containing pathogens (at 36 °C)	Cultivation	Important sum parameter for plausibility testing of measurements	
Total anaerobic bacteria	All	Potentially also containing pathogens	Cultivation		Chai et al. 1997
Thermophilic bacteria	All	Potentially also containing pathogens	Cultivation	Special measurement parameter in VDI standards	
Gram-negative bacteria	All	Endotoxins as cell-wall component, potentially also containing pathogens	Cultivation	Sum parameter in VDI standards, have thin cell wall, sensitive to dehydration, air concentrations are therefore relatively low	e.g. Matkovic et al. 2006, Zucker et al. 2000, Bakutis et al. 2004
Enterobacteriaceae	All	Indicator for faecal contamination, potentially also containing pathogens	Cultivation	Indicator parameter in VDI standards, are often damaged during sampling and therefore only detected airborne in very low concentrations	e.g. Gordon 1963, Duan et al. 2006, Zucker & Müller 2002
Coliforms	All	Indicator for faecal contamination, potentially also containing pathogens	Cultivation	Special measurement parameter in VDI standards, not a taxonomically but a historically evolved classification	e.g. Benham & Egdell 1970, Baĭkov & Petkov 1986
Escherichia coli	All	Several strains known to be pathogenic (EHEC)	Cultivation	Special measurement parameter in VDI standards	e.g. Chai et al. 2003
"Extended-spectrum beta-lactamase" (ESBL)-forming bacteria	Poultry, cattle, pigs	Antibiotic-resistant (e.g. *E.coli* serotype O157:H7)	Cultivation		e.g. Hering et al. 2015, Dohmen et al. 2017, Laube et al. 2014

Bacteria continued					
Salmonella spp.	Poultry, cattle, pigs, sheep	Pathogen of salmonellosis	Cultivation	Special measurement parameter in VDI standards	e.g. Oliveira et al. 2006, Chinivasagam et al. 2009, Okraszewska-Lasica et al. 2014, Harbaugh et al. 2006
Legionella spp.	Pigs (exhaust air cleaning systems)	Pathogen of Legionnaires' disease	Only molecular biologically to date	indicator parameter in VDI standards	Walser et al. 2017
Campylobacter spp.	Poultry	Gastroenteritis	Only molecular biologically to date	Special measurement parameter in VDI standards, most important pathogen of gastroenteritis worldwide in 2010, the most commonly registered zoonosis in the EU	e.g. Gilpin et al. 2008, Olsen et al., 2009, Chinivasagam et al. 2009, Søndergaard et al. 2014
Leptospira spp.	Cattle, pigs	12 pathogenic species with 250 pathogenic serovars	Unknown	Special measurement parameter in VDI standards, most widespread zoonosis pathogen worldwide	Adler & Moctezuma 2010, Monno et al. 2009
Pseudomonas aeruginosa	Drinking water, pasture	Pathogen of various infections (pneumonia, meningitis)	Cultivation	Special measurement parameter in VDI standards	
Pseudomonas spp.	Drinking water, pasture, exhaust air cleaning systems	Potentially also containing pathogens	Cultivation	Special measurement parameter in VDI standards	Anonymous 2013a
Staphylococcaceae	Horses, pigs, poultry, cattle, goats	Potentially also containing pathogens	Cultivation	Indicator parameter in VDI standards, in the opinion of most authors the most important indicator parameter for animal husbandry	e.g. Müller 1974, Hojovec et al. 1977, Hartung 1992, O'Bien et al. 2016, Curtis et al. 1975a
Staphylococcus aureus	Cattle, goats, sheep, pigs, horses	Pathogen of various infections (osteomyelitis, mastitis, endocarditis, pneumonia)	Cultivation and PCR	Indicator parameter in VDI standards, only 1 – 5% of cultivable staphylococci are *S. aureus*, therefore complicated to select	e.g. Chai et al. 2003, Alvarado et al. 2009, Zhong et al. 2009

Bacteria continued					
MRSA	Cattle, goats, sheep, pigs, horses	Antibiotic-resistant	Cultivation and PCR		e.g. Zhong et al. 2009, Tenhagen et al. 2008, Liu et al. 2012
Intestinal enterococci	All	Indicator for faecal contamination, potentially also containing pathogens	Cultivation	Indicator parameter in VDI standards, is only detected airborne in very low concentrations	e.g. Aarnink et al. 2012, Anonymous 2013a, Brooks et al. 2010
"Streptococci"	Cattle	Potentially also containing pathogens	Cultivation, FISH	Special measurement parameter in VDI standards, not a taxonomic classification but an auxiliary designation combining all aerotolerant gram-positive, catalase-negative cocci	e.g. Matkovic et al. 2006, Kristiansen et al. 2012, Angersbach-Hegers 2002
Streptococcus suis	Pigs	Pathogen of various infections (sepsis, meningitis, endocarditis, pneumonia)	Cultivation, PCR	Common zoonosis pathogen in China, Canada and the USA, only sporadic in the EU to date	Gauthier-Levesque et al. 2016, Bonifeit et al. 2014, Lun et al. 2007
Rhodococcus equi	Horses	Pathogen of various infections (osteomyelitis, pneumonia)	Cultivation	Rarely investigated	Kuskie et al. 2012, Takai 1997
Listeria monocytogenes	Cattle, sheep, pigs	Listeriosis	Cultivation	Rarely investigated	Okraszewska-Lasica et al. 2014
Bacillus anthracis	Cattle, goats, sheep, pigs, horses	Pathogen of anthrax	Cultivation	Special measurement parameter in VDI standards, in risk group 3 in the German biomaterial regulations, protection class 3 laboratory required	
Lactobacillus spp.	Pigs	Potentially also containing pathogens	Cultivation	Rarely investigated. Probably accounts for most of the anaerobic bacterial flora in animal stalls	Hill & Kenworthy 1970, Sauter et al. 1981
Sulphite-reducing clostridia		Potentially also containing pathogens	Cultivation	Special measurement parameter in VDI standards	
Clostridium perfringens	Calves	Exotoxin producer, pathogen of gas gangrene	Cultivation, microscopy		Draz et al. 1999, Chai et al. 1997

Bacteria continued					
Clostridioides (Clostridium) difficile	Pigs	Diarrhoea	Cultivation	Zoonosis pathogen and one of the most common nosocomial pathogens	e.g. Keessen et al. 2011, Hopman et al. 2010, Songer & Uzal 2005
Brucella spp.	Sheep, goats, pigs, cattle	Pathogen of brucellosis	Unknown	Particularly relevant at slaughter	Kaufmann et al. 1980, Monno et al. 2009
Mycobacterium bovis	Cattle	Bovine tuberculosis	Cultivation, PCR	Also transmissible to humans	Gannon et al. 2007
Non-tuberculous mycobacteria		Potentially also containing pathogens		Special measurement parameter in VDI standards, detection according to DIN 58943-3	
Mycoplasma hypopneumoniae	Pigs	Pneumonia	PCR	One of the most important pathogens of respiratory diseases in pigs	Otake et al. 2010, Dee et al. 2009
Clamydophila psittacosi	Poultry	Pathogen of psittacosis	Cell culture, molecular biologically	Special measurement parameter in VDI standards, intracellular reproduction in the host. Cultivation not possible. Molecular biological detection, cell culture	Dickx et al. 2010, Gaede et al. 2008
Coxiella burnetti	Cattle, goats, sheep	Pathogen of Q-fever	Cell culture, molecular biologically	Intracellular reproduction in the host. Cultivation not possible. Molecular biological detection, cell culture	e.g. Hackert et al. 2012, Smit et al. 2012, Schulz et al. 2005, van der Hoek et al. 2012
Saccharopolyspora rectivirgula	Dairy cows, pigs	Extrinsic allergic alveolitis (EAA)	Cultivation, PCR		e.g. Schäfer et al. 2011, Duchaine et al. 1999a, Cormier et al. 1990
Actinomycetes (mesophilic)	Litter, feed, biofilters	Potentially also containing pathogens	Cultivation	Special measurement parameter in VDI standards, group is too diverse from a phylogenetic point of view	Anonymous 2013a Angersbach-Hegers 2002,
Actinomycetes (thermophilic)	Litter, feed, biofilters	Potentially also containing pathogens	Cultivation	Special measurement parameter in VDI standards, group is too diverse from a phylogenetic point of view	e.g. Anonymous 2013a

Moulds					
Total moulds (25 °C)	Litter, feed, biofilters	Potentially also containing pathogens	Cultivation, microscopy	High concentrations in the case of poor litter quality or contaminated feed	
Aspergillus fumigatus	Litter, feed, biofilters	Aspergillosis	Cultivation, microscopy	High concentrations in the case of poor litter quality or contaminated feed	e.g. Arne et al. 2011, Schiek 1998
Aspergillus spp.	Litter, feed, biofilters	Potentially also containing pathogens	Cultivation, microscopy	High concentrations in the case of poor litter quality or contaminated feed	e.g. Pavan & Manjunath 2014
Penicillium spp.	Litter, feed, biofilters	Rarely also containing pathogens	Cultivation, microscopy	High concentrations in the case of poor litter quality or contaminated feed	e.g. Pavan & Manjunath 2014, Jo & Kang 2005
Cladosporium spp.	Litter, feed, biofilters	Rarely also containing pathogens	Cultivation, microscopy	High concentrations in the case of poor litter quality or contaminated feed	z.B. Jo & Kang 2005, Pavan & Manjunath 2014
Viruses					
PRRS virus	Pigs	Porcine reproductive and respiratory syndrome	PCR	Leads to considerable losses in pig stocks, is capable of covering long distances when airborne	e.g. Garcia-Mochales 2016, Otake et al. 2010, Dee et al. 2009, Murtaugh & Yeske 2008
CSF virus (classical swine fever)	Pigs	Classical swine fever	PCR	Animal disease that is difficult to control	Laevens et al. 1998, Laevens et al. 1999, Dewulf et al. 2000
ASF virus (African swine fever)	Pigs	African swine fever	PCR, cell culture	Sporadic outbreaks in Europe, not detected in Germany to date	Wilkinson et al. 1977, de Carvalho Ferreira et al. 2013
Porcine circovirus type 2 (PCV2)	Pigs	Postweaning multisystemic wasting syndrome (PMWS), porcine dermatitis and nephropathy syndrome (PDNS)	PCR	Widespread in pigs throughout the world, detected in Germany since 1962. For many years classified as harmless	e.g. Allan et al. 1998, Harding 2004, Jacobsen et al. 2009

Viruses continued					
FMD virus	Pigs	Pathogen of foot-and-mouth disease	PCR	Leads to considerable losses in pig stocks, is capable of covering long distances in the air	e.g. Garcia-Mochales 2016, Aleksanderse n et al. 2008, Donaldson et al. 1982
Influenza-A virus	Pigs	Swine influenza	PCR	Several subtypes detected in bioaerosol samples	e.g. Fraser et al. 2009, Khardori 2010, Al-Tawfiq et al. 2014
Porcine epidemic diarrhoea virus (PEDV)	Pigs	Porcine viral diarrhoea	PCR	Airborne spread of up to 15 km demonstrated	Pensaert & de Bouck 1978, Alonso et al. 2014
Suid herpesvirus 1 (SuHV-1)	Pigs, cattle, sheep, goats	Aujezsky's disease (pseudorabies)	PCR	Pigs as the primary host	Christensen et al. 1990, Christensen et al. 1993, Donaldson et al. 1983
Newcastle disease virus	Poultry	Newcastle disease, atypical avian influenza	PCR, ELISA, cell culture	Has long been known to be transmissible aerogenically	Hugh-Jones et al. 1973, Li et al. 2009
Avian influenza A virus (IAV)	Poultry	Avian influenza, bird flu	PCR	Highly infectious. Different types of course, depending on the subtype	Garcia-Mochales 2016, Jonges et al. 2015, Stegeman et al. 2004
IBD virus (infectious bursal disease)	Poultry	Infectious bursitis	Cell culture, PCR		Friese 2010
Others					
Endotoxins	All	Pyrogenic, general respiratory diseases	LAL test, ELISA	Cell wall component of Gram-negative bacteria. Endotoxins can accumulate in dust. Marker for organic dusts from animal husbandry	Zucker & Müller 2000, Myrna et al. 2017
Beta 1-3 glucans	All	Pyrogenic	Modified LAL test	Reference indicator for moulds	Lee & Liao 2014, Cyprowski et al. 2011

Which measurement parameter is investigated in the case of bioaerosols depends primarily on the question at hand. The sum parameter “Total bacteria” is almost always included in investigations as a sensible way of putting one’s own measurements in context and to test their plausibility. The investigation of “Total anaerobic bacteria”, i.e. those that can only be cultivated under the exclusion of oxygen, has naturally been of little interest in connection with bioaerosols to date. However, it may be assumed that they account for a large part of the aerogenic microflora in animal stalls, as they are excreted in large numbers via the faeces. Within the total bacteria, Gram-positive and Gram-negative bacteria are generally distinguished. In contrast to the Gram-positive bacteria, the latter have a thinner cell wall, are therefore more sensitive to dehydration and thus not viable in the air for a particularly long time. Therefore, they are only found in low concentrations in the air of animal stalls. The percentage share of the total bacteria is around 1 - 10% (Matkovic et al. 2006). However, the Gram-negative bacteria also include important pathogens, such as the salmonellae, *Campylobacter*, legionellae and *Leptospira*. Within the group of Gram-negative bacteria, the family of the Enterobacteriaceae is detected most frequently (Duan et al. 2006, Zucker & Müller 2002), its most well-known representative being *Escherichia coli*. Of particular medical relevance are the antibiotic-resistant strains (ESBL (extended-spectrum beta-lactamase) formers) of *E. coli,* e.g. the *s*erotype O157:H7 (Hering et al. 2015, von Salviati et al. 2015, Laube et al. 2014). Enterobacteriaceae are above all indicators for faecal contamination, as they are excreted in large numbers via the faeces. Bacteria from the group of the pseudomonads are mostly typical aquatic microorganisms and occur in the drinking water of the stalls or are carried in from outside. Their occurrence in animal stalls has been poorly investigated to date. As a result of their thicker cell wall and the accompanying greater “robustness” towards the airborne state, most of the bacteria from the air detected via cultivation methods are attributable to the Gram-positive bacteria (Zhao 2011). Within this group, in the opinion of most authors it is the *Staphylococcaceae* that are particularly specific for agricultural livestock farming and they are therefore considered to be one of the most important indicator parameters for microbial air contamination from animal stalls. In addition, *Staphylococcaceae* are easy to detect with commonly used cultivation methods and minimal laboratory standards. Within this group, there are several potential disease pathogens that are classified in risk group 2. The best known is *Staphylococcus aureus*, although its share of the total staphylococcal flora is mostly very small. Therefore, when cultivated on culture medium, typical colonies are often grown over, making quantification difficult. Of particular importance here are the antibiotic-resistant strains, such as *methicillin-resistant Staphylococcus aureus* (MRSA). In numerous studies from Germany, Denmark, Belgium, the Netherlands, Switzerland, Japan, Canada and the USA, poultry, pigs, cattle and horses were colonised with MRSA in between 1% and 93% of the animals of a herd (Kraemer et al. 2017, Persoons et al. 2009, Nemati et al. 2008, Spohr et al. 2011, Graveland et al. 2010, Lozano et al. 2011, Khanna et al. 2008, Alt et al. 2011, deNeeling et al. 2007, van Dujikeren et al. 2008, van Loo et al. 2007, Guardabassi et al. 2007, Smith et al. 2009, Shimizu et al 1997, Weese et al 2005, Schulz et al. 2012). MRSA have also been detected in raw meat from animals that were designated for food production (Kelman et al. 2011, Pu et al. 2009). Particularly high concentrations were found in the air of pig fattening stalls (Chapin et al. 2004, Gibbs et al. 2004, Clauß et al. 2013c). MRSA have also been detected in the

exhaust air plumes from the stalls and on the soils in the vicinity (Gibbs et al. 2006, Green et al. 2006, Friese et al. 2012, Friese et al. 2013, Schulz et al. 2012). Beside ESBL formers and MRSA, there are further antibiotic-resistant zoonosis pathogens for which transmission via the air cannot be ruled out. Vancomycin-resistant enterococci, for example, have been detected in farm animals in the USA and in Europe (Barbara 1997, van den Bogaard & Stobberingh 1999, Kuhn et al. 2005). Antibiotic-resistant salmonellae are zoonosis pathogens that have been found mainly in poultry, but also in cattle and pigs (White et al. 2004, Brichta-Harhay et al. 2011, Alam et al. 2009, Dargatz et al. 2002). Further important groups within the Gram-positive bacteria are enterococci and streptococci, both of which may also contain pathogenic species, as well as important disease pathogens like *Bacillus anthracis* or *Clostridium difficile*, the latter being an enteropathogen for humans and animals (Keessen et al. 2011). Another important disease pathogen is the bacterium *Coxiella burnetii*, which has led to an increase in local Q-fever epidemics in Germany over the past few years, particularly in connection with sheep housing, as well as to more serious outbreaks in the Netherlands (Hackert et al. 2012, Smit et al. 2012, Schulz et al. 2005, van der Hoek et al. 2012).

Moulds in the air of animal stalls have been investigated intensively especially in the tropical countries. As a result of the climatic conditions that prevail there, generally with high temperatures and air humidity throughout the year, they have optimal growth conditions and thus pose a greater problem than in our temperate latitudes. Even here in Germany, more than one hundred different species have been detected in animal stalls to date, although the concentrations are relatively small. The level of the concentrations and the composition of the mould species in the stalls are mostly dependent on the presence and the quality of litter (Hartung 1992). The groups most commonly detected in cattle, pigs, poultry and rabbits are the moulds *Aspergillus* sp., *Alternaria* sp., *Cladosporium* sp., *Penicillium* sp., *Fusarium* sp., *Scopulariopsis* sp., as well as the yeasts of the genus *Candida* (Wang et al. 2007, Zhao 2011, Chang et al. 2001a, Cormier et al. 1990, Martin et al. 1996, Matkovic et al. 2009a, b, Wilson et al. 2002, Miao et al. 2010). Above all several pathogenic aspergillus species are of relevance to health, e.g. *A. flavus, A. fumigatus* and *A. niger* (Wang et al. 2007).

Beside bacteria and moulds, aerogenically transmissible viruses have become a focus of bioaerosol research in agricultural livestock farming over the past few years. This is also due to the fact that new collection and detection methods have now been established for airborne viruses (Friese 2010, Alonso et al. 2016, Andersen et al. 2017, Gloster et al. 2010, Otake et al. 2010, Verreault et al. 2008, Corzo et al. 2013). In Germany, viruses occurring in bioaerosols have barely been investigated in agricultural livestock farming to date, although the international literature contains reports that many viruses are aerogenically transmissible, are carried long distances through the air and can lead to at times devastating outbreaks of disease.

A further important medically relevant component of bioaerosols are the endotoxins. They represent the most significant proinflammatory bioaerosol component, i.e. the component that has the most inflammatory action. The faeces of the animals is the most important source of

endotoxins in the stalls (Eckhardt 2008). Endotoxins are localised in the outer cell membrane of Gram-negative bacteria and they are largely responsible for the organisation, stability and barrier properties of the cell wall. The term endotoxin is used to mean the entirety of toxic cell wall products of the microorganisms concerned, whereby it is basically a question of lipopolysaccharides (LPS) from a biochemical point of view (Linsel & Kummer 1998). They consist of a hydrophilic polysaccharide component, which is responsible for the water solubility of the molecule, and a hydrophobic lipid component, which primarily determines the biological activity and the toxic properties of the molecule. For example, an *E. coli* cell contains around 10^6 lipopolysaccharide molecules, of which living cells continuously release small amounts into the environment. If cells die and decay, the endotoxins are released in large numbers. They are relatively stable and can remain active for a long time, even in the airborne state or in sedimentation dust (Pomorska et al. 2009).

Given the large number of different bioaerosol components found to date, the question is which of them is particularly characteristic for emissions from agricultural livestock farming. In the unanimous opinion of many authors, these are the staphylococci, as they originate directly from the animals and have almost always been detected in large numbers in the air of the stalls. The results from studies that determined both the concentrations of total bacteria and those of the staphylococci in the stalls or in emissions are summarised below (Tab. 2). These figures were used to calculate the share of staphylococci as a percentage of the total bacteria found for the individual animal species.

Tab. 2: Share of staphylococci as a percentage of total airborne bacteria in the stalls and in emissions from animal stalls, calculated from the results of different studies (CFU: colony-forming units)

Animal species	Reference	Country	Total bacteria in CFU/m³	Staphylococci in CFU/m³	Percentage share
Pigs	Hojovec et al. 1976	Czech Republic	1.0×10^6	3.0×10^5	30
	Fiser 1978	Czech Republic	2.2×10^7	7.4×10^4	3
	Spirin & Mikhaĭlova 1991	Russia	2.2×10^5	9.5×10^4	43
	Butera et al. 1991	Canada	4.5×10^5	3.8×10^3	1
	Eliot et al. 1976	USA	2.0×10^5	1.0×10^5	50
	Geburek et al. 2005	Germany	5.0×10^5	5.0×10^3	1
	Anonymous 2013a	Germany	8.1×10^4	1.7×10^4	21
	Anonymous 2013a	Germany	7.6×10^4	5.1×10^3	7
	Anonymous 2013a	Germany	8.7×10^4	3.8×10^3	4
	Bayrisches Landesamt 2015b	Germany	2.0×10^5	5.0×10^4	25
	Clauß (own data)	Germany	1.0×10^5	6.5×10^4	70
				Total:	**23**
Cattle	Karowska 2005	Poland	1.6×10^3	3.4×10^2	21
	Clauß (own data)	Germany	2.6×10^5	5.3×10^3	2
				Total:	**11**
Goats	Clauß (own data)	Germany	3.6×10^5	2.4×10^5	**67**
Horses	Fritz 2017	Austria	3.5×10^3	3.9×10^2	**11**
Chickens	Brooks et al. 2010	USA	6.7×10^3	1.5×10^4	224

	Brooks et al. 2010	USA	4.0×10^6	3.7×10^6	92
Chickens	Chai et al. 2001a	China	2.7×10^5	3.3×10^4	12
continued	Schulz et al. 2004	Germany	1×10^7	1×10^7	100
	Witkowska & Sowińska 2013	Poland	1×10^6	1×10^5	10
	Saleh 2006	Germany	2.5×10^5	1×10^5	40
	Saleh 2006	Germany	4.7×10^7	4.6×10^7	98
	Agabou 2009	Algeria	1.0×10^2	3.0×10^1	30
	Angersbach-Hegers 2002	Germany	2.8×10^6	2.2×10^6	77
	Anonymous 2012	Germany	3.0×10^6	3.0×10^6	100
	Anonymous 2012	Germany	1×10^5	2.5×10^4	25
	Blomberg et al. 2009	Germany	1×10^5	1.2×10^6	120
	Blomberg et al. 2009	Germany	9.4×10^5	7.7×10^5	82
	Springorum et al. 2015	Germany	6.5×10^5	2.7×10^5	42
	Springorum et al. 2015	Germany	7.5×10^5	2.5×10^5	33
	Springorum et al. 2015	Germany	2.0×10^5	1.5×10^5	75
	Springorum et al. 2015	Germany	1.6×10^5	1.5×10^5	63
	Popescu et al. 2013	Romania	7.7×10^4	2.0×10^4	26
	Popescu et al. 2013	Romania	4.8×10^6	3.1×10^6	65
	Schrader et al. 2013	Germany	4.3×10^6	3.2×10^6	74
	Popescu et al. 2010	Romania	2.3×10^5	3.5×10^5	156
	Popescu et al. 2010	Romania	2.2×10^6	1.3×10^6	59
	Lippmann et al. 2016	Germany	7.4×10^6	7.4×10^6	100
	Lippmann et al. 2016	Germany	1.5×10^6	1.5×10^6	100
	Gärtner et al. 2011	Germany	4.8×10^6	2.4×10^6	50
	Gärtner et al. 2011	Germany	5.7×10^6	2.3×10^6	40
	Gärtner et al. 2011	Germany	8.4×10^6	6.2×10^6	74
	Gärtner et al. 2017	Germany	4.9×10^7	2.7×10^7	55
	Bayrisches Landesamt 2015a	Germany	5.0×10^7	1.0×10^7	20
				Total:	**70**

The percentage shares calculated from the results of the studies vary considerably, and sufficient data are not available for all animal species. However, for chicken farming and pig farming it can be deduced that a respective share of on average approx. 23% and 70% of the total bacteria can be attributed to the *Staphylococcaceae*. The large differences are probably due to the different collection and detection methods for staphylococci, above all the different culture media and the type of analysis. If one only considers the results obtained with standardised methods in Germany, the share of *Staphylococcaceae* as a percentage of total bacteria is markedly higher and is approx. 90% in poultry and approx. 70% in pigs. According to these figures, the group of the *Staphylococcaceae* would be very well suited for chicken farming and still well suited for pig farming as a characteristic indicator parameter for emissions from agricultural livestock farming.

