AIRBORNE MICROORGANISMS AND DUST FROM LIVESTOCK HOUSES

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SUMMARY

The objective of this study was to evaluate the efficiencies and suitability of samplers for airborne microorganisms and dust, which could be used in practical livestock houses. Two studies were performed: 1) Testing impaction and cyclone pre-separators for dust sampling in livestock houses; 2) Determining sampling efficiencies of four bioaerosol samplers for bacteria and virus.

Study 1. The overloading problem of the EU reference impaction pre-separator (IPS) was tested in layer houses and compared with cyclone pre-separators (CPS) for sampling PM_{10} and $PM_{2.5}$. Study 2. Physical and biological efficiencies of Andersen 6-stage impactor, all glass impinger (AGI-30), high air flow rate sampler OMNI-3000, and MD8 with gelatin filter were investigated for collecting aerosolized bacteria, *Enterococcus faecalis*, *Escherichia coli*, *Campylobacter jejuni* and *Mycoplasma synoviae* and live Gumboro vaccine virus. A tracer (uranine) was used to determine physical efficiencies and bioaerosol deposition. The study was done in a HEPA isolator (volume: 1.3 m^3).

The results show the PM_{10} IPS did not become overloaded in 24 h measurements in layer houses, whereas $PM_{2.5}$ IPS became overloaded within 1 h. CPS did not become overloaded during 48 h sampling of both dust fractions. The OMNI-3000 (62%) had lower physical efficiency than the MD8, while the other samplers had similar efficiencies as MD8. All the bioaerosol samplers had high biological efficiencies for all four bacterial species, except for *C. jejuni* (1%) when measured with the OMNI-3000 and for *E. coli* (38%) and *C. jejuni* (2%) when measured with the MD8. The biological efficiencies of the Andersen impactor (61%), the AGI-30 (90%) and the MD8 (163%) were not significantly different from 100% for collecting the aerosolized virus. However, the biological efficiency (23%) of the OMNI-3000 was significantly lower than 100%.

INTRODUCTION

Airborne microorganisms in livestock houses attach to dust particles. They can emit to the ambient air through the ventilation exhausts. Emission of pathogenic microorganisms pose infection risk to animals in other nearby livestock units and/or to humans. Lab-scale experiments have confirmed short distance airborne transmission of some microorganisms from animal to animal: it was found that healthy animals kept physically but not aerially separated from infected animals became infected (Berthelot-Herault et al. 2001; Brockmeier and Lager 2002). Also, the porcine reproductive and respiratory syndrome virus (PRRSV) was collected kilometers away from the source farm (Otake et al. 2010). However, to date these findings still have not incontrovertibly been linked to long distance airborne transmission of microorganisms between farms. Furthermore, there is still lack of knowledge about the role of dust in airborne transmission. Knowledge gaps in airborne transmission need to be filled and effective transmission control technologies require to be developed (Zhao et al. 2011a). Therefore, investigations on airborne microorganisms and dust should be carried out, such as source identification, suspension, physical and biological decay in airborne transportation, deposition in respiratory tracts, and infection in recipients. Almost all the above mentioned investigations cannot be performed without accurately and precisely measuring airborne microorganisms and dust from livestock production systems.

Measurements of airborne microorganisms is performed with bioaerosol samplers applying different principles, including impaction, impingement, cyclone forces and filtration. Because airborne microorganisms may either be physically miss-collected or biologically inactivated by various sources of stresses during sampling, the efficiencies of these samplers are generally known to be imperfect. To date, the efficiencies of the samplers for collecting different microbial species have not been well established. Notably, there is no standard protocol for sampling airborne microorganisms that specifies the requirements for hardware and also the procedures immediately prior, during and after the sampling. The lack of a protocol makes it difficult to interpret and compare the results of different studies. The sampling protocol for collecting PM_{10} and $PM_{2.5}$ in ambient air has been legislated by the EU commission and US EPA (European Commission 1998; 2005a; US EPA 2010). These sampling techniques, however, might not be suitable to sample dust in livestock houses, because the concentrations and particle sizes of dust in livestock houses are profoundly different from those in the ambient air, and this may compromise the efficiency and accuracy of the sampling. There is therefore an urgent need to develop a technique and eventually a protocol suitable for sampling dust in livestock houses.