4 Measurement of bioaerosols

Bioaerosols can be measured online and offline, whereby, due to the complexity of bioaerosols in agricultural livestock farming, the classical methods tend to be best suited, i.e. sampling on site with subsequent evaluation via culture-based or molecular biological methods in the laboratory.

In principle, there are two ways of measuring bioaerosols: online and offline. Methods for the online measurement of bioaerosols have rarely been used in agricultural livestock farming up to now. Mostly for military purposes, various LIDAR (light detection and ranging) systems are used in order to detect above all large collections of bioaerosols from a great distance (NATO 2010). In contrast, various laser particle spectrometers, such as the ultraviolet aerodynamic particle sizer (UVAPS) or the waveband integrated bioaerosol sensor (WIBS), enable the measurement of bioaerosols on site online in the airflow of a measuring cell (Hairston et al. 1997, Kaye et al. 2004, Kaye et al. 2005a, Kaye et al. 2005b, Chang et al. 2007). Such systems have already been used to detect bioaerosols in animal stalls (Agranovski et al. 2004, Agranovski et al. 2007). The measuring principle is based on the fact that proteins and DNA can be stimulated to fluoresce at defined laser wavelengths. The specific wavelengths at which they emit are detected. Depending on the system, information can also be collected about the size and shape of the particles. This information can be used to identify a bioaerosol particle to a certain degree. For artificial bioaerosols of known composition, these systems function very well (Toprak & Schnaiter 2013). They are also being successfully applied in atmosphere research for the detection of small and simple primary bioaerosol particles that are relevant, e.g. in cloud formation (Toprak 2014). However, in animal stalls, bioaerosol particles are usually larger, consisting of a wide variety of different biological substances, and contain many bacterial cells from different species, which are often present embedded in an organic matrix (Clauß et al. 2011a, b). A quantitative and qualitative detection of microorganisms in such particles is not possible with such systems. They can only give an indication of the rough composition of a bioaerosol. Thus, e.g. Agranovski et al. in 2007 only found that 80% of the airborne particles occurring in broiler stalls fluoresced, i.e. were of biological origin.

In the case of “offline measurement” of bioaerosols, they first have to be collected in a sample. The sample is analysed subsequently, mostly via cultivation and/or various different biochemical and molecular biological methods.

4.1 Sampling of bioaerosols

Due to the large number of different collection methods available for bioaerosols, standardised systems that have high physical and biological collection efficiency should be fallen back on, always depending on the question at hand.

Throughout the world, numerous different sample collection systems are available for detecting bioaerosols. The most important collection methods are sedimentation, filtration, impaction, impingement and centrifugal separation (cyclones). Fig. 2 shows the percentage distribution of the systems used from 313 publications in which bioaerosol samples were taken and analysed in agricultural livestock farming.

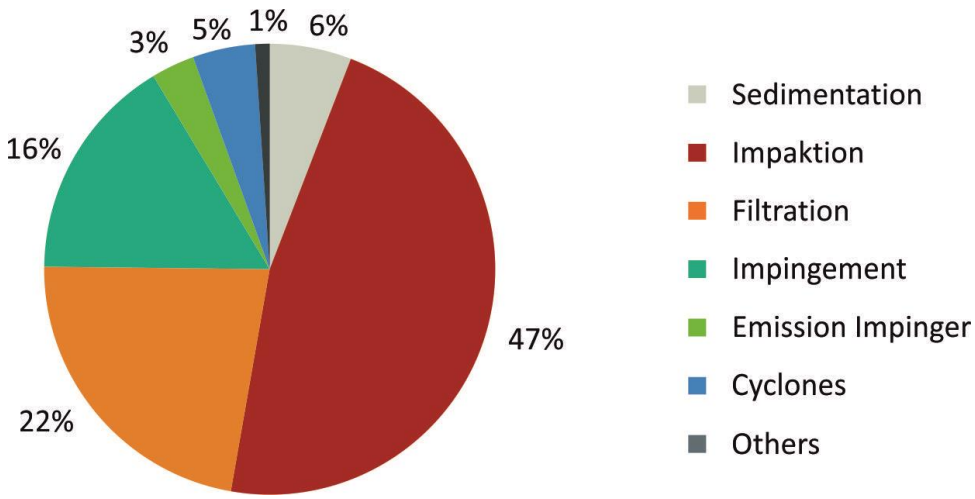


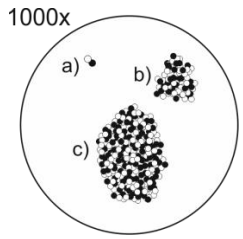
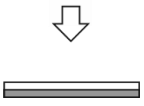
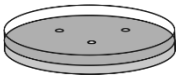
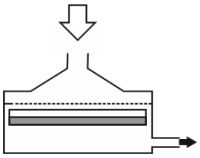
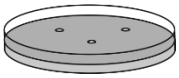
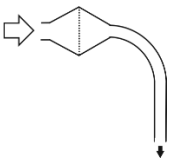
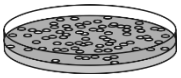
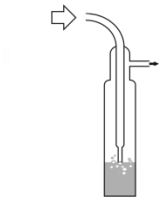
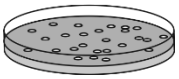
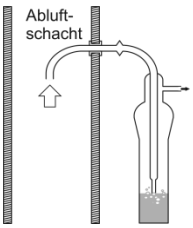
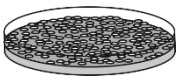
Fig. 2 Percentage distribution of the systems used for collecting bioaerosols in agricultural livestock farming from 313 publications.

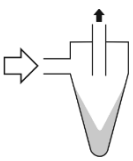
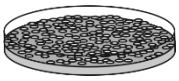
Worldwide, impactors were used as bioaerosol collectors in almost every second study. They are followed by various filter systems, at 22%, which were used above all for the collection of endotoxins and moulds. 16% of the authors applied impingers, in most cases the AGI-30 (all glass impinger). This is recommended in Germany for the collection of bioaerosols in immissions (VDI 4252 Sheet 3). In contrast, the emission impinger is a standardised system in Germany for sampling bioaerosols in emissions (Gärtner et al. 2008a, b, VDI 4257 Sheet 2). It was used for this purpose in 3% of cases and has only been applied in Germany to date. Cyclones are becoming increasingly popular, with a share of 5% at present. Above all due to their high air throughput, they are advantageous for the collection of viruses, as well as microorganisms that only occur in very small concentrations in the air (Clauß 2016). In as many as 6% of the studies, sedimentation on culture medium plates was still applied after 1960 as the simplest collection method. The remaining 1% are accounted for by other methods, such as that of Sauter et al. (1981), who collected microorganisms on culture media with the aid of an impactor, but then washed them

from the agar plates into a liquid. Olsen et al. (2009) used an electrostatic collection method, and Steiger & Stellmacher (1977) collected bioaerosols from cattle stalls in the former GDR with the aid of a thermoprecipitator.

When collecting airborne bacteria, the different sampling methods alone can lead to deviations in the results of several powers of ten. One reason for this is that the bacteria in the air of animal stalls are predominantly present in larger aggregates (Hesse 1884, Hesse 1888). The influence of the sampling systems on the results is illustrated in Tab. 3. Both sedimentation and impaction are relatively simple collection methods, in which the airborne microorganisms are usually impacted directly onto culture medium plates. After sampling, the culture media are incubated in an incubator in the laboratory. Each particle that carries microorganisms capable of reproduction, regardless of their number, produces just one countable colony. This method can thus only be used to determine the number of particles in the air carrying microorganisms and not the total number of microorganisms. In the case of sedimentation, the statement of a concentration, e.g. CFU/m³, is only possible to a limited degree, since a defined volume flow through the collection system is missing. The sedimentation methods in particular cause difficulties, since the unit of measurement CFU/m²*s does not allow a direct comparison with results from volumetric measurements and, additionally, problems arise in the interpretation of the results (Erwerth et al. 1983). In the case of the impactors, the relatively low physical collection efficiency is often a disadvantage, caused by the in part high wall loses intrinsic to the system. In the case of the impactor most commonly used internationally, the Andersen collector, the losses are 10% for particles with an aerodynamic diameter (AD) of 5 µm and 41% for particles with 15 µm AD (McFarland 1977, Wedding et al. 1977). And it is virtually impossible to collect particles > 20 µm AD with this collection system. Impactors that were originally developed for the size-fractionated collection of dust are sometimes used for the sampling of bioaerosols. After collection, the exposed filters are eluted in a liquid in order to wash off the collected dust along with the microorganisms and then the wash liquid is further analysed. In the filtration methods, too, bioaerosol particles are collected on filters and the particles are washed off the filters after sampling. This breaks up cell aggregates and microorganisms present are isolated. If the eluate is smeared onto culture medium plates, all cultivable cells can be detected. However, this method has a relatively low biological collection efficiency and, as a result of the risk of dehydration during sampling on the filter medium, is only suitable for resistant microorganisms such as staphylococci or fungal spores. Sensitive microorganisms can easily die here (Fallschissel 2011). In the case of impingement, bioaerosols are collected directly in a liquid. Here, too, cell aggregates are broken up and microorganisms are isolated. However, the curved inlet pipe acts as a pre-separator, in which the larger particles collect and thus do not find their way into the sample. Therefore, e.g. in the case of the impinger AGI-30, only around 20% to 30% of the 10 µm sized particles are collected, and virtually all 20 µm sized particles are retained in the bend of the inlet pipe. The bend of the impinger was originally intended to imitate the human respiratory tract. The intention was to only collect particles that penetrate into the lower respiratory tract. Above all, this is relevant in the case of measurements in the workplace for assessing potential health risks of bioaerosols. When investigating other questions,

Tab. 3: Examples of the influence of the collection system on the result of the sampling of airborne bacteria

<table>
<tr><td> 
</td><td colspan="3">Example
3 bioaerosol particles/m³, all finding their way into the collection system:
a) ø = 1 µm, 2 cells, of which 1 is cultivable
b) ø = 5 µm, 50 cells, of which 25 are cultivable
c) ø = 20 µm, 500 cells, of which 250 are cultivable</td></tr>
<tr><td colspan="2">Collection method</td><td>Fictitious result</td><td>Comment</td></tr>
<tr><td>  </td><td>Sedimentation</td><td>3 CFU
  </td><td>Only the number of bacteria-bearing particles is detected, a true concentration/m³ cannot be stated</td></tr>
<tr><td>  </td><td>Impaction, e.g. Andersen collector</td><td>2 CFU/m³
  </td><td>Only the number of bacteria-bearing particles is detected</td></tr>
<tr><td>  </td><td>Filtration</td><td>137 CFU/m³
  </td><td>All particles are separated on the filter, but there are losses as a result of cell death due to dehydration</td></tr>
<tr><td>  </td><td>Impingement, e.g. AGI-30</td><td>26 CFU/m³
  </td><td>Particles > 5 µm are separated in the bend and therefore go largely undetected</td></tr>
<tr><td> 
</td><td>Emission impinger</td><td>276 CFU/m³
  </td><td>All particles are detected in emission measurement if the probes and the bend are washed into the sample

[Abluftschacht = Exhaust air shaft]</td></tr>
<tr><td>  </td><td>Centrifugal separator (cyclone)</td><td>276 CFU/m³
  </td><td>If the inlet efficiency is sufficiently high, all particles are detected</td></tr>
</table>

it is also possible to rinse the bend in order to wash the particles separated there into the sample (Chinivasagam & Blackall 2005). Markedly higher concentrations are thus achieved with washing of the bend than without washing (Tesseraux et al 2015). In the case of emission measurements with the emission impinger according to VDI 4257 Sheet 2, the standardised procedure involves washing both the bend of the impinger and the sampling probes after the collection of samples. By combining the wash and collection liquid, theoretically all cultivable microorganisms in all particle fractions are detected. This achieves a very high physical and biological collection efficiency. It makes sense to use this system for emission measurements in particular, as it enables the detection of the entire emission of airborne microorganisms, and not only microorganisms in just one particular particle size fraction or only the number of microorganism-bearing particles. In the case of centrifugal separators (cyclones), the bioaerosols are suctioned into the collection system, where they are forced into a circular path and separated by centrifugal forces, usually in a liquid. Since they operate with a high air throughput and can thus process a relatively large volume of air samples, these systems are mainly used where very low concentrations of microorganisms are to be expected, e.g. in immission measurements, background measurements (Clauß 2016) and for the detection of viruses (Alonso et al. 2016, Andersen et al. 2017, Gloster et al. 2010, Otake et al. 2010, Corzo et al. 2013). A further advantage here is the direct separation into a liquid, as in the case of the impingers. If the inlet efficiency is sufficiently high, centrifugal separators can theoretically detect all airborne microorganisms individually. However, compared with other collection systems, cyclones have yet to be tested sufficiently with regard to their inlet efficiency for different particle size fractions.

Isokinetic sampling

Bioaerosols are particles. For the sampling of particles from flowing gases, sampling under isokinetic conditions is generally recommended, in order to avoid sampling-related changes in the particle-size spectrum, which can considerably influence the result. The effect is size- and mass-dependent and increases with the size (mass) of the particles. Particularly in the air of animal stalls, bacteria form large aggregates or are attached to large dust particles (Clauß et al. 2011a, b). Many investigations have shown that, depending on the measurement parameters (CFU, cell counts, gene copies), a high percentage occurs in the particle fraction > 10 µm (see section 5.4.2) (Adell et al. 2011a, b, Aarnink et al. 2012, Chai et al. 2001, Chinivasagam & Blackall 2005, Cormier et al. 1990, Lecours et al. 2012, Lenhart et al. 1982, Liu & Ma 2010, Predicala et al. 2002, Sowiak et al. 2011, Siggers et al. 2011, Zheng et al. 2013, Clauß 2015a, Gärtner et al. 2017). Fig. 3 shows how important the influence can be for the measurement of particles in this size range.

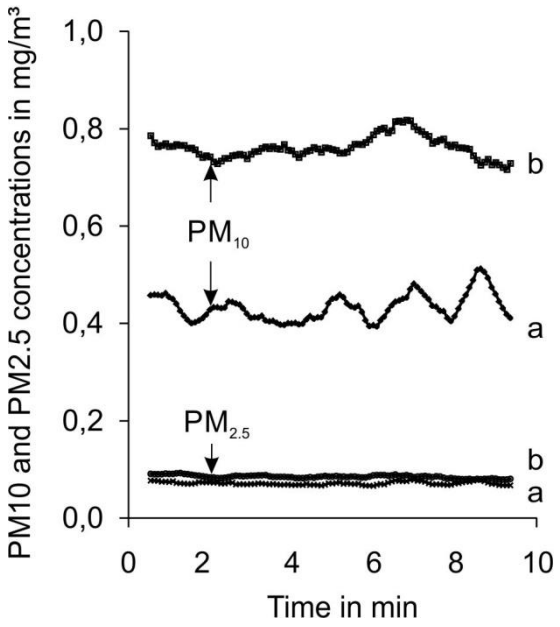


Fig. 3: PM 2.5 and PM 10 concentrations measured in relation to velocity (a = isokinetic, b = hypokinetic) (from Clauß & Hinz 2014)

In an aerosol measurement section, PM 2.5 and PM 10 test dusts were collected from the air under iso- and hypokinetic (velocity in the inlet nozzle of the measuring probe approx. 50% of the air velocity in the measurement section) conditions and the dust concentrations were determined with a particle spectrometer in mg/m³. In the case of PM 2.5 dust, hardly any differences in the result can be seen between the two samplings. In contrast, in the case of PM 10 dust, hypokinetic conditions lead to almost double the concentrations. Although the sample air volume is only half as much here, all large particles nevertheless migrate on the basis of their inertia alone from the inlet nozzle into the sampling probe and are detected with the others. It has been shown that these large particles can contain hundreds of bacteria in animal stalls (Clauß et al. 2011a, b). In the case of airborne microorganisms, it must also be taken into consideration that, beside the deviations in grain size distribution, mass or number generally known for particles, the species spectrum may also be falsified (Fig. 4).

As a result of the different characteristics and the various advantages and disadvantages of the individual collection methods, the choice of sampling system must always be made primarily according to the question at hand. Then, standardised or at least sufficiently characterised systems should be fallen back on, in order to ensure comparability of the results. Above all, the promising cyclones need to be better evaluated for the collection of bioaerosols in the future.

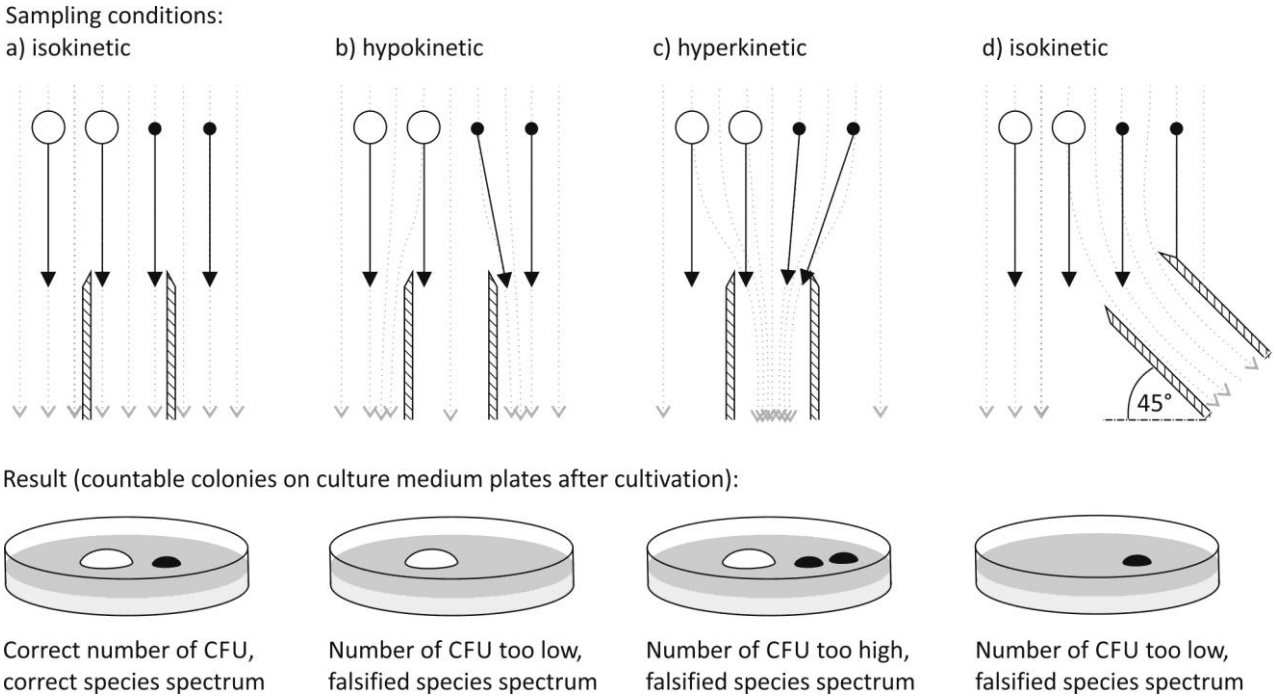


Fig. 4: Theoretical influence of sampling conditions on the result in the collection of airborne microorganisms by suction of a partial flow from the exhaust gas flow (from Clauß & Hinz 2014)

4.2 Evaluation

After the bioaerosols have been collected in a sample, they are evaluated usually via cultivation and/or various different biochemical and molecular biological methods.

4.2.1 Cultivation

Cultivation on culture media and counting of the colonies that have grown remains the gold standard for quantification of airborne microorganisms.

The gold standard for quantification of airborne microorganisms remains their cultivation on culture media and counting of the colonies that grow. However, detection via cultivation means that only organisms capable of reproduction can be determined and, in addition, only the proportion of microorganisms that are capable of growth under the selected conditions. On top of this, under unfavourable environmental conditions, many bacteria enter into a so-called VBNC (viable but not culturable) state (Bogosian et al. 2001, James 2010, Oliver 2005). Here, the metabolic activity is reduced to a minimum and the bacteria may no longer be cultivable and thus detectable using standard methods. There are also obligatory intracellular bacteria, e.g. chlamydiae or coxiellae, which cannot be grown on culture media as a result of the way they live. Therefore, any type of cultivation is selective to a certain extent. Even the culture media referred to as “non-selective”, which, for example, are used to determine total bacteria, always only reflect a part of the bacterial spectrum originally present in the bioaerosol. On the other hand, on culture media for the selective detection of specific bacterial groups, microorganisms of the selected target group do not grow exclusively. Additionally, most of the commercially available culture media have been developed and tested for human-medical purposes. In hospitals, the pharmaceutical and food industry, they are used for the culture-based detection of a limited number of mostly disease-related pathogens that generally do not occur with any notable accompanying flora. It is therefore often insufficiently clear whether these culture media can also be used without restrictions for environmental samples, where the inhibition of the accompanying flora is much more important due to the large number of microorganisms present. The metabolic properties of bacteria vary greatly, being adapted to the way they live. Many selective culture media are therefore based on the detection of specific metabolic properties, e.g. whether a certain sugar can be utilised or not. Others select via resistances, e.g. to antibiotics. Many bacteria have short generation times, above all under optimal growth conditions (*Escherichia coli,* e.g. 20 minutes). As a result of the selective pressure and natural mutations that prevail in the environment, variations of metabolic properties can come about very rapidly within a species and even within a single strain. Metabolic properties or resistances can also be passed on across species by means of plasmids, e.g. antibiotic resistance genes. This can additionally limit the selectivity of some culture media. There is thus a not insignificant number of

publications that report very questionable results, probably as a result of incorrectly evaluated selective culture media. This is why a standardisation of cultivation conditions for the detection of bioaerosols is particularly important. A major step in this direction has been taken with the appearance of the VDI 4253 Sheet 3. There, cultivation and detection methods that have proved effective for the identification and quantification of bioaerosols are stated for many of the microorganisms presented here in section 3. As a whole, cultivation-dependent methods can give a good overview of the occurrence of bacterial groups, but a much more detailed insight is provided by cultivation-independent, molecular biological methods (Fallschissel 2011).

4.2.2 Molecular biological methods

Molecular biological methods such as PCR, particularly in combination with the classical culture-based procedures, make it possible to gain a more detailed insight into the composition of bioaerosols, whereby a further standardisation of procedures is necessary here.

Molecular biological methods are being applied increasingly frequently in the analysis of airborne microorganisms, including those from agricultural livestock farming (Olsen et al. 2009). Beside the identification of organisms that cannot be detected by cultivation-dependent methods, a quantification of these organisms is also of interest (Fallschissel 2011). The fundamental method of nucleic acid-based analytical systems is PCR (polymerase chain reaction). Two basic procedures have to be differentiated here: qualitative and quantitative PCR.

Qualitative PCR is used to test samples for the presence of certain microorganisms. This procedure specifically detects certain species-specific DNA segments with a known target sequence. To begin with, microorganism cells from a sample are lysed. Then, the DNA is extracted and purified. In the PCR, the double-stranded DNA is then denatured and the target sequence is selectively enzymatically amplified by applying taxon-specific primers. The specific PCR product is detected and thus confirms the presence of the respective microorganisms in the sample. Most commonly used for this purpose are group-specific sequence differences on the bacterial 16S rRNA gene, as, beside highly conserved areas, it has very variable areas, whose sequence varies for each genus or even species. Quantitative PCR methods (qPCR) such as real-time PCR, also exploit the procedure described above for amplifying specific DNA sequences. However, additionally, the specific PCR products (amplicons) are already detected during the amplification process. This takes place during the procedure either through unspecific binding of fluorescent stains to double-stranded DNA or with the aid of specific DNA probes that are coupled with fluorescent stains. After fluorescence marking, the signal can be detected by stimulation of certain wavelengths. The fluorescence signal increases proportionally to the product amount. With the aid of a standard curve with known DNA concentrations, it is then possible to conclude the initial concentration of the sequence and thus approximately the

number of respective microorganisms in the sample. A problematic aspect of this procedure is that the number of detectable genes per cell can vary (Farrelly 1995) and also fluctuate (Bremer & Dennis 1987), depending on the species. In addition, the DNA of dead cells is also detected. Therefore, the results of this method mostly yield higher concentrations than those achieved with cultivation methods. However, a great advantage is that molecular biological quantification and identification methods enable a considerably more detailed insight into the composition of bioaerosols. Thus, a much higher species diversity was found in many areas with PCR methods than with the classical culture-based methods (Brodie et al. 2007, Despres et al. 2007). PCR methods are constantly yielding new findings. Thus, Martin et al. (2010a, b), found that up to 39% of all bacterial cells in poultry farming belong to the genus *Jeotgalicoccus*, a bacterium that was isolated for the first time just a few years ago from Korean seafood (Yoon et al. 2003). In pig stalls, Nehme et al. (2009) found high concentrations of archaea, an archetypal group of microorganisms, which had not been detected in animal stalls by cultivation up to that point. A further advantage of PCR is that it can be used to classify microorganisms, e.g. in the immission of a stall, directly to a source. Thus, for example, Duan et al. (2009) found that a high percentage of the *E. coli* found in the immission of a pig stall originated from the faeces of the animals.

The development of new PCR methods is advancing constantly, and in combination with other procedures like HPLC (high-performance liquid chromatography) (Nieguitsila et al. 2010) or MALDI-TOF (time-of-flight mass spectrometry) (Szponar & Larsson 2001, Druckenmüller et al. 2017), they are also being used for the detection of bioaerosols in animal stalls. What is missing at present are standardised methods. Once they are available, the detailed description of species spectra using sequence-based procedures can be a helpful complement to the cultivation-dependent measurement of indicator organisms in bioaerosol analysis of the future (Schneider et al. 2015).

4.2.3 Detection of endotoxins

The test most commonly used for detecting endotoxins is the LAL test, although it is relatively prone to interference and has yet to be uniformly standardised.

A comprehensive review of the measurement of endotoxins in bioaerosol samples is provided by Duquenne et al. (2013). The standard test for the measurement of airborne bacterial endotoxins is the limulus amoebocyte lysate test (LAL test). It is based on the observation of Levin & Bang (1964) that very small amounts of bacterial endotoxins elicit a coagulation of the haemolymph of horseshoe crabs (genus: *Limulus*). The coagulation cascade is set in motion by the so-called factor C, which is activated by the endotoxin. There are several modifications of the test. One disadvantage of the LAL test, however, is its susceptibility to numerous substances that act as interference factors and are regularly to be found in bioaerosol samples, first and foremost the ß-

glucans, e.g. from the cell wall of fungi. A further problem is posed by the varying sensitivities of different LAL tests from different suppliers or even batches.

Over the past few years, a recombinant rFC test has been developed. Here, DNA with the factor-C gene of the horseshoe crab is introduced via a virus into insect cells, which then express an activatable rFC product. This test appears to be much less susceptible to interference, while yielding results comparable to the LAL test, and will probably become established as the standard method in the future (Alwis & Milton 2006, Lohmeyer et al. 2017, Thorne et al. 2010, Uribe 2007), also because horseshoe crabs are in the meantime in danger of extinction and would no longer be needed as a source of whole blood.

The molecular structure of the endotoxins or also the lipopolysaccharides of different species and strains of Gram-negative bacteria vary. Accordingly, the strength of coagulation induced by comparable amounts of endotoxin can vary. Therefore, the results cannot be attributed to substance concentrations, but to activities of the endotoxins in the sample. These are then compared with a chemically purified standard endotoxin (usually from *E. coli*). Therefore, in order to describe the activity of endotoxins as pyrogenic in the body or in a test system, an activity is attributed to a certain amount, expressed in endotoxin units (EU) and defined via an international standard. Normally, 1 ng of standard endotoxin corresponds to around 10 endotoxin units (EU).

There are already numerous standards available for the measurement and the detection of endotoxins, e.g. the international standard EN 14031 (2003), in Canada “Method 332, IRSST” (IRSST 2009), in France “MetroPol method 089/V2, INRS” (INRS 2010), or in Germany “Method 9450, BGIA” (BGIA 2002). The latter operating procedure has also become established for the detection of endotoxins from animal stalls (e.g. Anonymous 2013a). Currently, the VDI standard 4254 Sheet 2 is being drafted, in which a further standard for the measurement of endotoxins in bioaerosol samples is to be established for Germany. A harmonisation with the existing standards is considered to be expedient.

5 Results of bioaerosol measurements

Most of the data are available from the USA and Germany, with the majority of investigations having been performed in the stalls of pigs, cattle and chickens.

For the following presentations of the results of bioaerosol measurements in and from agricultural livestock farming, 601 datasets from a total of 313 publications from 34 countries in the period from 1960 to today were evaluated (Fig. 5). This compilation makes no claims to completeness. Results from publications before 1960 are difficult to compare with the more recent values, as almost exclusively the sedimentation procedure was used for the sampling of airborne microorganisms back then, which does not allow precise quantification. In addition, the housing procedures for the animals and the stock densities have changed over the course of time.

	1960	1961	1962	1963	1964	1965	1966	1967	1968	1969	1970	1971	1972	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
Algeria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Australia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	1	1	1	0	0	0	0	0	0	0	0
Austria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Brazil	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Bulgaria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Canada	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2	1	2	2	0	1	0	0
China	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	4	1	2	2	0	1	1	0	1	1
Croatia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	3	2	1	3	0	0	0	1	0	0	0
Czech Republic	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Denmark	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0	1	0	0
Egypt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
Finland	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
France	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0
Germany	0	0	0	0	0	0	0	1	0	2	2	3	0	1	2	1	3	1	0	0	0	1	0	2	2	0	1	0	0	0	1	0	1	1	1	1	0	1	4	1	2	0	3	1	2	7	3	4	2	2	2	6	2	3	4	6	4	2
Great Britain	0	0	0	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	3	0	2	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Hungary	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
India	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	1	0	1
Iran	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0
Ireland	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0
Italy	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
Korea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	1	1	1	1	0	0	0	1	0	2	0
Latvia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lithuania	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Mexico	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Netherlands	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	3	2	0	0	1	2	0
Norway	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Poland	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	2	0	0	1	1	0	3	1	4	3	1	0	0	0
Romania	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0
Russia	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
South Africa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Spain	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0
Sweden	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Switzerland	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0	0	1
Taiwan	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	1	1	0	0	1	0	0	0	0	0
USA	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	2	1	0	1	0	0	2	0	1	2	0	0	2	0	0	0	0	1	0	0	2	0	1	1	0	0	1	1	0	1	1	2	0	1	4	5	3	3	2	2	2	4	2

Fig. 5: Number of evaluated publications that contain results of bioaerosol measurements in agricultural livestock farming, arranged according to country and year of publication.

A further 25 publications that were believed to contain measurement results on bioaerosols from livestock farming, particularly from the Middle East and the former Soviet Union, could no longer be obtained, as the journals concerned were no longer available. Most of the publications were identified in Germany and the USA, whereby articles from these countries are relatively easy to obtain and the script does not represent a barrier for an online search, in contrast to, e.g. Asian or Cyrillic characters. Therefore, even more investigations probably exist in the other countries.

As a result of the large number of different test conditions, a weighting of the results, e.g. according to the amount of air volume sampled, the number of samples taken as a basis (n), the number of evaluated culture medium plates, the number of investigated animal stalls or the duration of the investigations, was not practicable. Therefore, for the following presentations, all published values on bioaerosol concentrations are given the same weighting. It must further be taken into account that some authors published geometric means, others arithmetic means. If solely the minimum and maximum concentrations were stated, these were used to form the arithmetic mean. These, too, were given equal weight in the following presentations. All endotoxin concentrations were presented in EU. If only available in ng, they were multiplied by a factor of 10, regardless of the standard endotoxin used (if stated at all), and thus converted into EU.

Worldwide, most investigations were performed on chickens (39%), pigs (33%) and cattle (18%) (Fig. 6).

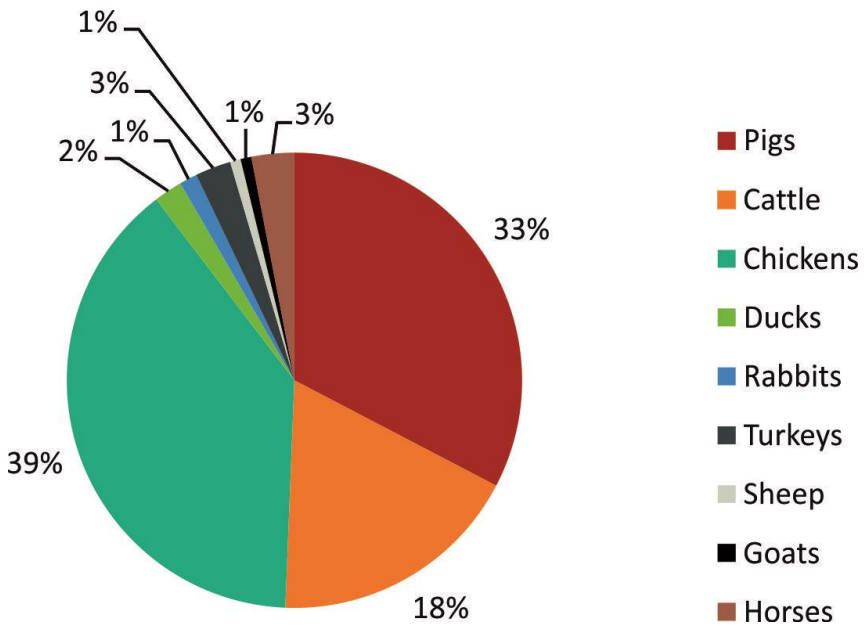


Fig. 6: Distribution of the bioaerosol investigations performed throughout the world according to the different animal species

The remaining 10% are distributed over ducks, rabbits, turkeys, sheep, goats and horses. The percentage of investigations in turkey farming is remarkably low, at just 2.5%. And yet, as a proportion of the total production of poultry meat, e.g. in Germany, turkey farming has a share of almost 50% currently (Deutscher Bauernverband 2016). Within the animal species pigs, cattle and chickens, in relation to the production stages in pigs, almost three quarters of the investigations were conducted in pig fattening (Tab. 4). The least data are available for sow farming. In the case of cattle, dairy cattle have been investigated most, at slightly over 50%, followed by beef cattle and calf farming. Mother cows have been investigated least here. In the case of chickens, 58% of the investigations were performed on broilers, 34% on laying hens and the remaining 8% on parent birds, hatcheries and rearing of young hens. Here, it must generally be borne in mind that the transitions between most production stages are fluid and are not regulated uniformly internationally. The details of the production stages in which the investigations were conducted have been taken from the publications.

Tab. 4: Number of publications on bioaerosol measurements in different production stages of pig, cattle, and chicken farming

Animal species	Production stage	No. of publications	Percentage (%)
Pigs	Mother sows	7	5
	Weaning piglets	9	7
	Piglet rearing	18	14
	Fattening pigs	95	74
	Pigs total	**129**	100
Cattle	Dairy cows	37	52
	Mother cows	1	1
	Beef cattle	20	28
	Calves	13	18
	Cattle total	**71**	100
Chickens	Parent birds	1	1
	Hatchery	4	3
	Young hen rearing	6	4
	Broilers	90	58
	Laying hens	53	34
	Chickens total	**154**	100

In relation to the site of measurement, most of the measurements were performed in the animal stalls, at 78%, and they were primarily conducted with a specialist background, above all work

protection but also animal welfare (Fig. 7). Immission measurements were conducted in 16% of the cases. The goal here was mostly the determination of the distance of bioaerosol spread from animal stalls. Only 6% of the samplings conducted at animal stalls were emission measurements, primarily with the aim of determining emission factors for bioaerosols, which can be used above all as entry data for immission prognoses.

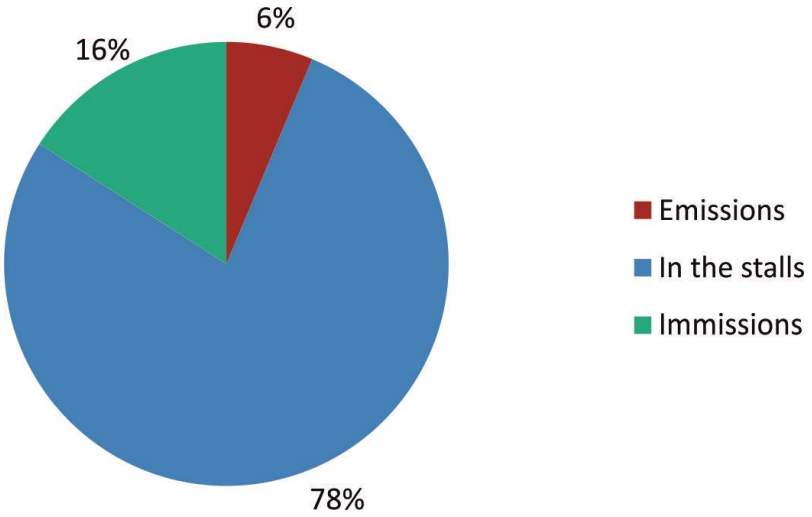


Fig. 7: Shares of emission and immission measurements as well as measurements in the stalls as a percentage of bioaerosol measurements performed throughout the world in agricultural livestock farming

5.1 Measurements in the stalls

With a relatively large range of fluctuation for all animal species, the highest concentrations of airborne bacteria were found in the stalls of chickens, followed by turkeys, ducks, sheep, goats, pigs, cattle, horses and rabbits, whereby the various housing procedures and production stages have a marked influence.

In agricultural livestock farming, most bioaerosol measurements were performed in the animal stalls. The air quality there is an important factor both for work protection and for animal welfare. From the worldwide literature, for each animal species regardless of the housing form and the collection system, the mean concentrations of total bacteria and total fungi were determined via cultivation, as well as the concentrations of endotoxins (Figure 8).

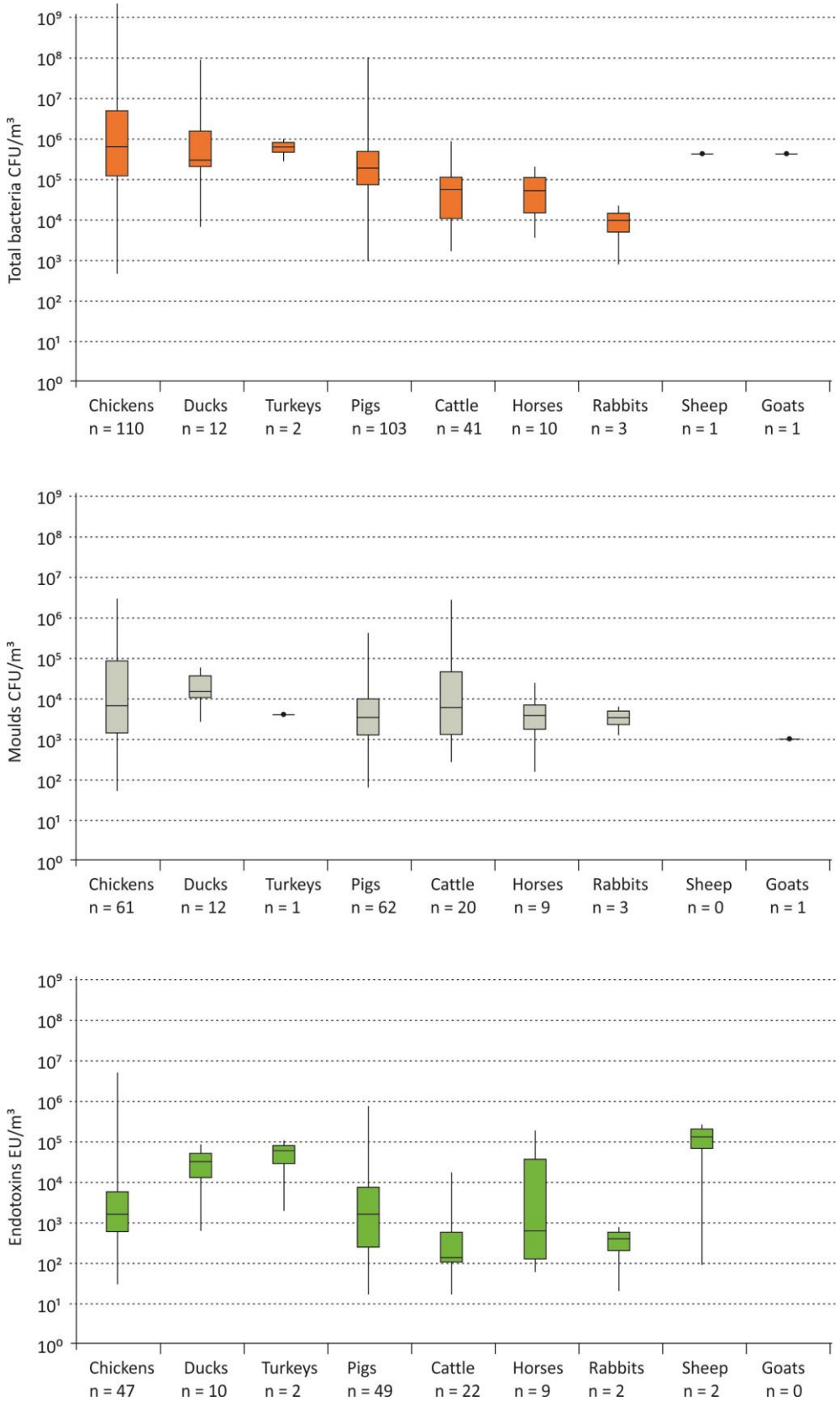


Fig.: 8 Box-and-whisker plots of the mean concentrations of total bacteria and total fungi determined via cultivation, as well as the concentrations of endotoxins, for each animal species regardless of the housing form and the collection system, from the worldwide literature, and the number of datasets incorporated.

In the case of total bacteria, the highest mean concentrations were found in chickens, with a median of 7×10^5 CFU/m³. The most datasets were also available on investigations in chicken farming, at n = 110. The range of fluctuation is relatively high here, probably as a result of the different housing forms. In the case of ducks, the median was 3×10^5 CFU/m³ from 12 datasets from 7 publications (Crook et al. 2008, Martin et al. 2010a, Seedorf et al. 1998b, Yu et al. 2016a, b, Martin et al. 2015, Martin & Jäckel 2011). The keeping of ducks has only been investigated more intensively over the past few years. There, even new bacterial species such as *Leucobacter aerolatus* sp. nov have been described (Martin et al. 2010c). In addition, it was determined that 4 – 18% of the bacterial genes found in duck farming can be classified to the genus *Jeotgalicoccus*, a representative of the *Staphylococcacea* (Martin et al. 2010b) only found a few years ago. *Jeotgalicoccus* spp. appear to be widespread in poultry farming as a whole (Martin et al. 2010b). Fallschissel et al. (2009) used molecular biological methods to investigate the occurrence of *Salmonella* spp. in duck stalls and found between 2.5×10^1 genes/m³ and 3×10^6 genes/m³. Martin et al. (2015) determined total cell counts of 5×10^7 cells/m³ and 2×10^7 cells/m³ for duck hatcheries and duck fattening, respectively, much higher than those detected via culture methods. Schäfer et al. (2011) found the health-relevant bacterium *Saccharopolyspora* spp. at concentrations of 2.7×10^5 cells/m³ using PCR. The turkeys, with a mean concentration of 6×10^5 CFU/m³ found in the stalls, are between the chickens and ducks, although only 2 datasets were available here (Fallschissel 2010, 2011). Further studies were conducted in turkey farming, in which specific microorganism groups were investigated. Fulleringer et al. (2006) investigated turkey stalls in France for the toxin-forming moulds *Aspergillus fumigatus* and *Aspergillus flavus* and found concentrations of 10 CFU/m³ and 37 CFU/m³ there. In the USA, Mulhausen et al. (1987) also found 73 CFU/m³ *Aspergillus* spp. in the air of turkey stalls. In a turkey stall in the Netherlands, Jonges et al. (2015) investigated the occurrence of avian influenza A viruses and found 8.5×10^4 genome copies/m³ air. In Germany, Brauner et al. (2016) investigated airborne enterococci in a turkey hatchery and found 1×10^5 CFU/m³ there. In pig farming, a mean concentration of 2×10^5 CFU/m³ total bacteria in the air was calculated from n = 103 datasets. The concentrations are thus markedly lower than those in poultry farming. In cattle, the mean concentration from n = 41 datasets was again markedly lower, at 5×10^4 CFU/m³ air. A similar mean concentration of 4×10^4 CFU/m³ was determined in horse farming, on the basis of n = 10 datasets from 5 publications (Eckhardt 2008, Fritz 2017, Samadi et al. 2009, Zeitler 1986, Dutkiewicz et al. 1994). Rabbit housing was investigated above all in China and India, a median of 1×10^4 CFU/m³ being determined from n = 3 datasets (Yao et al. 2007a, b, Duan et al. 2006). For airborne total bacteria in housing for sheep, just one value was found from Germany: 4×10^5 CFU/m³ (Eckhardt 2008). In addition, air samples from sheep stalls were found to be positive for salmonellae in Ireland (Okraszewska-Lasica et al. 2014). For goat housing, data of our own that have not yet been published were taken as a basis, yielding a mean concentration of 4×10^5 CFU/m³ total bacteria from n = 24 daytime and nighttime measurements with an automatic bioaerosol collector in the air of a goat stall with 2000 animals.

When considered as a whole, the highest concentrations of total bacteria in the air in stalls were found in chickens, followed by turkeys, ducks, sheep, goats, pigs, cattle, horses and rabbits.

The concentrations of airborne moulds are markedly lower than those of the bacteria. In chicken farming, a mean concentration of 6×10^3 CFU/m³ was calculated from n = 61 datasets, whereby the range of fluctuation was again very high here. In the ducks, the concentration was 1.5×10^3 CFU/m^3 (Crook et al. 2008, Yu et al. 2016a, b). In the turkeys, a value for moulds, of 6.3×10^3 CFU/m³, was only found in Debey et al. (1995) in the USA . In the pigs, n = 62 datasets produced a mean concentration of 3×10^3 CFU/m^3, in the cattle n = 20 datasets yielded 7×10^3 CFU/m^3. In horses, the figure is slightly lower at 4×10^3 CFU/m^3 (Fritz 2017, Nardoni et al. 2005, Samadi et al. 2009, Dutkiewicz et al. 1994). At the same level were the concentrations of moulds in rabbits in South-East Asia (Miao et al. 2010, Wang et al. 2007, Pavan 2015). No published mould concentrations were found for sheep. For goat housing, our own as yet unpublished data were again taken as a basis here, yielding a mean concentration of 1×10^3 CFU/m³ moulds. Considered generally, differences in the mean concentrations of bacteria in the stalls of up to two powers of ten were produced, whereas the differences in the moulds between the animal species are lower and in the range of only approx. one power of ten.

For endotoxins, a mean concentration of 2×10^3 EU/m³ was determined in the air of chicken stalls from n = 47 datasets. In the case of ducks it was a power of ten higher, at 3×10^4 EU/m³ (Crook et al. 2008, Seedorf et al. 1998b, Yu et al. 2016). In the turkeys it was a little higher, at 5×10^4 EU/m³, whereby only two datasets were available here and the values differed strongly in the two publications, at 1×10^6 EU/m³ and 2×10^3 EU/m³, respectively (Jonges et al. 2015, Schirl et al. 2007). In the pigs, a mean concentration of 2×10^3 EU/m³ was calculated in the stall air from n = 49 datasets, the same as in chicken farming, but with a slightly greater range of fluctuation. The concentration of endotoxins in cattle farming from n = 22 datasets was comparatively low, at 1×10^2 EU/m³. Endotoxins in the air of horse stalls were found at a mean concentration (n = 9) of 6×10^2 EU/m³, with a relatively high range of fluctuation (McGorum et al. 1998, Pomorska et al. 2007, Samadi et al. 2009, Dutkiewicz et al. 1994). Endotoxins in rabbits were 4×10^2 EU/m³ (Duan et al. 2006) and in sheep stalls in Germany and Poland 1×10^5 EU/m³. No values were found for goats. Considered generally, the endotoxins are found to have the greatest fluctuations. The concentrations found do not reflect the concentrations of total airborne bacteria. The consideration of the individual animal species above was independent of the collection system employed. In order to reveal any possible influence of sampling on the results, as a result of the amount of available data, the results for total bacteria and moulds for the animal species chickens, pigs and cattle were presented graphically as box-and-whisker plots, dependent on the collection system (Fig. 9).