The objective of this study was to investigate the suitability and efficiency of bioaerosol and dust samplers for measuring airborne microorganisms and dust from livestock production systems. In details, experiments were carried out to:

- investigate the overloading problem of EU reference dust sampler with an impaction pre-separator (IPS), when used for measuring PM_{10} and $PM_{2.5}$ in the dusty environment of layer houses; evaluate the cyclone pre-separator (CPS) as a reference equivalent pre-separator for PM sampling in the dusty environment of livestock houses following the EU standard procedure.
- assess the sampling efficiencies of four bioaerosol samplers (Andersen 6-stage impactor, AGI-30, OMNI-3000, and MD8 with a gelatine filter) on measuring aerosolized *E. faecalis*, *E. coli*, *M. synoviae*, *C. jejuni*, and Gumboro vaccine virus.

MATERIAL AND METHODS

Evaluation of IPS and CPS in livestock houses

Sampler and pump

EU reference dust sampler consists an IPS and a filter holder. In the IPS, a flat impaction plate was rubbed with grease and placed under eight impaction nozzles. Larger particles strike the plate at speed and are retained on the impaction plate because of their inertia. The smaller target particles (PM_{10} or $PM_{2.5}$) are carried along in the air stream and are collected on the downstream filter. The airflow rate through the inlet head of an IPS is 2.3 m^3 h^{-1}. More detailed descriptions of the EU sampler can be found in EU documentations (European Commission 1998; 2005a).

The candidate sampler consists of an air inlet head, a CPS (URG corp., US) and a filter holder. The CPS uses the centrifugal principle to separate large particles trapped in a dust collector. PM_{10} or $PM_{2.5}$ are conveyed in the air stream and collected by a glass fibre filter in the filter holder. The airflow used for a CPS is set at 1 m^3 h^{-1}.

Charlie HV pumps (Ravebo Supply b.v., Brielle, the Netherlands) were used to suck air through the two types of samplers. These pumps are able to maintain constant airflow (< 2% nominal value) during dust sampling.
Mass of each filter before and after sampling was measured. The PM_{10} or $PM_{2.5}$ concentration was calculated by dividing the mass difference by the total volume of air passing through the filter. The unit of dust was expressed as μg m^{-3}.

Overloading of IPS and CPS

When a pre-separator becomes overloaded, it's greased plate (of IPS) or dust collector (of CPS) is no longer able to separate larger particles from the incoming air stream. Therefore, dust particles in the whole size range are transported to the downwind filter. This results in an overestimation of PM_{10} or $PM_{2.5}$ concentration. Understanding above mentioned phenomenon, the overloading of a pre-separator was determined in this study by comparing the PM concentration collected with a sampler without cleaning the pre-separator during sampling (control) to that collected by a sampler with regular cleaning the pre-separator (treatment). When the dust concentration measured by a control pre-separator is higher than that measured by a treatment pre-separator, the pre-separator was overloaded. See the study by Zhao et al. (2009) for details.

Validating CPS

To be qualified as the reference equivalent device, CPS should be able to perform precise and accurate measurements. The equivalent test was carried out following the EU standard procedure as required (European Commission 1998). Ninety-six pairs of 24 h measurements, 48 for PM_{10} and 46 for $PM_{2.5}$, were conducted in various environments: livestock houses (three fattening pig houses, one broiler house and one dairy barn); an industrial workplace; and in the ambient air. For each pair of measurements we used one IPS (as the reference sampler) and two CPSs (as the candidate sampler).

Sampling efficiency of bioaerosol sampler

Sampling efficiency includes physical and biological efficiency. The physical efficiency of a sampler reflects how well the sampler aspire, transport and retain the airborne particles from the ambient air to its collection medium. The biological efficiency reflects how well the viability of the microorganisms is maintained during sampling. In this study, the physical and biological efficiencies of four bioaerosol samplers (Andersen 6-stage impactor, AGI-30, OMNI-3000, and MD8 with gelatin filter) on collecting five microbial species (*E. faecalis*, *E. coli*, *C. jejuni*, *M. synoviae*, and Gumboro vaccine virus) were investigated. This was done by aerosolizing the microbial suspensions (with or without a physical tracer) in an HEPA isolator, and by collecting the aerosolized microorganisms with the samplers. The physical efficiency was calculated based on the amount of tracer collected; and the biological efficiency was calculated based on the microorganisms/tracer ratio. More details can be found in Zhao et al. (2011b; 2011c; 2011d).