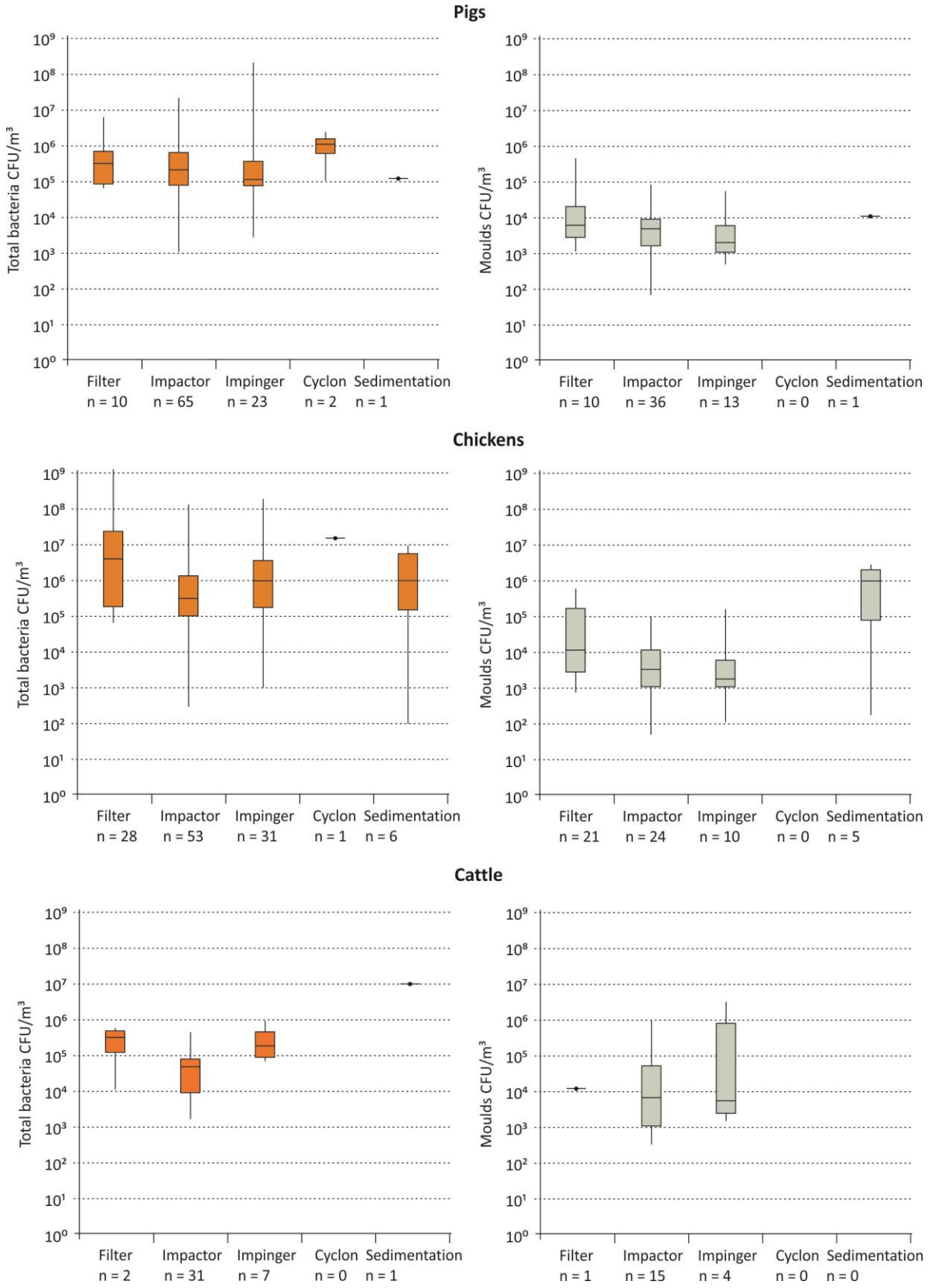


Fig. 9: Box-and-whisker plots of the mean concentrations of total bacteria and total moulds from the literature, determined via cultivation, independent of the housing form and dependent on the collection method, for pigs, chickens and cattle, and the number of available datasets.

The endotoxins are not included here, as, with few exceptions (e.g. Duchaine et al. 2001), they were always collected on filters. The highest concentrations were found with cyclones in pigs and chickens. This suggests a high biological and physical collection efficiency of the systems. As a result of the small number of investigations (Bonifeit et al. 2014, Hartmann et al. 1986, Ahmed et al. 2013), however, further measurements are needed here. Cyclones were also used by Nieguitsila et al. (2011) for the collection of Aspergillus (30 CFU/m³) in broiler stalls in France and by Ahmed et al. (2013) for the detection of *Campylobacter* in broiler stalls. However, the latter could not be detected airborne via cultivation but only via PCR. Bonifeit et al. (2014) found *Streptococcus suis* in concentrations of 4×10^5 CFU/m³ to 1×10^6 CFU/m³ using cyclone separators in fattening pigs in Canada, Otake et al. (2010) PRRSV and *Mycoplasma hyopneumoniae* in the air of pig fattening stalls in the USA. O'Brien & Nonnemann (2016) used this system to collect swine influenza viruses. In the total bacteria and the moulds, in all of the animal species considered, the second highest mean concentrations were found with the filtration method. This is probably due to the high physical collection efficiency of the filters. The sedimentation method was only used in a few cases, yielding higher calculated values, but a precise quantification is barely possible for methodological reasons. Impaction was the most commonly used method, but lower concentrations overall were determined than with cyclone separators and filters. Measurements with impingers yielded the lowest concentrations, whereby the mean value in chickens and cattle for total bacteria was higher than in the case of impaction. However, comparison of the values proves difficult, also due to the great differences in the baseline data. At any rate, it appears that impingement is less well suited for the collection of moulds, compared with the other methods. The hydrophobic fungal spores probably cannot be sufficiently well separated in a liquid (Grinshpun et al. 1997). All in all, cyclone collectors appear to be very well suited for the collection of bioaerosols, although they have yet to be sufficiently evaluated.

As a result of the large amount of available data, the various production stages in pigs, chickens and cattle will be considered in more detail below. In the pigs, the lowest mean concentrations of total bacteria (approx. 1×10^5 CFU/m³) were determined in the air of sow housing (Fig. 10). Here, the lowest concentrations of moulds were also found, at 8×10^2 CFU/m³ (Fig. 11) and endotoxins, at 8×10^1 EU/m³ (Fig. 12). In the weaning piglets, the concentrations of moulds and endotoxins are also very low, at 7×10^2 CFU/m³ and 2×10^2 EU/m³, respectively. The mean total bacteria concentration was relatively high, at 3×10^5 CFU/m³. In piglet rearing and in the fattening pigs, this figure is 2×10^5 CFU/m³ in each case. In the case of the moulds, the concentrations for piglet rearing are relatively similar, at 4×10^3 CFU/m³ for piglet rearing and 3×10^3 CFU/m³ for the fattening pigs. In the case of the endotoxins, the mean concentration is markedly higher in piglet rearing and, at 1×10^4 Eu/m³, has a relatively high range of fluctuation. In the fattening pigs the corresponding figure is 3×10^3 Eu/m³.

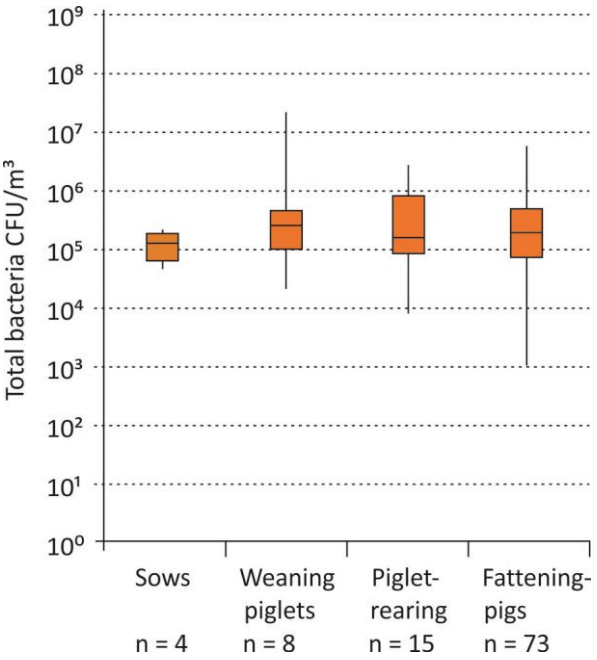


Fig. 10: Box-and-whisker plots of the mean concentrations of total bacteria from the literature, determined via cultivation for various production stages in pigs, and the number of datasets incorporated. References: sows (Spirin & Mikhaĭlova 1991, Banhazi et al. 2008a, Chang et al. 2001b, Sächsisches Landesamt 2002), weaning piglets (Witek 1974, Müller et al. 1976, Fiser 1978, Banhazi et al. 2008a, Chang et al. 2001b, Chang et al. 2001a, Fiser 1978, Spirin & Mikhaĭlova 1991), piglet rearing (Attwood et al. 1987, Banhazi et al. 2002, Chinivasagam & Blackall 2005, Cormier et al. 1990, Hađina et al. 2009, Fiser 1978, Kim et al. 2008, Pavicic et al. 2007, Spirin & Mikhaĭlova 1991, Yao et al. 2010, Dutkiewicz et al. 1994, Banhazi et al. 2008a), fattening pigs (Banhazi et al. 2008a, Agranovski et al. 2004, Attwood et al. 1987, Bakutis et al. 2004, Bonifeit et al. 2014, Butera et al. 1991, Chang et al. 2001b, Clark et al. 1983, Clauß unpublished, Coggins et al. 2007, Cormier et al. 1990, Curtis et al. 1975a, Donham et al. 1989, Dutkiewicz et al. 1994, Eckhardt 2008, Eliot et al. 1976, Ferguson 2012, Geburek et al. 2005, Gordon 1963, Green et al. 2006, Gutzmirtl et al. 2004, Hartmann et al. 1986, Heederik et al. 1991, Hill & Kenworthy 1970, Jo & Kang 2005, Fiser 1978, Müller et al. 1976, Karowska 2005, Kim et al. 2005, Kim et al. 2006, Kim et al. 2007, Ko et al. 2008, Ko et al. 2010, Letourneau et al. 2009, Liu & Ma 2010, Pavicic et al. 2006, Platz et al. 1995, Hojovec et al. 1976, Zucker et al. 2005, Predicala et al. 2001, Predicala et al. 2002, Radon et al. 2002, Rautila et al. 2003, Schulz et al. 2013, Siggers et al. 2011, Sowiak et al. 2011, Szadkowska-Stańczyk et al. 2010, Thorne et al. 1992, Letourneau et al. 2009, Chiba et al. 1987, Zucker et al. 2000)

Representatives of many different microorganism groups were found in the air of pig housing, e.g. Gram-negative bacteria (e.g. Rautila et al. 2003, Heederik et al. 1991, Popescu et al. 2010, Dutkiewicz et al. 1994, Cormier et al. 1990), staphylococci (e.g. Spirin & Mikhaĭlova 1991, Hojovec et al. 1976, Fiser 1978, Spirin & Mikhaĭlova 1991, Butera et al. 1991, Eliot et al. 1976, Geburek et al. 2005), MRSA (e.g. Schulz et al. 2012, 2013, Ferguson 2012), enterococci (Geburek et al. 2005), haemolytic cocci (Spirin & Mikhaĭlova 1991), streptococci (Popescu et al. 2010), Enterobacteriaceae (Spirin & Mikhaĭlova 1991, Fiser 1978), coliform bacteria (Green et al. 2006, Yao et al. 2010, Pavicic et al. 2006, 2007), *E. coli* (Yao et al. 2010, Yuan et al. 2010, Eliot et al. 1976, Coggins et al. 2007), salmonellae (Okraszewska-Lasica et al. 2014, Elliot et al. 1976), Clostridium perfringens

(Zucker et al. 2005), thermophilic actinomycetes (Dutkiewicz et al. 1994, Letourneau et al. 2009), thermophilic fungi (Rautila et al. 2003, Letourneau et al. 2009), or moulds of the genera *Aspergillus, Cladosporium* and *Penicillium* (Jo & Kang 2005, Cormier 1990). In addition, Keessen et al. (2011) detected 6.3 × 10^2 CFU/m³ *Clostridium difficile* in air samples from piglet rearing in the Netherlands. Nehme et al. (2009) found large amounts of archaea in fattening pigs in Canada via PCR. Otake et al. (2010) found PRRSV and *Mycoplasma hyopneumoniae* and O'Brien & Nonnemann (2016) swine influenza viruses in the air of pig fattening stalls.

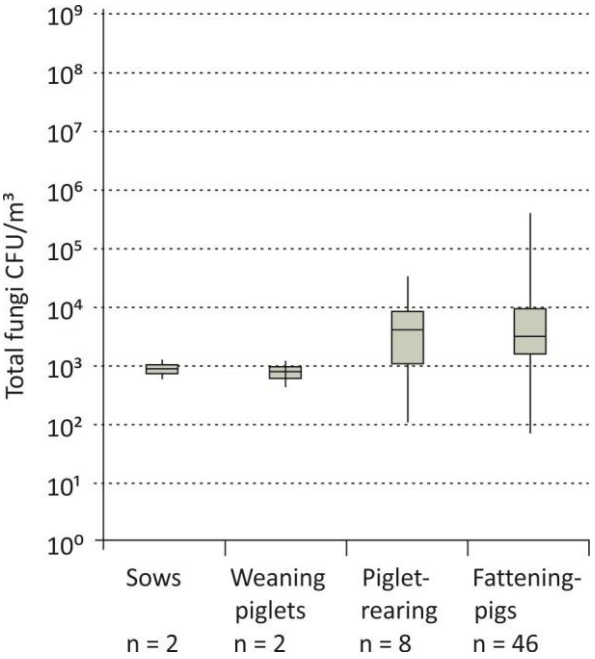


Fig. 11: Box-and-whisker plots of the mean concentrations of total fungi from the literature, determined via cultivation for various production stages in pigs, and the number of datasets incorporated. References: sows (Spirin & Mikhaïlova 1991, Sächsisches Landesamt 2002), weaning piglets (Chang et al. 2001b, Spirin & Mikhaïlova 1991), piglet rearing (Cormier et al. 1990, Kim et al. 2008, Pavicic et al. 2007, Spirin & Mikhaïlova 1991, Dutkiewicz et al. 1994), fattening pigs (Agranovski et al. 2004, Butera et al. 1991, Clark et al. 1983, Clauß unpublished, Coggins et al. 2007, Cormier et al. 1990, Diefenbach et al. 2007, Donham et al. 1989, Dutkiewicz et al. 1994, Geburek et al. 2005, Jo & Kang 2005, Kim et al. 2006, Kim et al. 2007, Ko et al. 2008, Ko et al. 2010, Lee & Liao 2014, Letourneau et al. 2009, Liu & Ma 2010, Pavan & Manjunath 2013, Pavan 2015, Pavicic et al. 2006, Radon et al. 2002, Rautila et al. 2003, Schulz et al. 2013, Siggers et al. 2011, Sowiak et al. 2011, Szadkowska-Stańczyk et al. 2010, Thorne et al. 1992, Wang et al. 2007, Letourneau et al. 2009, Masclaux et al. 2013, Zucker et al. 2000).

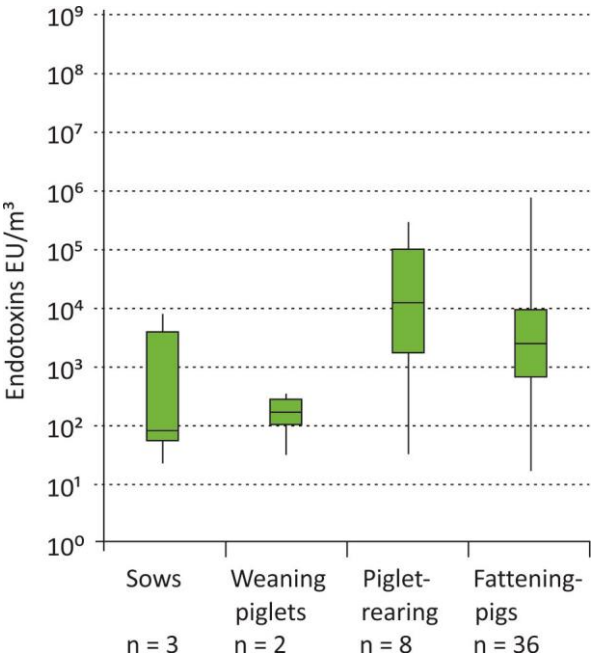


Fig. 12: Box-and-whisker plots of the mean concentrations of endotoxins from the literature, determined for various production stages in pigs, and the number of datasets incorporated. References: sow farming (Seedorf et al. 1998a, Banhazi et al. 2008a, Chang et al. 2001a), weaning piglets (Banhazi et al. 2008a, Chang et al. 2001a), piglet rearing (Attwood et al. 1987, Dutkiewicz et al. 1994), fattening pigs (Banhazi et al. 2008a, Attwood et al. 1987, Attwood et al. 1986, Bakutis et al. 2004, Butera et al. 1991, Chang et al. 2001a, Clark et al. 1983, Coggins et al. 2007, Diefenbach et al. 2007, Donham et al. 1989, Dutkiewicz et al. 1994, Geburek et al. 2005, Heederik et al. 1991, Ogink et al. 2016, Ko et al. 2010, Letourneau et al. 2009, Zucker et al. 2005, Pomorska et al. 2007, Radon et al. 2002, Roque et al. 2016, Schirl et al. 2007, Siggers et al. 2011, Szadkowska-Stańczyk et al. 2010, Thorne et al. 2009, Letourneau et al. 2009, Seedorf et al. 1998a, Masclaux et al. 2013).

A wealth of data can be found on the concentration of bioaerosols in the stalls of chickens for laying hens and broilers, but little data for the keeping of parent birds, for hatcheries and for chick rearing. Just one investigation from China was found for parent birds (Hao et al. 2014). Here, the concentration of total bacteria was 2×10^5 CFU/m³ (Fig. 13) and that of moulds 8×10^3 CFU/m³ (Fig. 14). Endotoxins were not investigated. The concentration of total bacteria in the air of hatcheries was investigated in Poland (Brodka et al. 2012, Chmielowiec-Korzeniowska et al. 2007, Dutkiewicz 1978). These were also 2×10^5 CFU/m³. The endotoxin concentrations in the air were very high, at 5×10^6 EU/m³ (Chmielowiec-Korzeniowska et al. 2007). In chick rearing, the concentrations of total airborne bacteria were very low, at a mean of 7×10^2 CFU/m³. Solely the value of 1×10^3 CFU/m³ was found for moulds (Sowiak et al. 2012). The mean concentrations of total bacteria and endotoxins are slightly higher in laying hens, at 1×10^6 CFU/m³ and 3×10^3 EU/m³, than in broilers, at 6×10^5 CFU/m³ and 1×10^3 EU/m³, respectively. In contrast, the mean mould concentrations are slightly higher in the broilers, at 1×10^4 CFU/m³, compared with the laying hens, at 3×10^3 EU/m³.

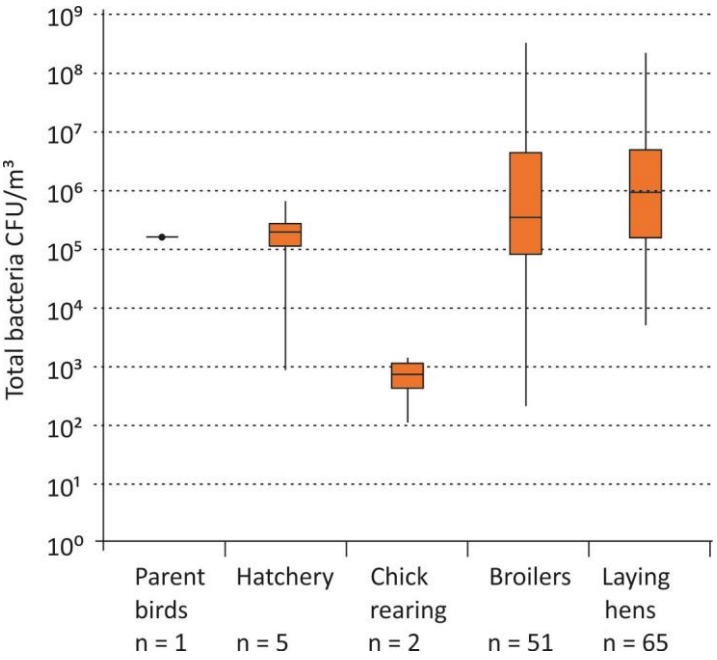


Fig. 13: Box-and-whisker plots of the mean concentrations of total bacteria from the literature, determined via cultivation for various production stages in chickens, and the number of datasets incorporated. References: parent birds (Hao et al. 2014), hatchery (Brodka et al. 2012, Chmielowiec-Korzeniowska et al. 2007, Dutkiewicz 1978), chick rearing (Agabou 2009, Sowiak et al. 2012, Berrang et al. 1995, Erman et al. 1989, Gentry et al. 1962), broilers (Fallschissel 2011, Popescu et al. 2010, Adell et al. 2011a, b, Agranovski et al. 2007, Awad et al. 2010, Baykov & Stoyanov 1999, Brodka et al. 2012, Brooks et al. 2010, Chai et al. 2001a, Chi & Li 2006, Crook et al. 2008, Dutkiewicz 1978, Hahne 2014, Hinz et al. 1994, Jones et al. 1984, Karowska 2005, Kostandinova et al. 2014, Lawniczek-Walczyk et al. 2013, Madelin & Wathes 1989, Mituniewicz et al. 2008, Nielsen & Breum 1995, Madelin & Wathes 1989, Nichita et al. 2010, Petkov & Tsutsumanski 1975a, Radon et al. 2002, Saleh et al. 2005, Schulz et al. 2004, Vucemilo et al. 2006, Vucemilo et al. 2007, Witkowska & Sowińska 2013, Witkowska et al. 2010, Wojcik et al. 2010, Saleh 2006, Jo & Kang 2005, Bakutis et al. 2004, Clark et al. 1983, Singh & Singh 1996), laying hens (Ahmed et al. 2013, Angersbach-Hegers 2002, Anonymous 2012, Baïkov & Petkov 1986, Bloomberg et al. 2009, Brodka et al. 2012, Chai et al. 2003, Clauß 2015, Crook et al. 2008, Eckhardt 2008, Gärttner 1975, Gebhardt 1973, Sauter et al. 1981, Jellen 1984, Quarles 1969, Kepmann 1970, Knoche 1971, Hilliger 1969, Hu et al. 2014, Hurtienne 1967, Just et al. 2011, Kösters & Müller 1970, Lippmann 2007, Lippmann 2014, Matković et al. 2013, Northcutt et al. 2004, Springorum et al. 2015, Zhao et al. 2016, Zheng et al. 2013, Zucker et al. 2000, Zucker & Müller 2000, Popescu et al. 2013, Schrader et al. 2013, Sarikas 1976, Venter et al. 2004, Woodward et al. 2004, Yao et al. 2007a, b)

Representatives of many other microorganism groups were found in the air of chicken housing, such as anaerobic bacteria (Sauter et al. 1981), Gram-negative bacteria (Lippmann 2007, Venter et al. 2004, Clark et al. 1983, Lippmann 2014, Popescu et al. 2013, Brodka et al. 2012, Hinz et al. 1994, Chmielowiec-Korzeniowska et al. 2007), staphylococci (e.g. Chai et al. 2001, Witkowska & Sowińska 2013, Popescu et al. 2013, Chinivasagam et al. 2009, Nonnenmann et al. 2010,

Angersbach-Hegers 2002, Saleh 2006, Bloomberg et al. 2009, Agabou 2009, Schulz et al. 2004, Schulz et al. 2011, Springorum et al. 2015, Brooks et al. 2010), *Staphylococcus aureus* (Zhong et al. 2009, Chai et al. 2003), MRSA (Liu et al. 2012), enterococci (Brodka et al. 2012, Bloomberg et al. 2009, Schrader et al. 2013, Springorum et al. 2015, Brooks et al. 2010), haemolytic cocci (Baĭkov & Petkov 1986), streptococci (Popescu et al. 2013, Angersbach-Hegers 2002, Chai et al. 2003), Enterobacteriaceae (Witkowska & Sowinska 2013, Angersbach-Hegers 2002, Berang et al. 1995, Whyte et al. 2001), coliforms (Petkov & Tsutsumanski 1975, Northcutt et al. 2004, Baĭkov & Petkov 1986, Kostandinova et al. 2014), *E. coli* (Yao et al. 2007, Chinivasagam et al. 2009, Chai et al. 2003, Whyte et al. 2001, Laube et al. 2014), salmonellae (Duan et al. 2008, Venter et al. 2004, Fallschissel et al. 2009, Chinivasagam et al. 2009a), *Coxiella burnetii* (Søndergaard et al. 2014), actinomycetes (Angersbach-Hegers 2002) and various different moulds, such as *Aspergillus, Cladosporium, Penicillium* (Jo & Kang 2005, Sowiak et al. 2012).

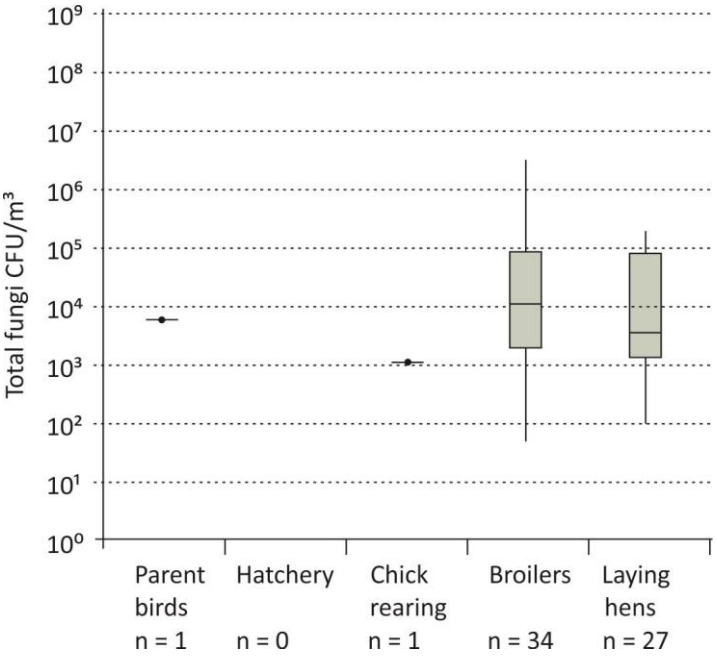


Fig. 14: Box-and-whisker plots of the mean concentrations of total fungi from the literature, determined via cultivation for various production stages in chickens, and the number of datasets incorporated. References: parent birds (Hao et al. 2014), chick rearing (Sowiak et al. 2012), broilers (Popescu et al. 2010, Agranovski et al. 2007, Ajoudanifar et al. 2011, Chai et al. 2007, Crook et al. 2008, Gigli et al. 2005, Hahne 2014, Jones et al. 1984, Lee & Liao 2014, Madelin & Wathes 1989, Mituniewicz et al. 2008, Nonnenmann et al. 2010, Pavan 2015, Madelin & Wathes 1989, Nichita et al. 2010, Petkov & Tsutsumanski 1975a, Radon et al. 2002, Shokri 2016, Vucemilo et al. 2007, Wang et al. 2007, Wojcik et al. 2010, Saleh 2006, Jo & Kang 2005, Clark et al. 1983), laying hens (Angersbach-Hegers 2002, Baĭkov & Petkov 1986, Bloomberg et al. 2009, Crook et al. 2008, Sauter et al. 1981, Hu et al. 2014, Lippmann 2014, Matkovic et al. 2009a, Matković et al. 2013, Northcutt et al. 2004, Springorum et al. 2015, Popescu et al. 2013, Sowiak et al. 2012)

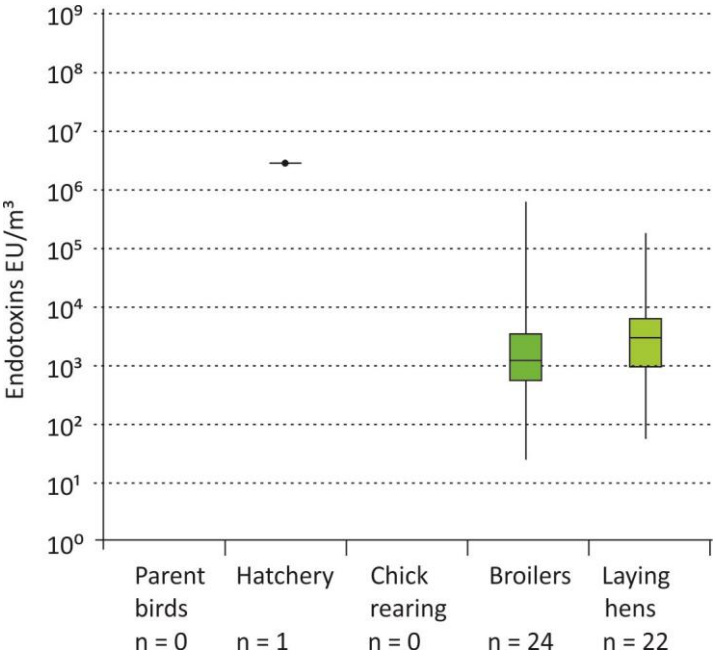


Fig. 15: Box-and-whisker plots of the mean concentrations of endotoxins from the literature, determined for various production stages in chickens, and the number of datasets incorporated. References: hatchery (Chmielowiec-Korzeniowska et al. 2007), broilers (Brooks et al. 2010, Crook et al. 2008, Hahne 2014, Hinz et al. 1994, Jones et al. 1984, Whates et al. 1997, Ogink et al. 2016, Nielsen & Breum 1995, Nieuwenhuijsen et al. 1999, Pomorska et al. 2007, Radon et al. 2002, Roque et al. 2016, Saleh et al. 2005, Vucemilo et al. 2008, Wiegand et al. 1993, Seedorf et al. 1998a, Bakutis et al. 2004, Clark et al. 1983, Singh & Singh 1996), laying hens (Angersbach-hegers 2002, Anonymous 2012, Crook et al. 2008, Eckhardt 2008, Whates et al. 1997, Ogink et al. 2016, Just et al. 2011, Lippmann 2007, Lippmann 2014, Schirl et al. 2007, Seedorf et al. 1998a)

For the laying hens, various alternative housing forms became established after the abolition of cage rearing in Germany. Clauß (2014) determined bioaerosol concentrations in barn keeping, free-range keeping, aviary keeping and keeping in small groups (Fig. 16). The lowest concentrations were found for keeping in small groups, although this is to be abolished in Germany in the medium term. This was followed by free-range keeping and, as systems with the highest concentrations, aviary keeping and barn keeping. Other authors arrive at the same results (Nimmermark et al. 2009, Saleh et al. 2006, Kirychuk et al. (2006).

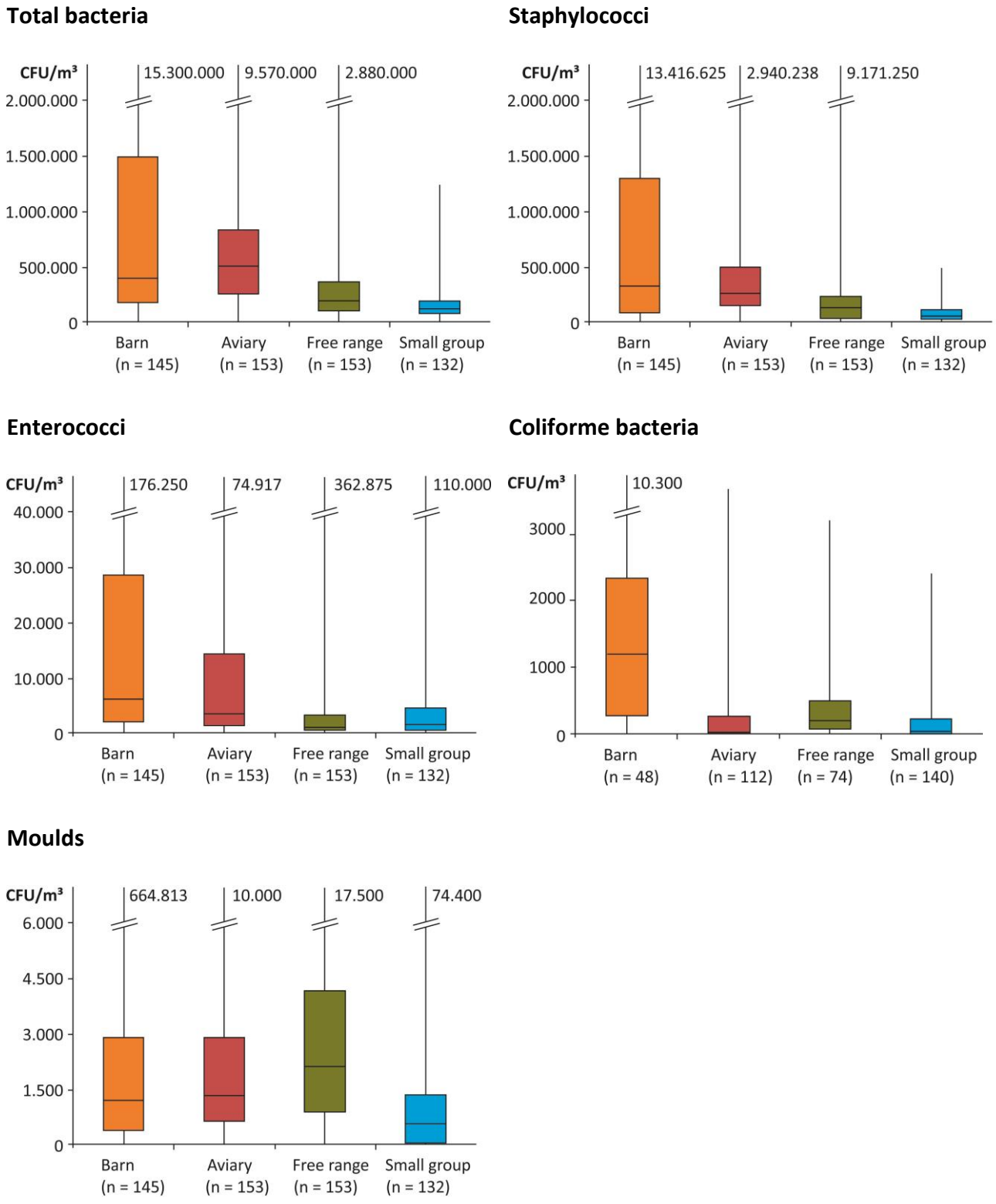


Fig. 16: Box-and-whisker plots of the concentrations of total bacteria, staphylococci, enterococci, coliforme bacteria and moulds determined by cultivation in various systems for keeping laying hens (from Clauß 2014).

In cattle farming, the mean concentrations of total airborne bacteria in calves and dairy cattle were roughly the same, at approx. 5 × 10^4 CFU/m³, respectively, but in beef cattle they were much higher, at 1 × 10^5 CFU/m³. In the case of moulds, the mean concentrations were also very similar in calves and dairy cattle, at 6 × 10^3 CFU/m³ and 8 × 10^3 CFU/m³, respectively, in contrast to beef cattle where they were markedly lower, at 1 × 10^3 CFU/m³. The endotoxin concentrations are on the same level in calves, dairy cattle and beef cattle, at 1 × 10^2 EU/m³, 2 × 10^2 EU/m³ and 1 × 10^2 EU/m³, respectively. Many other microbiological parameters have also been investigated in cattle

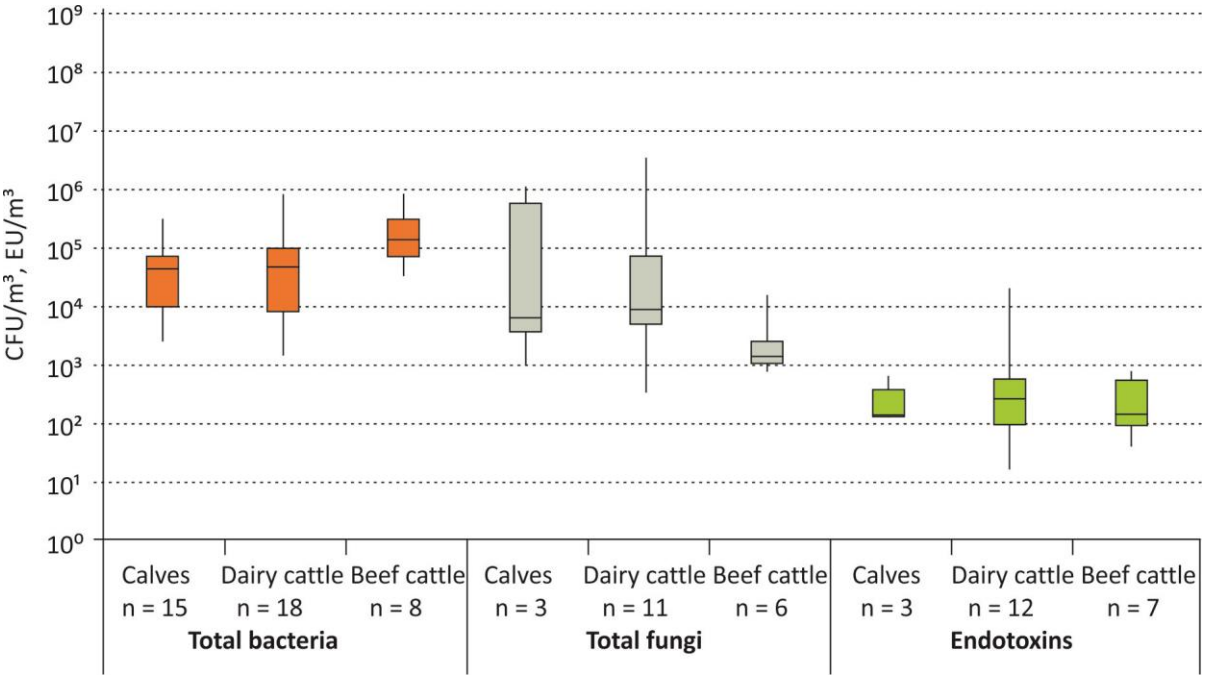


Fig. 17: Box-and-whisker plots of the mean concentrations of total bacteria and total fungi, determined via cultivation, as well as endotoxins, for various production stages in cattle, from the literature, and the number of datasets incorporated. References: total bacteria calves (Zucker et al. 2000, Chai et al. 1997, Wathes et al. 1984, Chai et al. 1999, Blom et al. 1984, Dutkiewicz et al. 1994, Beer 1973, Marschang & Crainiceanu 1971, Steiger & Stellmacher 1977), total bacteria dairy cattle (Zucker et al. 2000, Samadi et al. 2012, Karowska 2005, Matkovic et al. 2006, Dutkiewicz et al. 1994, Hanhela et al. 1995, Eckhardt 2008, Lang et al. 1997, Duchaine et al. 1999b, Abd-Elall et al. 2009, Matković et al. 2007, Banhazi et al. 2008a), total bacteria beef cattle (Clauß unpublished, Duan et al. 2013, Bakutis et al. 2004, Abd-Elall et al. 2009, Alvarado et al. 2009, Kullmann et al. 1998), moulds calves (Blom et al. 1984, Dutkiewicz et al. 1994), moulds dairy cattle (Pavan 2015, Pavan & Manjunath 2014, Karowska 2005, Matkovic et al. 2006, Matkovic et al. 2009a, b, Dutkiewicz et al. 1994, Lang et al. 1997, Duchaine et al. 1999b, Abd-Elall et al. 2009), moulds beef cattle (Adhikari et al. 2004, Wang et al. 2007, Ajoudanifar et al. 2011, Abd-Elall et al. 2009, Alvarado et al. 2009, Kullmann et al. 1998), endotoxins calves (Dutkiewicz et al. 1994, Seedorf et al. 1998a), endotoxins dairy cattle (Evans 2017, Samadi et al. 2012, Dutkiewicz et al. 1994, Pomorska et al. 2007, Schirl et al. 2007, Duchaine et al. 1999b, Seedorf et al. 1998a, Banhazi et al. 2008a), endotoxins beef cattle (Roque et al. 2016, Bakutis et al. 2004, Berger et al. 2005, Schirl et al. 2007, Seedorf et al. 1998a, Kullmann et al. 1998).

stalls, such as anaerobic bacteria (Chai et al. 1997, Chai et al. 1999), Gram-negative bacteria (Zucker et al. 2000, Dutkiewicz et al. 1994), staphylococci (Karowska 2005), *Staphylococcus aureus* (Alvarado et al. 2009), actinomycetes (Dalphin et al 1991, Dutkiewicz et al. 1994) moulds of the genera *Aspergillus, Cladosporium* and *Penicillium* (Pavan & Manjunath 2014) and yeasts (Kullmann et al. 1998, Lang et al. 1997). Some authors determined the number of bacterial cells in cattle stalls, which were between 5×10^4 cells/m³ and 1×10^7 cells/m³ (Eglite et al. 1989, Erman et al. 1989, Larsson et al. 1988). Dalphin et al (1991) found 5.6×10^1 CFU/m³ thermophilic actinomycetes. Lecours et al. (2012) found up to 10^6 archaea and 10^8 bacterial 16S rRNA genes/m³, and *Saccharopolyspora rectivirgula* at 10^6 16S rRNA genes/m³ air, in Canadian dairy cows. Okraszewska-Lasica et al. (2014) found Salmonella-positive air samples in cattle in Ireland. Chai (1998) investigated the occurrence of *Clostridium perfringens* in cattle.

Apart from the influence of the animal species, the stages of production, the housing systems (Protais et al. 2003) and the collection methods, there are further parameters that have an influence on the bioaerosol concentrations measured in animal stalls. Generally, the concentrations of bioaerosols in the stalls increase with increasing animal age and animal weight. This has been demonstrated particularly well for poultry (Madelin & Wathes 1989, Brodka et al 2012, Vucemilo et al. 2005, 2006, 2007, Opplinger et al. 2008, Sauter et al. 1981, Witkowska et al 2010, Baykov & Stoianov 1999, Saleh et al. 2005, Lonc & Plewa 2010). The bacterial spectrum also changes over this period (Vucemilo et al. 2005, 2006, 2007). This is not only caused by the growth of the animals but also by the concentration of residues of faeces, litter and feed in the stalls. This can be shown particularly well for broilers during fattening. In ducks, too, the highest bioaerosol concentrations are found shortly before the end of fattening (Yu et al. 2016a, b). The seasons also have an influence (Witkowska et al. 20012, Kumari & Choi 2014). With the aid of PCR, Masclaux et al. (2013) found almost twice as many total bacteria and staphylococci in pig stalls in winter than in summer, as did Wojzic et al. (2010) via cultivation. In contrast, Spirin & Mikhaĭlova (1991) generally found higher bioaerosol concentrations in pigs in summer. Most of the moulds are found in autumn (Sowiak et al. 2012). This is consistent with the course of the natural background concentration for moulds (Kolk et al. 2009, Clauß et al. 2013b).

5.2 Emissions

Emission factors published for airborne microorganisms differ in part considerably for the same animal species and housing form, the causes lying in different sampling conditions and different methods for determination of the concentrations.

Bioaerosols find their way into the environment with the exhaust air from the barns. These emissions are preferably measured at the interface between the stalls and the environment. In the results of such measurements, a simple statement of the concentration of airborne

microorganisms in the exhaust airflow does not generally make sense, as the amount of bioaerosols emitted is also dependent on the air volume flow and in this connection also on time. Therefore, for the characterisation of emissions, it is better to state specific loads, e.g. CFU/s, or emission factors, which reveal e.g. the number of bioaerosols (mostly microorganisms) per unit of time, in relation to the animal place or livestock unit. The conduct of emission measurements is regulated in Germany by VDI 4257 Sheet 1. Emission factors thus determined can be found for poultry farming in VDI 4255 Sheet 3 and for pig farming in VDI 4255 Sheet 4. These are intended to provide a representative mean of the emissions in relation to the year and relate to measurements with the emission impinger. Here, it is particularly important to bear in mind that these emission factors are only based on the results of measurements that were taken during the day. However, markedly lower emissions are registered during the night (see section 5.3), which means that the convention values for emission factors stated in the currently valid VDI standards are too high as annual means. Compared with the emission factors valid up to now from the previous standards, they are as much as 2 powers of ten above the older values. However, this is also mainly due to the fact that the emission impinger was used for sampling in the new values, with which all microorganisms in the emission are detected individually and not just microorganism-bearing particles, which was the case in the old values. Taking into account the different sampling conditions and the fact that measurements were taken exclusively during the day, the new higher values appear to be plausible (Clauß & Hinz 2014).

In livestock farming, the quantity and quality of bioaerosol emissions are dependent on the animal species, the keeping method and the stall system (Banhazi 2008c). They are further subject to considerable daily and seasonal fluctuations, which in turn are dependent on the outside temperature, stall management, animal activity, animal age and animal mass. Table 5 shows emission factors from the literature for different groups of airborne microorganisms in relation to animal species and housing form. Most of the emission factors relate to microbiological sum parameters, such as total bacteria or total fungi and, in the meantime, also to the stall-specific indicator parameters staphylococci, enterococci and enterobacteria specified in the VDI standards. Although representatives of the latter two groups may also be considered to be stall-specific, they are to be found in markedly lower concentrations in the air compared with staphylococci. Therefore, from a practical point of view, they are not as well suited for characterising the emissions from a livestock facility. The emission factors available from various publications (Table 5) differ in part considerably from each other. And yet they are really supposed to provide a representative mean of the emissions in relation to the year, which is specific for the animal species and the housing form. A general challenge in this respect are the large differences in stall systems, stall management, hygiene, animal activity, animal age and animal mass. Above all for poultry, there is a marked influence of the housing form on the level of emissions. Likewise, the results differ considerably for the same animal species and housing form if different collection systems were used. This underscores the importance of a standardisation of techniques and methods for the sampling of bioaerosols. This is particularly important if it is a question of approval-relevant issues or of legislation in connection with the environmental effect of livestock farming. With regard to the time and duration of measurement, it must be taken into

account for all animal species that there are both seasonal and diurnal influences on the level of emissions, as well as those related to the housing form and stall management. Therefore, representative measurements for the determination of annual means should ideally be distributed uniformly over the year, in all seasons, with repeat measurements, both during the day and at night, over sufficiently long periods, and taking into account keeping-specific parameters. This has been handled very differently up to now. In future, it should also be taken into account that, beside annual means, hourly means can also be important, as even briefly elevated emissions can be critical for some health-relevant issues. Thus, a single briefly elevated emission of mould spores of the species *Aspergillus fumigatus* from an edible mushroom farm led to major losses of birds in a neighbouring falcon farm (Lierz 2017).

Tab. 5: Emission factors of microbiological parameters for different animal species and housing forms from the literature, and the collection systems used for their determination. For standardisation purposes, if the original reference was to animal place (AP), this was converted to livestock unit (LU) using the factor stated for the respective animal species (from the LU-key of the Kuratorium für Technik und Bauwesen in der Landwirtschaft).

Animal species	Country	Housing form	Result	Collector	Reference
Chickens					
Young hens (0.0014)	D	Aviary keeping	Staphylococci: 1.4×10^6 CFU/LU*s Enterococci: 1.4×10^4 CFU/LU*s	Emission impinger	VDI 4255 Sheet 3
	D	Aviary keeping, manure belt, 80,000 AP	Total bacteria: 1.6×10^6 CFU/(LU*s) Moulds: "generally very low" Endotoxins (PM_{10}): 1.7×10^2 EU/(LU*s)	Emission impinger, impactor	Bayrisches Landesamt 2011
Broilers (0.0015)	D	Barn keeping	Staphylococci: 4.7×10^6 CFU/LU*s Enterococci: 1.3×10^4 CFU/LU*s	Emission impinger	VDI 4255 Sheet 3
	D	27,000 animals	Total bacteria: 5.9×10^6 CFU/LU*s Staphylococci: 3.6×10^6 CFU/LU*s Moulds: 4.6×10^4 CFU/LU*s Total cell count: 3.2×10^8 cells/LU*s	Emission impinger	Gärtner et al. 2009
	D	41,400	Total bacteria: 4.8×10^6 CFU/LU*s Staphylococci: 2.4×10^6 CFU/LU*s Moulds: 5.6×10^4 CFU/LU*s Total cell count: 2.2×10^8 cells/LU*s	Emission impinger	Gärtner et al. 2011
	D	n.s.	Total bacteria: 5.7×10^6 CFU/LU*s Staphylococci: 2.3×10^6 CFU/LU*s Enterococci: 1.6×10^4 CFU/LU*s Moulds: 4.8×10^3 CFU/LU*s Total cell count: 7.8×10^7 cells/LU*s	Emission impinger	Gärtner et al. 2011

Chickens continued Broilers (0.0015)	D	Barn keeping, 39,500 AP	Total bacteria: 3.5×10^6 CFU/LU*s to 3.7×10^7 CFU/LU*s Staphylococci: 2.5×10^6 CFU/LU*s to 1.9×10^7 CFU/LU*s Enterococci: 9.8×10^2 CFU/LU*s to 1.0×10^4 CFU/LU*s	Emission impinger	Bayrisches Landesamt 2015a
	D	n.s.	Total bacteria: 8.4×10^6 CFU/LU*s Staphylococci: 6.2×10^6 CFU/LU*s Enterococci: 2.2×10^4 CFU/LU*s Moulds: 3.6×10^4 CFU/LU*s Total cell count: 6.7×10^7 cells/LU*s	Emission impinger	Gärtner et al. 2011
	D	Chicken fattening stalls with forced ventilation (n = 8)	Total cell count: 6×10^6 cells/LU*s to 8×10^7 cells/LU*s	Emission impinger	Gärtner et al. 2014
	D		Staphylococci: 8×10^2 CFU/LU*s	AGI-30	Schulz 2007
	D	8 broiler stalls	Total bacteria: 1×10^3 CFU/LU*s Moulds: 2×10^1 CFU/sGV*s Enterobacteria: 6×10^1 CFU/LU*s	Automatic slit impactor	Seedorf 2004
	D	8 broiler stalls	Total bacteria: 1×10^3 CFU/LU*s Moulds: 2×10^1 CFU/LU*s Enterobacteria: 8×10^1 CFU/LU*s	Automatic slit impactor	Seedorf et al. 1998a
	NL	2 broiler stalls, 44500, 13500 AP	Endotoxins: 7.8×10^2 EU/LU*s Markedly lower values at night	Filter	Ogink et al. 2016
	AUS	Tunnel-ventilated stalls	Total bacteria: max. 3×10^5 CFU/s Moulds: max.: 5.3×10^4 CFU/s	AGI-30 and Andersen impactor	Agranovski et al. 2007
Laying hens (0.0034)	D	Aviary keeping	Staphylococci: 5.9×10^6 CFU/LU*s Enterococci: 8.8×10^3 CFU/LU*s	Emission impinger	VDI 4255 Sheet 3
	D	Barn keeping	Staphylococci: 9.7×10^5 CFU/LU*s Enterococci: 5.9×10^4 CFU/LU*s	Emission impinger	VDI 4255 Sheet 3
	GB	Cage rearing	Endotoxins: 1.4×10^2 EU/LU*s	Filter	Whates et al. 1997
	D	Small group 8600	Total bacteria: 6.4×10^4 CFU/LU*s	Emission impinger	Lippmann 2014
	D	Small group 31000	Total bacteria: 3.1×10^6 CFU/LU*s Endotoxins 4.5×10^2 CFU/LU*s	Emission impinger	Lippmann 2014
	D	Aviary 8000	Total bacteria: 4.1×10^7 CFU/LU*s	Emission impinger	Lippmann 2014
	D	Aviary 20000	Total bacteria: 2.3×10^5 CFU/LU*s Endotoxins 1.9×10^3 CFU/LU*s	Emission impinger	Lippmann 2014
	D	15,000, Aviary	Total bacteria: 8.0×10^5 CFU/LU*s Staphylococci: 9.7×10^5 CFU/LU*s Moulds: 2.1×10^3 CFU/LU*s Endotoxins: 4.3×10^2 EU/LU*s	Emission impinger	Lippmann et al. 2016

Laying hens continued (0.0034)	D	8 stalls	Total bacteria: 1×10^{1} CFU/LU*s Moulds: 9×10^{-1} CFU/LU*s Enterobacteria: 9×10^{0} CFU/LU*s	Automatic slit impactor	Seedorf 2004
	D	8 stalls	Total bacteria: 8×10^{0} CFU/LU*s Moulds: 3×10^{0} CFU/LU*s Enterobacteria: 3×10^{-1} CFU/LU*s	Automatic slit impactor	Seedorf et al. 1998a
	D	Keeping in small groups / manure belt ventilation, 390,000 AP	Total bacteria: 1.4×10^{5} CFU/LU*s Moulds: "generally very low" Endotoxins (PM_{10}): 8.6×10^{1} EU/LU*s	Emission impinger, impactor	Bayrisches Landesamt 2011
	D	20,000, aviary	Total bacteria: 4.3×10^{6} CFU/LU*s Staphylococci: 3.9×10^{6} CFU/LU*s Moulds: 3.2×10^{3} CFU/LU*s Endotoxins: 1.6×10^{3} EU/LU*s	Emission impinger	Lippmann et al. 2016
	GB	Emission in all three	Endotoxins: 6.9×10^{2} EU/LU*s	Filter	Whates et al. 1997
	GB	Cage	Endotoxins: 8.3×10^{1} EU/LU*s Endotoxins: 30 µg/h*LU	Filter	Whates et al. 1997
	NL	2 stalls, 12125, 17460 AP	Endotoxins: 2.3×10^{3} CFU/LU*s Markedly lower values at night	Filter	Ogink et al. 2016
	USA	Cage rearing	Total bacteria: 5.6×10^{3} CFU/LU*s	Agi-30	Zhao et al. 2016
	USA	Aviary keeping	Total bacteria: 1.1×10^{5} CFU/LU*s	Agi-30	Zhao et al. 2016
	USA	Enriched cage	Total bacteria: 5.6×10^{3} CFU/LU*s	Agi-30	Zhao et al. 2016
Turkeys (0.0125)	D	Barn keeping, 1,700 AP	Total bacteria: 7.8×10^{5} CFU/LU*s Staphylococci: 4.7×10^{5} CFU/LU*s Moulds: 8.7×10^{3} CFU/LU*s Endotoxins: 1.0×10^{3} EU/LU*s	Emission impinger	Lippmann et al. 2016
Pigs					
Sows (0.3)	D	16 stalls	Total bacteria: 4×10^{3} CFU/LU*s Moulds: 3×10^{2} CFU/LU*s Enterobacteria: 8×10^{1} CFU/LU*s	Automatic slit impactor	Seedorf et al. 1998a
	D	16 stalls	Total bacteria: 5×10^{3} CFU/LU*s Moulds: 2×10^{2} CFU/LU*s Enterobacteria: 2×10^{1} CFU/LU*s	Automatic slit impactor	Seedorf 2004
	AUS		Total bacteria 4×10^{3} CFU/LU*s	Andersen impactor	Banhazi 2012
Weaning piglets (0.03)	D	Fully/partially slatted flooring in group keeping, 1400 piglets	Total bacteria: 7.8×10^{2} CFU/LU*s Moulds: "generally very low" Endotoxins (PM_{10}): 2.8×10^{1} EU/LU*s	Emission impinger, Filter	Bayrisches Landesamt 2011

Weaning piglets continued (0.03)	D	16 stalls	Total bacteria: 6×10^2 CFU/LU*s Moulds: 2×10^2 CFU/LU*s Enterobacteria: 2×10^0 CFU/LU*s	Automatic slit impactor	Seedorf 2004
	D	Liquid manure procedure	Staphylococci: 6.7×10^2 CFU/LU*s Enterococci: 6.7×10^1 CFU/LU*s	Emission impinger	VDI 4255 Sheet 4
	D	8 stalls	Total bacteria: 1×10^2 CFU/LU*s Moulds: 5×10^0 CFU/LU*s Enterobacteria: 6×10^1 CFU/LU*s	Automatic slit impactor	Seedorf et al. 1998a
	AUS		Total bacteria: 4×10^3 CFU/LU*s	Andersen collector	Banhazi 2012
Fattening pigs (0.15)	D	Liquid manure procedure	Staphylococci: 2.0×10^4 CFU/LU*s Enterococci: 2.0×10^3 CFU/LU*s	Emission impinge	VDI 4255 Sheet 4
	D	8 stalls	Total bacteria: 2×10^3 CFU/LU*s Moulds: 7×10^1 CFU/LU*s Enterobacteria: 3×10^2 CFU/LU*s	Automatic slit impactor	Seedorf et al. 1998a
	D	Fully slatted flooring, three-stage exhaust air cleaning system	Clean gas: Total bacteria: 1×10^2 CFU/LU*s Moulds: 2×10^0 CFU/LU*s Staphylococci: 2×10^1 CFU/LU*s Enterococci: 2×10^0 CFU/LU*s Raw gas: 1 power of ten higher	AGI-30	Anonymous 2013a
	D	Fully slatted flooring, three-stage exhaust air cleaning system	Clean gas: Total bacteria: 4×10^1 CFU/LU*s Moulds: 2×10^0 CFU/LU*s Staphylococci: 2×10^1 CFU/LU*s Enterococci: 2×10^0 CFU/LU*s Raw gas: 1 power of ten higher	AGI-30	Anonymous 2013a
	D	Investigation on biofilters	Total bacteria: 6×10^1 CFU/LU*s Staphylococci: 3×10^0 CFU/LU*s Enterococci: 4×10^0 CFU/LU*s	Filter	Geburek et al. 2005
	D	8 stalls	Total bacteria: 1×10^3 CFU/LU*s Moulds: 3×10^1 CFU/LU*s Enterobacteria: 6×10^1 CFU/LU*s	Automatic slit impactor	Seedorf 2004
	NL		Endotoxins: 3.9×10^2 CFU/LU*s	Filter	Ogink et al. 2016
	AUS	On straw	Total bacteria 2×10^4 CFU/LU*s	Andersen impactor	Banhazi 2012
	AUS	Fully slatted flooring	Total bacteria: 2×10^3 CFU/LU*s	Andersen impactor	Banhazi 2012
	ROK	15 stalls	Total bacteria: 0.015 CFU/LU*s Moulds: 0.009 CFU/LU*s	Andersen impactor	Kim et al. 2007, Kim et al. 2008

Cattle					
Mother cows (1.2)	D	Slatted flooring, liquid manure, daily floor cleaning, 22/60	Total bacteria: 3.3×10^2 CFU/LU*s Moulds: "generally very low" Endotoxins (PM_{10}): 1.3×10^0 EU/LU*s	Emission impinger, impactor	Bayrisches Landesamt 2011
Calves (0.3)	D	16 stalls	Total bacteria: 6×10^2 CFU/LU*s Moulds: 2×10^2 CFU/LU*s Enterobacteria: 2×10^0 CFU/LU*s	Automatic slit impactor	Seedorf 2004
	D	16 stalls	Total bacteria: 2×10^3 CFU/LU*s Moulds: 3×10^2 CFU/LU*s Enterobacteria: 1×10^2 CFU/LU*s	Automatic slit impactor	Seedorf et al. 1998a
Dairy cows (1.2)	D	8 stalls	Total bacteria: 6×10^2 CFU/LU*s Moulds: 4×10^2 CFU/LU*s Enterobacteria: 3×10^0 CFU/LU*s	Automatic slit impactor	Seedorf 2004
	D	8 stalls	Total bacteria: 2×10^3 CFU/LU*s Moulds: 3×10^2 CFU/LU*s Enterobacteria: 7×10^2 CFU/LU*s	Automatic slit impactor	Seedorf et al. 1998a
Fattening bulls (0.7)	D	10 stalls	Total bacteria: 5×10^2 CFU/LU*s Moulds: 1×10^2 CFU/LU*s Enterobacteria: 2×10^0 CFU/LU*s	Automatic slit impactor	Seedorf 2004
	D	10 stalls	Total bacteria: 6×10^2 CFU/LU*s Moulds: 1×10^4 CFU/LU*s Enterobacteria: 2×10^2 CFU/LU*s	Automatic slit impactor	Seedorf et al. 1998a

5.3 Diurnal cycles

The differences in bioaerosol concentration between the day and night can be up to a power of ten in the stalls in agricultural livestock farming, depending on the animal species, and that of emission factors even up to 3 powers of ten, which must be taken into account in future when calculating annual mean values and emission factors.

The concentrations of bioaerosols at a measuring site can vary strongly. Clauß et al. (2012) determined the level of the changes in concentration of bacteria and mould spores at various different sites in the outside air as well as the influence of these changes on the results of different sampling procedures (filter, impinger, coriolis@ collector, impactor, bioaerosol spectrometer). Within one hour, the concentrations found for bacteria fluctuated in part by more than three powers of ten, in contrast to moulds with a maximum factor of 3. This is primarily due to the fact that bacteria are present as aggregates and moulds rather as individuals (section 5.4.2). Thus, a single bioaerosol particle can contain thousands of bacterial cells. If such a particle finds it way e.g. into an impinger, in which otherwise only small aggregates with few cells were collected, this has a major influence on the level of the concentrations determined. However, if such a particle is collected with an impactor on a culture medium plate, at this point just one

colony is formed and the result is barely influenced at all. The resolution over time also plays a role here. For example, even at low concentrations of airborne microorganisms, values can be generated by the minute with the Andersen collector, as longer sampling times would lead to the culture medium plates being occupied by too many colonies, making them impossible to count. In comparison, with the AGI-30 impinger, longer collection times of e.g. half an hour would be needed as a result of the lower airflow though the sampling system, so that the result would consequently represent a half-hour mean.

Beside the short-time concentration fluctuations, considerable differences of e.g. a power of ten are also found between the day and the night (Clauß 2015). Nevertheless, almost all investigations on bioaerosols throughout the world were exclusively conducted during the day (Clauß & Springorum 2017). There are thus considerable deficits in knowledge with regard to nighttime bioaerosol concentrations, including the field of livestock farming. Particularly in animal stalls, very many microorganisms occur and above all bioaerosol aggregates find their way into the air in large numbers as a result of the activity of the animals (Clauß et al. 2011, a, b). If the animals are at rest, e.g. at night, the concentrations in the stalls drop markedly. This is also dependent on the animal species. At the Thünen Institute of Agricultural Technology, the concentration of airborne bacteria was measured continuously for 48 h in various different forms of animal housing (Clauß, in preparation), using an automatic bioaerosol collector (Clauß 2015b). It was found that the nighttime concentrations in chicken stalls are a power of ten below the daytime concentrations. In goats it is a factor of 5, in cattle a factor of 3 and in pigs the nighttime concentrations are a factor of 2 lower. This also has marked effects on emission. Thus, first measurements of the Thünen Institute of Agricultural Technology within the context of a project funded by the Sächsische Landesamt für Umwelt, Landwirtschaft und Geologie (Office for the Environment, Agriculture and Geology of the State of Saxony), emission factors for airborne bacteria were three powers of ten lower at night than during the day in the emission from poultry stalls (Clauß, in preparation). Similarly in the Netherlands, markedly lower concentrations of other bioaerosol components such as endotoxins were determined in animal stalls at night (Ogink et al. 2016). In Great Britain, the concentrations were reduced by half at night (Whates et al. (1997).

The markedly lower bioaerosol concentrations at night, which together with the lower air rates lead to reduced emission loads in the emission, must be taken into account in the future when calculating an annual mean value for emission factors.

5.4 Transmission

In transmission, i.e. transport through the air, the possible distance of spread of airborne microorganisms is primarily determined by their tenacity, i.e. their capacity to survive the airborne state, and the particle size.

After the bioaerosols have been emitted from the livestock buildings, they are transmitted through the air. Here, they are unprotected, exposed to the wind and weather, so that many bioaerosol components are relatively quickly sedimented, washed away by rain, or inactivated by dehydration and sunlight. The possible distances of spread can vary considerably, depending on the bioaerosol in question (Bovallius et al. 1978). For example, in 1981, the FMD virus travelled hundreds of kilometres through the air from France to southern England and led to an outbreak of foot-and-mouth disease there (Donaldson et al. 1982). In contrast to this, Davies & Morishita (2005) did not find any cultivable pathogens in the air at a distance of more than 20 m from stalls of laying hens that were positive for salmonella. The results are also dependent on the detection method. Thus, airborne salmonellae, confirmed to be from the faeces of the animals, were found at a distance of up to 200 m from the stalls with molecular biological methods by Yuan et al. (2010) in pigs, and by Duan & Chai (2008) as well as Duan et al. (2008) in chickens. Zhong et al. (2008) also used molecular biological methods to confirm S. aureus at a distance of 400 m from a chicken house. In the transmission of airborne microorganisms, the possible distance of spread is primarily determined by their tenacity, i.e. their capacity to survive the airborne state, and the particle size of the bioaerosol, which among other things determines how quickly the microorganisms are sedimented.

5.4.1 Tenacity

How long bacteria remain viable in the air is primarily determined by their tenacity, which depends on many factors and, due to the test systems used, has yet to be investigated adequately.

Moulds mostly spread via the air. Their spores are adapted to ensure that they do not lose their ability to germinate even after covering long distances through the air. In the case of bacteria, the air is not their natural habitat. How long bacteria remain viable in the air, and can thus be detected using the classical culture-based methods, primarily depends on their tenacity. This term describes the ability of a microorganism to survive even under suboptimal conditions, e.g. outside of its normal habitat (Rolle & Mayr 2002). The tenacity of bacteria is known to some degree for only a few species. For example, *Mycoplasma hyopneumoniae* from a pig house remained infectious over a distance of 4.7 km (Dee et al. 2010). Legionellae can also spread over a distance of several km (Nygård et al. 2008).

A current review on the tenacity of microorganisms is provided by Springorum & Clauß (2016). The tenacity of different bacterial species and even of strains and isolates within the same species can vary enormously (Cox 1966, Müller et al. 1981, Hatch & Wolocho 1969). Also, the tenacities of vegetative forms and permanent forms (e.g. spores) differ within a species. Thus, bacterial spores of the genus *Bacillus* can even survive the extreme conditions of outer space for prolonged periods, in contrast to their vegetative forms (Nicholson et al. 2000). In addition, the literature contains often contradictory data for the tenacity of individual species (Müller et al. 1981). However, Gram-positive bacteria generally appear to be more resistant than Gram-negative bacteria (Müller et al. 1981). Apart from this, many other factors influence the tenacity of bacteria (Mitscherlich & Marth 1984), e.g. the preceding cultivation or growth conditions, the production of a bioaerosol (Stersky et al. 1972, Marthi et al 1990, Dunklin & Puck 1948, Cox & Goldberg 1972, Dark & Collow 1973, Hess 1965, Müller & Dinter 1986, Hatch & Wolochow 1969), the method or type of aerosolisation (Cox 1966, Cox 1976, Dimmick 1960, Marthi et al 1990, Heidelberg et al. 1997), temperature (Marthi et al 1990, Kethley et al. 1957, Ehrlich et al. 1970a, Ehrlich & Miller 1973, Ehrlich et al. 1970b, Dinter & Müller 1984, Wright et al. 1969, Harrison et al. 2005), air humidity (Won & Ross 1966, Williamson & Gotaas 1942, Wells & Zappasoid 1942, Goldberg et al. 1958, Ehrlich et al. 1970b, Dinter & Müller 1984, Dimmick 1960, Anderson 1966, Cox 1976, Wells & Wells 1936, Dunklin & Puck 1948, Wathes et al. 1986, Wright et al. 1968, Müller & Gröning 1981, Müller et al. 1981), UV radiation (Ko et al. 2000, Kundsin 1968, Chi & Li 2007), the content of toxic gas (including oxygen) (Wells & Zappasoid 1942, Cox 1976, Hess 1965, Lighthart 1973, Müller et al. 1981), or also the collection technique applied (Cox 1966, Wathes et al. 1986, Henningson & Ahlberg 1994) and the subsequent storage and processing of the samples (Won & Ross 1966, Cox 1976). According to current knowledge, the greatest influence on the bacteria in the air is exerted by the meteorological factors temperature, air humidity and global or UV radiation (Beebe 1959, Xue & Nicholson 1996, Kaplan 1955, Riley & Kaufmann 1972), as well as the concentrations of certain biocidal substances in the air, such as free radicals, ozone and ozone-olefin reaction products. Together, these are referred to as “open air factor” (OAF) (Druett & May 1968, Druett & May 1969, Hood 1971, Dark & Nash 1970). Not only do all factors influence each other physically, their effects in the bacterial cells also depend on each other. The precise relationships are complicated and the effects on different microorganism groups can vary greatly (Lighthart 1973, Mitscherlich & Marth 1984, Tang 2009).

In the outdoor air, most of the cultivable bacteria are detected at temperatures of between 8 °C and 24°C (Rüden et al. 1978). Generally, the death rate increases with increasing temperatures (Ehrlich et al. 1970a, Wright et al. 1969, Müller & Gröning 1981, Tang 2009). Survival at different temperatures is also dependent on the relative air humidity (Wathes et al. 1986, Wright et al. 1968, Müller & Gröning 1981, Müller et al. 1981). For example, the mean survival time of *Escherichia coli* at 50% rel. air humidity and a temperature of 15 °C is approx. 14 minutes, as opposed to just 3 minutes at 30 °C. At 85% rel. air humidity, the survival time is 83 minutes at 15 °C, compared with 14 minutes at 30 °C (Whates et al. 1986). The influence of air humidity on bacteria in the airborne state is more complex than that of temperature and is also very strongly dependent on the species (Tang 2009). However, generally, very high and very low air humidities

(> 85%, < 20%) are considered to reduce viability (Müller & Gröning 1981, Müller et al. 1981). A strong change in air humidity during the airborne state also leads to a reduction in viability (DeOme 1944, Hatch & Dimmick 1966, Hatch et al. 1970). The influence of UV radiation on airborne microorganisms strongly depends on the water content of the cells during irradiation (Kaplan 1955, Riley & Kaufmann 1972). Dehydrated cells are already effectively killed by UV-A and UV-B radiation, whereas moist cells are hardly affected at all (Kaplan 1955). Photoreactivation also only takes place in the moist state (Kaplan 1955, Riley & Kaufmann 1972). In this light-dependent process (approx. 300 nm – 500 nm wavelength), bacteria can repair damage caused by UV radiation extremely effectively with the aid of the enzyme photolyase, so that inactivated germs are reactivated after a certain time (Clauß et al. 2005, Goosen & Moolenaar 2008). Otherwise, little research has been conducted on the influence of natural UV radiation on airborne bacteria. Paez-Rubio & Peccia (2005) found a significant influence of sunlight on the survival rate of *Mycobacterium parafortuitum* at moderate air humidity in a UV-A and UV-B permeable chamber.

Regardless of meteorological parameters, bacteria generally survive worse in the outdoor air than in indoor spaces, even if the conditions are otherwise similar (Hood 1971, Hood 1974). OAF is assumed to be the cause. Particularly nucleic acid and coat proteins of microorganisms are strongly damaged by this. However, the substances responsible for OAF such as free radicals, ozone and ozone-olefin reaction products are extremely unstable and are broken down by reactions with particles or surfaces within a few minutes. On top of this, the concentrations of the individual substances are extremely variable and are not associated with a time of day or time of year (Druett & May 1968). Hood 1971 found that, in a closed system, an air exchange of at least 12x per hour is required to maintain the OAF (Hood 1971). For *E. coli,* survival rates of 72% after 5 min and 7% after 60 min were found. In addition, the OAF appeared to have a greater influence on tenacity at low air humidity here than at higher air humidity. In contrast, Dark & Nash 1970 found the greatest influence at a moderate air humidity (60%) for *E. coli,* compared with 80% for *Micrococcus albus.* In addition, for both species they found survival rates of 0 – 100% after 10 min at 20 °C and 78% RH, depending on the type of olefin and the ozone concentration.

Many authors also found a positive correlation between the survival of airborne bacteria and viruses (Alonso et al. 2015) and the particle size fraction in which they were detected (Marthi et al 1990, Dinter & Müller 1984, Hood 1971, May & Druett 1968, Kundsin 1968, Lighthart & Shaffer 1997). They are probably better protected in the larger particles.

Most of the investigations on determination of the tenacity of airborne microorganisms were conducted under controlled conditions in closed bioaerosol chambers. In most cases, only the influence of individual factors on survival was investigated. The least consideration has been given to OAF up to now, as the substances that cause the effect break down within just a few minutes in the closed chambers (Druett & May 1968). An alternative is offered by Clauß et al. (2016). They report on a bioaerosol chamber made of a UV-permeable foil balloon, which is

continuously filled with fresh outdoor air. In this system, it was possible to maintain the ozone concentration and thus also the OAF at a level of 75% for 20 minutes. Some authors have attached bacteria to thin spider threads ("microthread technique") in order to investigate their tenacity in the air (May & Druett 1968, Dark & Nash 1970). However, the cultivation of the spiders and the harvesting of the threads was very complicated and the subsequent evaluation difficult, as the bacteria have to be washed off the threads after the experiments. In addition, many cells were lost before and during the experiments. Ultimately, particles that adhere to a surface are only comparable to a limited degree to those present in the airborne state (Hood 1971). Up to today, it has thus not been possible to investigate the tenacity of airborne bacteria under true outdoor air conditions.

Investigations conducted to date can provide at most indications regarding the tenacity of bacteria in the airborne state. However, the various different experimental conditions and the large number of influencing parameters mean that the experimental results are often contradictory. As a result of the great differences between the individual species, few conclusions can be drawn about the possible transmission distances. Further research is necessary here. Beside tenacity, infectiousness should also be considered in the risk analysis of bioaerosols and the estimation of the potential of bacterial infectious diseases to spread through the air.

5.4.2 Size of bioaerosols

In agricultural livestock farming, most microorganisms in the air are found in much larger particle size or mass fractions (> PM_{10}) than to be expected from the size of the individual cells of the organisms, whereby the distribution of different bioaerosol components can vary and does not correlate uniformly with the distribution of the dust fractions.

Microorganisms are generally very small, bacteria thus typically having a diameter of between half and just a few µm. Since the century before last, however, it has been known that bacteria in the air predominantly occur in aggregates or on larger dust particles (Hesse 1884, Hesse 1886). Therefore, most microorganisms in the air are found in much larger particle size fractions than to be expected from the size of the individual cells of the organisms. This particularly applies to agricultural livestock farming (Clauß et al. 2011a, b). On this subject, Clauß (2015a) provides a comprehensive overview concerning the particle size distribution of airborne microorganisms in different environmental areas. Measurements on particle size distribution were conducted most frequently with Andersen collectors. However, only the number of “cultivable microorganism”-bearing particles in the range of 0.65 µm to 12 µm aerodynamic diameter (AD) can be determined more or less reliably with this method. Fig. 18 shows the mean incidence in percent of cultivable bacteria- and mould-bearing particles in the different particle size fractions of 0.65 µm to 12 µm in the air of animal stalls in agricultural livestock farming, determined with the 6-

stage Andersen collector, from the literature. The different widths of the box-and-whisker plots represent the different widths of the particle size fractions in the Andersen collector.

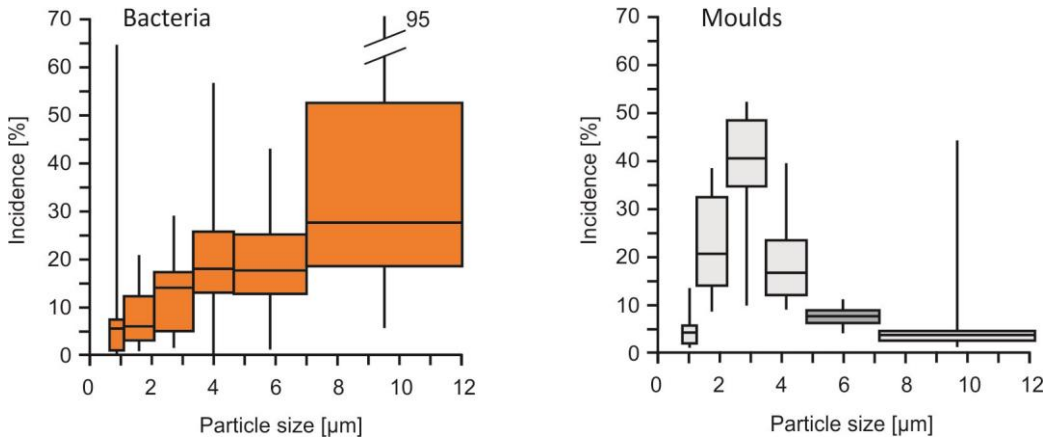


Fig. 18: Mean incidence in percent of cultivable bacteria- and mould-bearing particles in the different particle size fractions of 0.65 µm to 12 µm determined with the 6-stage Andersen collector in the air of animal stalls in agricultural livestock farming, from the literature. References on bacteria: Aarnink et al. 2012, Adell et al. 2011a, b, Chai et al. 2001, Chinivasagam & Blackall 2005, Lenhart et al. 1982, Liu & Ma 2010, Sowiak et al. 2011, Siggers et al. 2011, Zhao 2011, Zheng et al. 2013 (n = 26 datasets, 155 individual measurements). References on moulds: Chien et al. 2011, Liu & Ma 2010, Siggers et al. 2011 (n = 10 datasets, 23 individual measurements).

At approx. 30%, the most bacteria-bearing particles were found here in the impactor stage 7 µm to 12 µm. In comparison, in the case of moulds, most spores were found in the particle size fraction between 2 µm and 4 µm. In contrast to the bacteria, the ratios found represent the size distributions of the spores of the most commonly found s species (Clauß 2015a). This confirms the postulation of Hesse (1884, 188), that bacteria in the air predominantly occur in aggregates or also on larger dust particles. Mould spores, on the other hand, are largely present individually (Heikkilä et al. 1988, Pasanen et al. 1989). The number of all microorganisms (colony-forming units, cell count, number of gene copies) in a certain air volume has barely been investigated up to now. Similarly, as a result of the collection systems used to date, there are marked deficits in our knowledge on the particle size distribution of bioaerosols in the range > 12 µm AD. Therefore, Clauß (2015) recommended that, in future, collection systems should be used with which not only the number of microorganism-bearing particles in the range < 12 µm but also the number of all microorganisms in the environmentally and health-relevant particle size and mass fractions $PM_{2.5}$, PM_4, PM_{10} and total dust can be determined.

In the meantime, such measurements have been conducted by the Landesamt für Natur, Umwelt und Verbraucherschutz (LANUV) NRW (Office for Nature, the Environment and Consumer Protection of the State of North Rhine-Westphalia) (Gärtner et al. 2017) and the Thünen Institute of Agricultural Technology (Clauß et al., in preparation) in Germany (Clauß & Gärtner 2017). For

this purpose, both institutions use a combination of standardised emission impingers and impactors ($PM_{2.5}$ and PM_{10}) as pre-separators for the impingers. The first, in part preliminary, results are presented in Fig. 19.

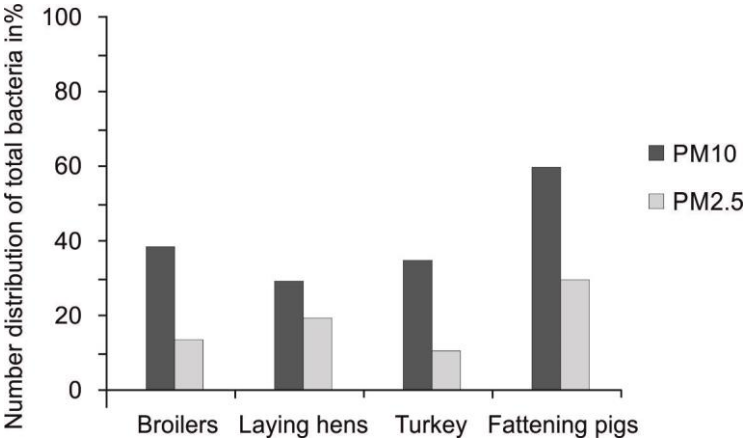


Fig. 19: Number distribution of total bacteria in the particle mass fractions PM_{10} and $PM_{2.5}$ in the emission from different animal stalls.

In the case of broilers, it was found that 39% of the airborne bacteria occur in the fraction PM_{10} and 14% in $PM_{2.5}$. The respective figures for laying hens were 30% and 20%. In turkeys, 35% of the airborne bacteria were found in the fraction PM_{10} and only 10% in $PM_{2.5}$. In the fattening pigs, the circumstances are slightly different than in poultry. Here, 60% of the airborne bacteria were in PM_{10} and 30% in $PM_{2.5}$. Further data are available for staphylococci, whose distribution is similar. In the case of fattening pigs and broilers, in parallel to the bioaerosols, dust measurements were conducted with the Johnas II impactor according to VDI 2066 Sheet 10. In the pigs, 65% of the particles were in the $PM_{2.5}$ fraction and 85% in PM_{10}, so the distribution differs considerably from the distribution of the airborne bacteria in the dust. In the broilers, in contrast, the distribution of the bacteria is roughly in line with that of the dust particles, at 12% in the $PM_{2.5}$ fraction and 45% in PM_{10}. According to the current state of knowledge, the concentrations of dust and airborne bacteria in the PM fractions do not appear to be uniformly correlated. Little is known at present about the size distribution of other bioaerosol components in the PM fractions. Attwood et al. (1986) detected the most endotoxins in the particle size fraction between 3.5 µm – 8.5 µm. In dairy cattle farming, Schaeffer et al. (2017) showed that a large proportion of bacteria, including the genera *Staphylococcus, Pseudomonas* and *Streptococcus,* are to be found on particles of greater than 10 µm. Alonso et al. (2015) showed that influenza A viruses (IAV), “porcine reproductive and respiratory syndrome” viruses (PRRSV) and “porcine epidemic diarrhoea” viruses (PEDV) are distributed over a broad range of particle sizes.

5.5 Immissions

The immission concentrations of bioaerosols drop exponentially with increasing distance from the source of emission, primarily dependent on the particle size (sedimentation) and on meteorological conditions.

The bioaerosols emitted from animal stalls are transmitted, primarily depending on meteorological parameters, into the area surrounding the stalls, where they lead to immissions. Therefore, particularly in regions with a high livestock density, elevated concentrations of airborne bacteria (Schaper 2004) and endotoxins (Myrna et al. 2017) can occur, compared e.g. with the situation in towns. Bioaerosols can additionally accumulate in sedimentation dust or in the soil in the surrounding area of animal stalls (Williams et al. 2016, Schulz et al. 2012). Depending on the animal species, the bioaerosols can also contain pathogens. In the Netherlands, 28% of the outdoor air samples around goat farms tested positive for Q-fever pathogen *Coxiella burnetii*, which is also harmful to humans (Rooij et al. 2016). In the USA, Cohen et al. (2012) found up to 29% of air samples in the surroundings of horse stables to be positive for *Rhodococcus equi*, a pathogen that causes pneumonia in foals. The stall-specific indicator parameter "Staphylococcaceae" is suitable for illustrating the concentration distribution of airborne microorganisms in the surrounding area of the stalls. Many authors have investigated the spread of staphylococci around animal stalls by measuring their concentrations at different distances from the stalls. Fig. 20 shows data taken from various different literature sources concerning concentrations of staphylococci in the exhaust air plume of chicken stalls with forced ventilation, measured with AGI-30 impingers at different distances from the stalls at a height of 1.5 m above the ground. The majority of comparable data was available for this constellation.

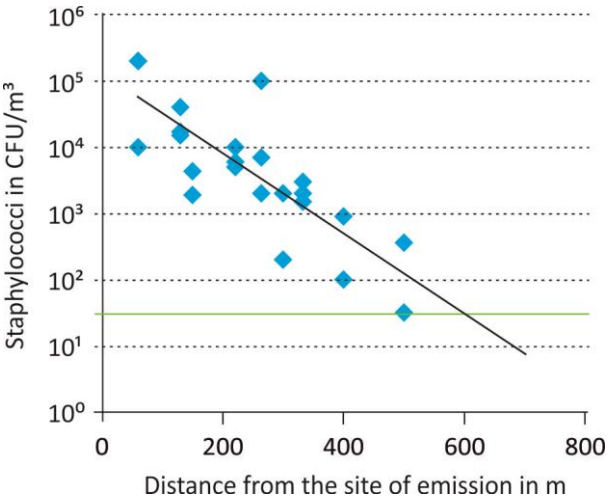


Fig. 20: Data taken from various different literature sources concerning concentrations of staphylococci in the exhaust air plume of chicken stalls with forced ventilation, measured at different distances from the stalls at a height of 1.5 m above the ground. Green bar: background concentration from Clauß et al. 2013b, references: Schulz et al 2004, Schulz et al. 2005, Lippmann et al. 2016.

A marked exponential drop in the concentrations can be seen with increasing distance from the source of emission. In this illustration, not until 600 m distance from the stalls are concentrations of staphylococci found that correspond to the rural background concentration determined by Clauß et al. (2013b). However, a marked range of fluctuation can also be seen, as the distance of spread of bioaerosols from animal stalls depends on many parameters. Beside the emission load (the amount of bioaerosols per unit of time) (section 5.2), the tenacity (section 5.4.1) and the particle size (section 5.4.2), these are mainly the wind speed and direction as well as the terrain conditions. Also the type of source (height above the ground, area sources such as biofilters, or point sources such as exhaust air shafts) has a marked influence on the form and size of the exhaust air plume and thus the possible distance of spread. In this respect, Agranovski et al. (2007) consider tunnel-ventilated stalls with ventilators in the side walls to be particularly problematical, as they produce the most concentrated exhaust air plumes.

In the case of immission measurements, the conduct of sampling has a major influence on the measurement results. In the ideal case, the immission measuring system should be located in the exhaust air plume for the entire period of measurement. In practice, this is rarely possible, due to changing wind directions and the mostly meandering exhaust air plume. Therefore, in VDI 4251 Sheet 1, plume measurements were suggested in which the immission concentrations should be measured on three legs in the lee of the stalls at different distances from the emission source. However, this entails a very high outlay in terms of logistics, staff and finance and has proved to be barely feasible in practice (Gladtke & Gessner 2017, Mietke-Hoffmann 2017, Winkler 2017). Therefore, measurements are mostly taken only on one leg against the main wind direction or at a fixed distance from the stalls on several legs. Further challenges are having suitable meteorological conditions during the measurement and difficult terrain on site. Buildings, trees or roads are often in the way, and there are frequently secondary bioaerosol sources in the vicinity, such as other stall buildings, but also compost heaps or woods.

In the VDI standards, the AGI-30 impinger is recommended as a collection system for immission measurements (4252 Sheet 3). However, this has a completely different collection efficiency than the emission impinger specified for emission measurements (section 4.1, Table 4). It would make sense to recommend the same collection system for emission and immission measurements. The influence of the various different systems on the results of measurements can namely, in theory and in practice, amount to a power of ten. Therefore, the results of emission measurements and immission measurements can only be compared to a limited degree. Also a comparison of immission measurements with the results of dispersion prognoses, e.g. according to VDI 4251 Sheet 3, is problematical in this respect. As entry parameters for the calculations, the VDI 4255 Sheet 3/4 suggests convention values for emission factors, which were obtained with emission impingers as the collection system. Therefore, the ensuing results of prognoses are not directly comparable with the results of immission measurements in which the AGI-30 impinger was used.

6 Dispersion prognoses for bioaerosols

Instead of conducting complicated measurements, the spread of bioaerosols can also be simulated with computer models, for the purpose of which, however, valid input data such as emission factors, particle size distributions and mortality constants have to be taken into account, which has not been the case to date.

The determination of the spread of airborne microorganisms in the vicinity of an emission source by concentration measurements on site is very complex and time-consuming. However, the spread of bioaerosols can also be simulated with computer models. For example, over the course of approval procedures, the spread of certain microbial indicator parameters is calculated, in order to be able to estimate and avert potential dangers and risks for people living in the vicinity of germ-emitting facilities. Beside the reduced amount of work involved and the time saved, the advantages are lower costs as well as the possibility to change variables in the models, e.g. meteorological conditions or the surrounding buildings. This means that prognoses can also be generated for alternative scenarios. Of particular interest here is the estimation of possible health risks resulting from a transmission of pathogenic germs through the air. Müller et al. (1978) already simulated the spread of bacteria from animal stalls with a simple mathematical model and also took into consideration the particle size distribution and tenacity using a mortality constant. Many different simulation models were developed in the past for the prediction of dispersion and deposition patterns of bioaerosols from point or area sources (Lighthart & Frisch 1976, Peterson & Lighthart 1977, Lighthart & Mohr 1987, Lighthart & Kim 1989, Ganio et al. 1995, Blackall & Gloster 1981, Gloster et al. 1981, 2003, 2007, Mikkelsen et al. 2003, Sørensen et al. 2001). However, little is known to date about the validity of the models used for the spread of microorganisms. In a few studies, a correlation between the modelled airborne pathogen transmission and the occurrence of diseases in the vicinity has been demonstrated. This has been achieved, for example for *Legionella pneumophila* (Nguyen et al. 2006), the Newcastle disease virus (Gloster 1983), foot-and-mouth disease (Sørensen et al. 2001), *Coxiella burnetii* (Wallenstein et al. 2010), the influenza virus (Liu & You 2012, Jonges et al. 2015) and the bird flu virus (Ssematimba et al. 2012). A comprehensive overview on this subject is provided by van Leuken et al. (2016). In an experiment with released *Bacillus* spores, Ganio et al. (1995) determined large differences between the concentrations detected in the vicinity of the source and the predicted values. In contrast, Mayer et al. (2008) found good agreement in the dispersion simulation of the FMD virus. In the area surrounding broiler stalls, Schulz (2007) found in part good agreement for staphylococci between concentrations measured on site and the germ counts calculated using a Gaussian model. In contrast, in a study of the Bayerisches Landesamt für Umwelt (Office for the Environment of the State of Bavaria, 2015b), only few cases of agreement at least in magnitude between the results of dispersion calculations and measured values. A further investigation on the agreement of predicted values of two dispersion models with actually measured concentrations of airborne staphylococci in the vicinity of livestock

farming facilities in turn showed marked deviations between the measured and the predicted concentrations at the site of immission (Springorum et al. 2014). The discontinuous underestimation observed here showed that further parameters probably need to be considered as input variables for the models. The reasons for deviations between the model values and the measured values may be found both in the measurements themselves and in the quality of the input parameters for the models. The transport mechanisms for aerosols are complicated and have yet to be fully understood (Zhang & Chen 2007). The transmission of stall air particles in the outdoor air is determined by the meteorological conditions, the orographic circumstances and the particle properties themselves (Schulz et al. 2011). With the dispersion class, roughness length, and sedimentation and deposition velocity of the particles, these parameters are incorporated in the computations of most dispersion models. The parameterisation of such models is pursuing a conservative approach in Germany at present. It is thus assumed that 100% of bacteria survive the airborne state, regardless of its duration. However, this is not in line with the latest scientific findings. In the dispersion prognosis of living microorganisms, the environmental factors that affect them, such as temperature, air humidity, UV radiation, components of the surrounding air as well as the so-called “open air factor” (OAF) must also be considered. These can take effect individually or synergistically and strongly affect the tenacity of the microorganisms (Burge 1995, Cox 1995). Prognoses from dispersion calculations in which airborne microorganisms are calculated as inert dust particles are therefore only transferrable to real life to a limited degree, since numerous factors are not taken into account. These include, e.g. natural UV radiation, as it immediately influences the tenacity of microorganisms. This could be taken into consideration either directly or indirectly (e.g. via the cloud class) in future computer models. Reproducible findings on the specific mortality coefficients of the relevant microorganisms in the airborne state would be particularly helpful. However, the necessary investigations are lacking at present. The same situation applies to the animal species-specific and husbandry type-specific mean particle sizes and their source strengths, which are subject to numerous influencing factors under practice conditions and can vary strongly. As long as these findings are lacking, in the case of immission prognoses for bioaerosols in the vicinity of livestock farming facilities it should be taken into account that the forecasts of dispersion models can deviate considerably from true conditions.

7 Health assessment of bioaerosols

From numerous publications it has long been known that, despite the lack of a dose-effect relationship, bioaerosols together with other air pollutants have a negative effect on the health of persons working in animal stalls. Up to now, it has not been possible to make a clear statement about the potential risk to people living in the vicinity of livestock facilities.

Many investigations from the field of veterinary and occupational medicine have demonstrated that elevated bioaerosol concentrations in animal stalls can have a negative effect on the health of the animals (Pauli et al. 1974, Wiseman et al. 1984, Sabo 2008, Huhn 1970, Jericho 1968, Kovgcs et al. 1967, Bækbo 1998) and of the persons who work there. Diseases of the respiratory tract are of primary concern here. In Germany, 417 respiratory diseases and 699 zoonoses were indicated as occupational diseases in the statutory agricultural accident insurance in 2013 (Riethmüller 2014). With regard to work-related respiratory diseases, pig farming has been investigated best worldwide (e.g. Coggins et al. 2007, Radon et al. 2000, Donham et al. 1977, Donham et al. 1982, Donham et al. 1984, Iversen et al. 2000, Cormier et al. 1991, Senthilselvan et al. 1997, Kirychuk et al. 1998, Donham et al. 1986a, b, Preller et al. 1995, Donham et al. 1989, Rylander et al. 1989, Malmberg & Larsson 1993, Zejda et al. 1994, Duchaine et al. 2000, Cormier et al. 2000, Jolie et al 1998). According to this research, pig farmers have the most work-related health problems, compared with other farmers (Attwood et al. 1986, Willems et al. 1984, Haglind et al. 1984, Butera et al. 1991, Donham et al. 1989, Holness et al. 1987). Thus, in the USA, an estimated 25% of the employees in pig farming have asthmatic diseases and 33% report health problems that are associated with organic dust toxic syndrome (ODTS) (Donham 2000). Likewise in cattle farming (Choudat et al. 1994) and poultry farming, work-related exposure to high concentrations of air pollutants lead to health impairments. In poultry farming, the estimated annual incidence of workplace-associated asthmatic diseases was 2.4% (Brooks et al. 2007). The prevalence of chronic bronchitis infections is between 12 and 25% (Danuser et al. 2001, Zuskin et al. 1995). Veterinarians specialised in poultry also report an increase in respiratory disorders (Elbers et al. 1996). Finally, cattle farmers also have a higher risk of dying from respiratory diseases (Choudat et al. 1994).

Bioaerosols can have a mechanical, infectious, toxic and/or sensitising effect in the respiratory tract, also synergistically with other agents that irritate the mucous membranes, e.g. ammonia or dust. Fundamentally, the impact on health is dependent on the composition (proportion of infectious pathogenic species, toxins, sensitising substances), the concentration (e.g. infectious dose), the exposure (frequency and duration) and the individual's constitution (health status), whereby the sensitising effect must be considered additionally. The most common diseases include allergic asthma, bronchitis, chronic obstructive pulmonary disease (COPD), exogenous allergic alveolitis (›Farmer's lung‹), MMI syndrome (mucous membrane irritation) and organic dust toxic syndrome (also called ›Thresher's fever‹), the latter primarily as a reaction to high

endotoxin exposures (Nowak 2016, Clark et al. 1983, Zhiping et al. 1996). Particular importance is attached above all to the endotoxins as a result of their high pro-inflammatory (inflammation-promoting) activity. Only around 15% of the pro-inflammatory activity is caused by other substances, such as β-glucans, toxins of Gram-positive bacteria, peptidoglycans and muramic acid (Eckhardt 2008), whose potential inflammatory effect has already been described by several authors (Hansen & Christensen 1990, Douwes et al. 2000). For endotoxins, it is even possible to deduce a dose-effect relationship (Donham et al. 1989). Concentrations of over 100 EU/m³ generally lead to irritations of the airways, more than 1000 EU/m³ to general respiratory symptoms and greater than 2000 EU/m³ to ODTS (Varnai & Macan 2004).

In the air of animal stalls, above all the number of airborne bacteria is generally strongly elevated and an increased number of disease pathogens are detected (Herr et al. 1999, Clauß 2014, Martin et al. 2015, de Rooije et al. 2016). According to the European Agency For Safety and Health at Work (2017), the most important agriculture-associated, aerogenically transmissible zoonoses are *Streptococcus suis* infections, psittacosis, leptospirosis, bovine tuberculosis and Q-fever. Beside these, there are many other parasitic, viral and bacterial diseases. When breathed in, the airborne pathogens can lead directly to diseases of the respiratory tract or be deposited in the mouth and pharynx and then swallowed, which e.g. in the case of *Escherichia coli, Salmonella, Campylobacter* and *Clostridium botulinum* can lead to infections of the gastrointestinal tract (Keessen et al. 2011, Pillai & Ricke 2002). Mackiewicz et al. (2015) attempted to deduce a dose-effect relationship for various different groups of airborne microorganisms in agriculture in a metaanalysis of data collected over 13 years in Poland. They found statistically significant correlations between the occurrence of work-related symptoms and the concentrations of total bacteria, Gram-negative bacteria and endotoxins. However, in a systematic review on correlations between exposure (air measurements) and effect (health effects) of bioaerosols on the basis of which it would be possible to deduce health-related assessment values for bioaerosols, the authors came to the conclusion that none of the human studies published to date (many of them from the field of occupational medicine in agricultural livestock farming) fulfil the criteria for the deduction of health-related assessment values or contain suitable dose-effect relationships (Gerstner et al. 2014, 2015). The role of the general microorganism content in stall air for the development of respiratory diseases in humans and animals thus cannot be clearly estimated at present. As long as no specific disease pathogens are present in infectious doses in the air, at most an unspecific burden on the respiratory tract occurs. This makes it difficult to specify a microbial “limit value”, as a high germ content on its own generally does not have consequences for health. On top of this, it has not been possible to clearly differentiate this from the effect of ammonia and the dust particle burden on the respiratory tract. However, the unsatisfactory estimation of the effect of the germ content is also linked to the fact that little is reported on the types of microorganisms that are present in the stall air. On the one hand, this is due to the very high total bacteria concentrations, under which the search for specific pathogens is difficult, on the other it has to do with the collection stress to which the microorganisms are exposed during sampling. This is the reason why many potential disease pathogens cannot be cultivated on culture media.

The microbiological loads not only lead to infections but also have toxic or sensitising effects, depending on the type of agent and the duration of exposure. While the number of acute diseases through extreme exposure has declined, the subacute and chronic respiratory diseases of employees in the interior area of stalls have increased (Donham 2000, von Essen & Donham 1999). Work-related allergic respiratory diseases have been well documented in agriculture and comprise both IgE-mediated diseases (bronchial asthma, hay fever) (Heutelbeck et al. 2007) and the IgG-mediated "farmer's lung" or "FHP" (farmer's hypersensitivity pneumonitis) (Imai et al. 2004). The potential triggers that come into question are exposure to animal hairs, feedstuff or grain dusts, or to moulds and actinomycetes (Skorge et al. 2005) of other origin, as has already been described (Lugauskas et al. 2004). In a meta-analysis of 44 publications, Goy (2007) found a prevalence for chronic bronchitis in employees in livestock farming of 17% (median). For the development of such chronic respiratory complaints, various different risk factors have additionally been identified, e.g. the size of the livestock facilities, the time spent in the stalls, and feeding and ventilation regimes (Radon et al. 2000, Radon et al. 2001). At the same time, the amount of sedimentation dust in the stalls represents a good approximation to the exposure to allergens at the workplace and in the household environment (Jacob et al. 2002, Chew et al. 2003). Only for a few allergens are the concentrations currently known for which health effects can be observed upon exposure (Baur et al. 1998). Above all, they were obtained in occupational medicine investigations with the outcome of asthmatic diseases (e.g. in bakeries, wood processing or the keeping of laboratory animals). At various other workplaces, beside moulds above all the allergens of the house dust mite (*Dermatophagoides* spp) have been investigated. The concentrations in animal housing are generally below the sensitisation limit (Macan et al. 2012), although poultry farming is excluded from this, as shown by Rimac et al. (2010). Lutsky et al. (1984) also found a connection between the development of allergic respiratory diseases and the exposure to allergens of the northern fowl mite *Ornithonyssus sylvarium*, which is also commonly encountered in livestock farming (poultry). Among the other allergens to be encountered in livestock farming, the cattle allergen Bos d 2 is particularly relevant, whereby concentrations in stalls, but also in living rooms and in mattresses, are above sensitisation thresholds. The so-called "farm effect", i.e. a lower incidence of allergic and – to a lesser extent – also asthmatic diseases in children who grew up on farms, is probably mainly attributable to a diversity of microbial exposures (Nowak 2016).

It is currently being discussed whether the negative health effects of stall dusts known from occupational medicine also occur in the population resident in the neighbourhood of animal stalls (Schlaud 1998). In two environmental-epidemiological cross-sectional studies in Lower Saxony, the question was investigated whether children (AABEL project: Hoopmann 2004) and adults (NiLS project: Radon 2004, Radon et al. 2007) living in the neighbourhood of animal stalls experience health impairments. For both studies, the exposure was quantified using dispersion calculations on the basis of the data available at the time for animal stall-related bioaerosols (dust, endotoxins). The health impairments were determined via school entry examinations (AABEL) and questionnaires as well as in a partial collective via clinical examinations including lung function analyses (NiLS). In both cases, the epidemiological evaluation came to the

conclusion that the individual bioaerosol components in the emission are strongly correlated with each other, so that it is barely possible to attribute an observed effect or a symptom association to a specific component. A deduction of dose-effect relationships and also effect thresholds was thus considered to be difficult. In a systematic review on bioaerosols and their health effects, O'Connor et al. (2010) also only found tendencies in people with allergies and no clear dose-effect relationship. In the Netherlands, it was discovered that people living in the vicinity of goat and sheep housing contract Q-fever more frequently (de Rooije et al. 2016). In the USA, an increase in child mortality in the vicinity of animal keeping was determined, probably primarily caused by increasing air pollution (Sneeringer 2009). In a further current study from the Netherlands, over a period of 7 years, van Dijk et al. (2016) investigated the health status of over 150,000 people living in areas with a high livestock density and compared them with 100,000 persons from areas with little livestock farming. In the areas with a high livestock density, they found a higher prevalence of diseases of the lower respiratory tract, chronic bronchitis and vertigo, but a lower prevalence of general respiratory symptoms and COPD. A shorter distance to the next farm was in turn associated with lower prevalences of the complaints mentioned, particularly in the case of cattle farms. Here, again, the effects of the individual bioaerosol components and accompanying air pollutants cannot be separated. Together with the bioaerosols, ammonia and dust may have a negative influence on health (Borlée et al. 2017). Van Dijk et al. (2016) additionally note that distortions can occur. Since people go to see the doctor less frequently in rural areas than in towns, an "under-reporting" of bioaerosol-associated symptoms in the vicinity of agricultural livestock farming may occur in epidemiological cross-sectional studies.

In conclusion, there are currently no scientifically tested threshold values for bioaerosols in the neighbourhood of livestock facilities above which the occurrence of health impairments is to be expected. Thus, the expert discussion "Germs from livestock and biogas facilities – effects on human health and the environment" in 2015 at the Ministry for Climate, the Environment, Agriculture, Nature Conservancy and Environmental Protection (North Rhine-Westphalia) came to the conclusion: "there is no evidence that living in the vicinity of livestock facilities poses a higher immediate health risk for the general population in relation to resistant bacteria (LA-MRSA and ESBL formers), compared with other places of residence" (Anonymous 2015). As a result of the complex composition of bioaerosols from livestock keeping, a direct dose-effect relationship cannot be deduced at present.

8 National and international regulations

Up to now, because of the lack of a dose-effect relationship, no generally valid threshold values have been formulated for bioaerosols, above which a damaging effect on health is to be expected. Therefore, based on the principle of a precautionary approach, a case-by-case analysis usually takes place.

In many countries there are regulatory requirements, e.g. in Germany the TA Luft, that are aimed at "protecting humans, animals and plants, the soil, the water, the atmosphere, as well as cultural and other material assets against harmful environmental influences and to prevent the development of harmful environmental influences". In Germany, the national implementation of the EU Directive 2000/54/EC "Biological agents at work", Biostoffverordnung (BioStoffV), regulates activities involving biological agents at the workplace. This does not contain any limit values for biological agents at work, only so-called "technical control values" ("technische Kontrollwerte", TKW) can be specified for workplaces according to TRBA 405 (2006) by the Committee for Biological Agents (Ausschuss für biologische Arbeitsstoffe, ABAS). These are to be determined individually for each workplace and depending on the respective state-of-the-art in technology at the workplace. These control values must be complied with and checked regularly. Beside the TA Luft, which is currently under revision, the determination and analysis of the emissions and immissions of particles is regulated by the Federal Immission Control Act (Bundes-Immissionsschutzgesetz, BImSchG) and its ordinances in Germany. The regulatory requirements also apply to facilities for the keeping or the rearing of livestock. Specifically for bioaerosols, the "Guide to the determination and analysis of bioaerosol immissions" (LAI-Leitfaden) of the Bund/Länderarbeitsgemeinschaft für Immissionsschutz (National/Federal Working Group for Immission Protection) also applies, which describes a nationally consistent, standardised methodology for the determination and analysis of bioaerosol loads. In addition, numerous technical rules have been drawn up in the field of work safety (Technical Rules for Biological Agents - TRBA) and environmental protection. One of the world's most comprehensive bodies of rules for the measurement and analysis of bioaerosols was created by the Association of German Engineers ("Verein Deutscher Ingenieure", VDI). As a result of its complexity, the subject area of "Bioaerosols and biological agents" is divided into several series of standards and currently comprises 21 guidelines and 8 European and international standards, while 5 further guidelines are in preparation at present. These rules are applicable in many of the federal states in Germany, especially in places where bioaerosols are an integral part of approval procedures for the construction or conversion of livestock housing. This affects operations with more than 2,000 fattening pigs or 750 sows and poultry farms with more than 40,000 animals. The immissions of certain airborne microorganisms to be expected in the surrounding area, which are referred to as "facility-specific indicator parameters" (see section 3), are determined using measurement methods or calculated via dispersion models, in order to subsequently analyse them from the point of view of environmental-medical aspects. If the bioaerosol concentration at a location in

the vicinity of the facility exceeds the level of the natural background, e.g. as a result of emissions from a facility to be assessed, this is considered to be an undesirable additional load. One difficulty in the analysis of the additional load is the broad spread of the natural background load. Therefore, so-called “orientation values” and “attention values” were introduced (VDI standard 4250 Sheet 1). For total bacteria, for example, the attention value is deduced from the 90th percentile of generally measured background concentrations. If these values are exceeded individually or collectively, an additional load can be estimated to be relevant.

In the VDI standard 4250 Sheet 3, a uniform orientation value is stated for the specified indicator parameters *Staphylococcus aureus*, staphylococci, enterococci and *Enterobacteriaceae*. This is the hypothetical (empirical) lower detection limit of the impinge procedure described in VDI standard 4253 Sheet 3 (wash bottle method for collection of the germs from air) of 80 CFU/m³, respectively, multiplied by a factor of 3. This factor serves as an adjustment for numerous possible uncertainties, such as insufficient measurement data collection, data not always being available as 6- or 8-hour means or only a few measurement data being available. For all four indicator parameters, this produces an orientation value of 240 CFU/m³. Here, it is often criticised that it is nonsensical to specify an orientation value based on a “lower detection limit” of a specified method, especially since the method described does not in principle have a lower detection limit, as has now been set out in the new version o0f the standard VDI 4253 Sheet 3. Compared with the background concentrations of staphylococci measured in various fields (Clauß et al. 2013b), the respective values from the LAI Guide nevertheless appear plausible. The other measurement parameters *Staphylococcus aureus*, enterococci and *Enterobacteriaceae* are less suitable as indicator parameters (cf. section 3). *S. aureus* can only be detected at great effort and expense with the standardised methods and is already contained in the measurement parameter “staphylococci”, where e.g. in chicken farming it accounts for a proportion of approx. 0.1% – 10%. Enterococci are present in very much smaller concentrations than staphylococci in the air and contaminations often occur on the culture media, e.g. of *Aerococcus* spp. The *Enterobacteriaceae* are so sensitive towards the airborne state that they already die before or during sampling and are thus barely detectable.

If the orientation values are exceeded, a special-case examination is conducted according to TA Luft. This examination is supposed to carry out an overall assessment of the available findings within an expert report. Not least as a result of the difficulties and influencing factors described above, the LAI Guide does not rate a concentration of staphylococci, enterococci or enterobacteria at the site of immission as critical until twice or three times the value, i.e. in excess of 480 to 720 CFU/m³. Below these limits, after individual overall assessment according to the TA Luft, a special-case examination can accordingly arrive at the outcome that no harmful effect is to be expected from the additional load. In other words, below these specified concentrations, no damage is expected. In accordance with section 4.2 of the LAI Guide to the determination and analysis of bioaerosol immissions (health analysis by expert report), with regard to the bioaerosols, the measured or predicted immissions, the germ spectrum and specific bioaerosol measurement parameters should be given primary consideration. In addition, the

assessment should also include the degree to which the orientation values were exceeded. If the orientation value for a facility-specific bioaerosol indicator parameter is exceeded by a factor of 2 to 3, but at most by a value of 10^3 CFU/m³, this is to be rated as very critical, as a harmful effect can then no longer be ruled out with sufficient probability.

A special feature of VDI 4250 Sheet 1 is that rather than taking the average citizen as a benchmark, sensitive (e.g. immune-suppressed) persons are used as a reference. In contrast to this, when deciding whether immissions are unreasonable for a neighbour, it is generally not a question of the subjective perceptions of the individual, but the perception of the average citizen (e.g. Münster Administrative Court – 10 L 199/09).

Also in other countries, there are various bodies of rules for the measurement and analysis of bioaerosols, mainly from the field of work safety. They sometimes also contain limit values, although these primarily apply to interior rooms. An overview of "Worldwide exposure standards for mold and bacteria" can be found in Brandys & Brandys (2003). With regard to bioaerosols in livestock farming, e.g. in Russia the "State Standard GOST 12.1.005-88" is applied for the keeping of poultry and defines general hygiene requirements for air at workplaces. This standard was also applied in Ukraine for the analysis of air in poultry stalls (Tsapko et al. 2011). In Australia, there is an unofficial limit value for the endotoxin concentration in the respirable dust fraction (limit value 10 mg/m³ air) of 50 EU/m³ (Banhazi 2008a, Cargill et al. 2002). For total bacteria, 1.0×10^5 CFU/m³ are recommended here. Also in Poland, a maximum concentration of 1.0×10^5 CFU/m³ is now accepted for workplaces (including in livestock farming), although this is frequently exceeded in practice (Dutkiexicz & Gorny 2002, Brodka et al. 2012). In Scandinavia, the same "tolerated background level" of 1.0×10^5 CFU/m³ total bacteria applies (Lavoie et al. 2007, Lavoie & Allard 2004, Goyer et al. 2001, Poulsen et al. 1995a, Malmros et al. 1992) and 1.0×10^3 CFU/m³ for Gram-negative bacteria (Lavoie & Allard 2004, Goyer et al. 2001, Poulsen et al. 1995a, Malmros et al. 1992). In Great Britain, the "Technical Guidance Note M9" (2017) was drafted. It is essentially based on VDI and CEN standards and describes the advantages and disadvantages of sampling systems such as impingers, Andersen impactors and personal filter collectors. It is focused on the standardised detection of mesophilic bacteria, thermotolerant moulds and *Aspergillus fumigatus*. In the USA, the "Institute of Inspection, Cleaning and Restoration Certification (IICRC)" has specified a limit value for moulds of 1.0×10^5 CFU/m³ spores in "Document S520, Standard and Reference Guide for Professional Mold Remediation". If this is exceeded, the wearing of personal respiratory protective equipment is recommended. In South Korea, the "Korean indoor bioaerosol guideline" states a concentration for total bacteria in interior rooms of 800 CFU/m³, which must not be exceeded (Jo & Kang 2005). Some EU countries have limit values for toxins that can be formed by certain organisms. In the Netherlands, the limit value for endotoxins was set at 90 EU/m³ in 2010 (Dutch Expert Committee on Occupational Safety 2010). In the meantime, the health committee recommends a limit of 30 EU/m³ (Winkel & Wouters 2016). The measurement protocols for emission measurements at Dutch livestock facilities are defined by Aarnink et al. (2015) in their "Measurement protocol for emissions of bioaerosols from animal houses".

9 Measures for prevention and reduction

A good stall management and hygiene concept, supported by technical solutions such as exhaust air treatment, enables a reduction of the emission of stall-specific bioaerosols by well over 90%.

A comprehensive and up-to-date review on the options for reducing and preventing bioaerosols in agricultural livestock farming is provided by Winkel et al. (2016). A reduction of emissions can be achieved both by measures in the stalls (process-integrated measures) and in the exhaust air. Fundamentally, it must be ensured that the selected measures do not increase the emissions of the other air pollutants, such as ammonia, methane, odours or dust. In the ideal case, emissions should already be reduced at source or even better avoided there. Here, the most sustainable method is a reduction in the number of animals and the stock density in the stalls (Petersen et al. 1978, Pavicic et al. 2006, Petkov & Tsutsumanski 1975, Sowiak et al. 2011). This also reduces the infective pressure in the stalls and the rates of disease and mortality drop (Spindler & Hartung 2009).

The housing form has a major influence on the amount and the composition of bioaerosols in agricultural livestock farming (Sowiak et al. 2011). In general, housing forms without litter are lower in emissions than those with litter. Relatively high concentrations of moulds and bacteria are often found in the latter (Kim et al. 2008, Letourneau et al. 2009). Housing forms that allow the animals species-appropriate activity are generally more prone to bioaerosols and dust. For example, comparatively high concentrations of airborne bacteria were found in the case of laying hens kept in barns and in aviary systems, both housing forms that offer the hens a relatively large freedom of movement compared with cage rearing or enriched cages (Just et al. 2011, Zheng et al. 2013, Clauß 2014). After the ban on cage rearing in Germany and Switzerland, an increased spread of diseases as a result of the poorer air quality in the alternative housing for laying hens and ensuing economic losses were therefore feared. However, these fears have proved unfounded to date, particularly as a result of the widespread adherence to good hygiene standards and regular vaccinations (Kaufmann & Hoop 2009).

Generally, it can be assumed that a reduction in the dust load in the air also leads to a reduction in the bioaerosol concentration, since bioaerosols represent the biological fraction of dust. One of the most important measures for the prevention of emissions of almost every type is the optimisation of hygiene in the stalls, e.g. by the regular cleaning of contaminated surfaces (Banhazi 2008b, Zucker et al. 2005) and the prompt removal of faeces from the stalls (Chang et al. 2001a). This is one of the strongest sources of bioaerosol components, e.g. also of endotoxins (Eckhardt 2008). In the case of chickens kept in barns, the removal of manure from the floor surface in conjunction with manure belt removal three times a week reduced the total number of airborne bacteria in the stalls by over 90% (Anonymous 2013b). The improvement in air quality

thus achieved also has a positive influence on the health and well-being of the animals (Duchaine et al. 2000, Hadina et al. 2003). In pig farming, stall systems that enable a separation of solid and liquid manure show lower emissions than the conventional systems (Chien et al. 2011, Létourneau et al. 2009). Further potential for reducing bioaerosol emissions is provided by optimised feeding (Sowiak et al. 2011). Various different feedstuffs can contain large amounts of microorganisms (Zhao 2011), a large number of which can also find their way into the air, depending on how the feed is administered (Chang et al. 2001a, Pearson & Sharples 1995). In this respect, above all manual feeding by the farmer can lead to high emissions (Kim et al. 2008). In the feeding of cattle, large amounts of actinomycetes and moulds can be released (Evans 2017). Here, a preceding drying of the feed can reduce emissions (Dalphin et al. 1991, Ferri et al. 2003). Generally, the feed should be prevented from being swirled up into the air. Here, an intelligent ventilation concept that ensures a uniform and gentle flow of air in the stalls can also contribute to the reduction of bioaerosols (Sowiak et al. 2011, Brodka et al. 2012, Hillmann et al. 1992).

Beside the influence of the different housing forms and the management-related reduction measures, there are further technical possibilities for reducing bioaerosol emissions (Aarnink et al. 2005). Various different spray and application systems for oils that are designed to create a film on the floor of the stalls have been reported to be particularly effective (Lemai et al. 2009). Above all aerosol formation from faeces-containing substances is to be prevented by such systems (Eckhardt 2008). The spraying particularly of essential oils has been found by various authors to markedly reduce bioaerosols (Bakutis et al. 2011, Kim et al. 2006, Siggers et al. 2011, Cravens et al. 1981). In particular, a reduction in the concentration of coliform bacteria and staphylococci in the air was determined (Witkowska & Sowinska 2013, Witkowska & Sowinska 2017). Beside oils and oil/water mixtures (Kirychuk et al. 1999), the atomisation of various different disinfectants (Shokri 2016) or the spraying of “slightly acidic electrolyzed water” are considered to be effective (Zheng et al. 2013). Exhaust air cleaning systems have proved to be the most effective method for reducing bioaerosol emissions up to now. Above all biological and chemical exhaust air cleaners certified according to the DLG Signum Test (DLG 2006) as well as combined systems, assuming that they are operated correctly, can be demonstrated to reduce not only stall-specific airborne microorganisms by over 90%, but also ammonia, odour and dust by more than 70% (Anonymous 2013a, Bayerisches Landesamt für Umwelt 2015b, Chmielowiec-Korzeniowska et al. 2007, Ottengraf & Konings 1991, Scharf 2004, Seedorf & Hartung 1999, Martens et al. 2001, Schirz et al. 2003, Clauß et al. 2013c, Sächsisches Landesamt 2017). Whereas there is a whole range of systems available from various different manufacturers for pig farming and an effective reduction of emissions has been demonstrated for ammonia, dust, airborne microorganisms and odour, suitable methods have yet to be developed for poultry farming (Chmielowiec-Korzeniowska et al. 2007, Hahne 2014). Initial orientational measurements of systems in the keeping of broilers yielded separation rates of between 70% and 90% and were thus in line with the values determined for the separation of dust. Due to the high volume flows and the resulting short retention times, exhaust air cleaning systems for broilers are most designed as single-stage chemowashers, whereby they operate with acidic wash water (pH value 3-5), in order to ensure reliable separation of ammonia (Clauß & Hahne 2017). However, acid-

tolerant fungi can grow in the acidic wash solutions, unless dust as a nutrient source has been separated in a preceding process stage or fungicides are added to the wash water. Above all in poultry farming, at high dust concentrations, dry filter walls are considered to be a sensible additional measure. A further area of research here for this purpose are electrofilters, although they need to be investigated further for application in animal stalls.

From the point of view of animal welfare, “end of pipe” exhaust air cleaning systems are not a desirable solution, as they do not reduce the concentrations of air pollutants in the stalls. A better solution would be retrofittable exhaust air cleaning systems that, e.g. can be integrated into available collection ducts, or small, e.g. 2-stage circulating air washers for integration into a stall compartment (Schulz et al. 2013). A circulating air washer with independent air recirculation must at the same time provide for dust removal (including the reduction of bioaerosols) as well as ammonia separation. The operating times of the systems should be organized in such a way that cleaning is synchronised with removal of the animals from the stalls. Such systems could thus contribute to an improvement of air quality in the stalls and also make a contribution to animal welfare (Clauß & Hahne 2017).

In future, the focus should be directed particularly towards exhaust air cleaning systems that can be retrofitted in existing animal stalls and can already reduce the air pollutant concentrations in the stalls, thus also making a contribution to animal welfare. Combined with intelligent ventilation technology and conditioning of incoming air, the bioaerosol emissions could be further reduced. A combination of the various different measures is considered to be particularly promising, starting with the housing methods, via the management and hygiene concept, feeding and manure removal techniques, up to the above-mentioned technical solutions in the stalls and in the exhaust air.

10 Future challenges

Clean air is our most vital resource. It must be our aim to protect it in the future. In the field of agricultural livestock farming, bioaerosols are relevant air pollutants. At the concentrations at which they occur there, above all in the stalls, they act synergistically with the other components of the stall air and can have a negative impact on health. Given the further increase in livestock numbers to be expected in the future, the problems will inevitably grow. We are a long way away from a valid health analysis or even the deduction of a dose-effect relationship, but the first steps have been taken in this direction and it is now a question of rigorously pursuing this path. The coming challenges vary in nature. From the viewpoint of measurement technology, the bioaerosol components must be further characterised in order to provide a more precise picture of the composition of the microbial community in the air of animal stalls. Here, the culture-based methods can increasingly make a contribution in combination with molecular biological methods in future. Advances are constantly being made in both techniques. Increased attention should be focused on the airborne viruses, which seem to have been almost completely neglected in Germany to date, as they include important disease pathogens that also have the potential to cause pandemics. In this respect, above all the zoonosis pathogens should be investigated. As a result of the high diurnal fluctuations in concentrations, bioaerosols should be measured not only during the day but also at night in the future. Virtually no data are available for the night. Likewise, the distribution of the bioaerosols in the health-relevant particle size fractions must be investigated further, as this is important both for the environmental-medical analysis and for dispersion prognoses. Under no circumstances may fractionated dust measurements of PM_{10} or $PM_{2.5}$ replace the measurement of bioaerosols in future, as the latest investigations have shown that only a small percentage of the bioaerosols from livestock farming are to be found in these two particle fraction. Processes that play a role in the transmission of bioaerosols from the emission source up to immission should also be investigated in more detail, of primarily interest here being the tenacity of microorganisms. A further standardisation or harmonisation of methods for better comparability of results in investigations of specific issues is desirable, on a national level, e.g. within the context of VDI standards, or also internationally, e.g. within the context of the VERA protocol. In the area of the whole chain of production in agricultural livestock farming, above all systems for keeping the animals have been investigated up to now. However, little is known about, e.g. what additional bioaerosols are released in the processing or the transport of feedstuffs, in meat production or the spreading of liquid manure. In view of its percentage share of total poultry production, there is also a marked deficit of knowledge on bioaerosols in turkey farming. In order to prevent the formation of bioaerosols already at source, good stall management is important. For emission reduction, exhaust air cleaning is currently the most effective procedure available to significantly reduce bioaerosols but at the same time also other relevant air pollutants. Here, in future, the focus should be directed towards the development of exhaust air cleaning systems that can be adapted and as far as possible retrofitted to existing facilities. These should preferably be installed in the stalls in order to

improve the air quality already at this point. Thus, these systems can make a contribution simultaneously to environmental protection and to animal welfare. Here, a combination of this technology with other available reduction methods, e.g. the conditioning of incoming air, is considered to be particularly promising.

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