RESULTS AND DISCUSSION

Dust sampler

Overloading of IPS and CPS

The results show that $PM_{2.5}$ IPS was overloaded within 8 h when used for sampling dust in a layer room. The overloading of $PM_{2.5}$ IPS was not solved even with a 1 h plate cleaning interval (Zhao et al. 2009), thus it cannot be used for PM sampling in such a dusty environment. Compared to IPS, $PM_{2.5}$ CPS was more resistant to high dust concentrations. It is shown that the $PM_{2.5}$ CPS did not become overloaded during 24 h sampling. Both PM_{10} IPS and PM_{10} CPS had no overloading problem.

Validating CPS

The results show that both PM_{10} and $PM_{2.5}$ CPSs were qualified as the reference equivalent pre-separator for the EU IPS, when these candidate samplers were used in environments with low dust concentrations (<100 μg m^{-3} for PM_{10}, and working place/ambient air for $PM_{2.5}$). The relative two side 95% confident interval (CI_{95}) of PM_{10} CPS (6%) is almost within the required value (5%); and the PM_{10} concentrations measured by the CPS was within the acceptance envelope: ($y = x \pm 10$) μg m^{-3}, where y is the PM concentration measured by CPS and x is the PM concentration measured by IPS. The absolute CI_{95} of $PM_{2.5}$ CPS (2.3 μg m^{-3}) is within the required value of 5 μg m^{-3}; and the $PM_{2.5}$ concentrations measured by the CPS was within the acceptance envelop: ($y = x \pm 10$) μg m^{-3}.

The PM_{10} concentrations measured by PM_{10} CPS were systematically lower than those measured by IPS in less dusty environments, and were higher in dusty environments. Therefore, the PM_{10} concentration measured by CPS should be corrected (Equations 1 and 2).

$$y = 1.09x \quad (x \le 223 \text{ μg m}^{-3}) \qquad (1),$$

$$y = 0.83x + 57.5 \quad (x > 223 \text{ μg m}^{-3}) \qquad (2)$$

y: is the calibrated concentration, μg m^{-3}; x: is the concentration measured with CPS, μg m^{-3}.

Bioaerosol sampler

Physical and biological efficiency

The physical efficiencies of the Andersen impactor and the AGI-30 were not different from the high efficient sampler - MD8. However, the physical efficency of the OMNI-3000 (62%) was signficantly lower than that of the MD8. The biological efficiencies of the samplers on collecting all microbial species were not different from 100%, except for C. jejuni (1 ± 1%) and Gumboro vaccine virus (23 ± 10%) when sampled by OMNI-3000, and for *C. jejuni* (2 ± 1%) and *E. coli* (38 ± 10%) when sampled by MD8. The significant lower efficiencies suggested that these microbial species were inactivated due to sampling stress from samplers.

The total sampling efficiency (combination of physical and biological efficiencies) and the detection limit were calculated from the efficiency data and are listed in Table 1. This information may be helpful for selecting samplers suitable for practical measurements. The Andersen impactor and the AGI-30 are suitable for sampling all microbial species because their total efficiencies are high. The MD8 is suitable for sampling *E. faecalis*, *M. synoviae* and Gumboro vaccine virus, but not *E. coli* and *C. jejuni*. Although the OMNI-3000 has low sampling efficiencies of 62% for *E. faecalis*, *E. coli* and *M. synoviae*, and 14% for Gumboro vaccine virus, it could still be a suitable sampler because its high air flow rate gives low detection limits. The OMNI-3000 cannot be used for *C. jejuni* because this species would be seriously inactivated by sampling stress. The Andersen impactor has high sampling efficiency on Gumboro vaccine virus (100%), however, its detection limit (4.1 $\log_{10}$ EID50 m^{-3}) is highest among all samplers, because virus was lost in air sample handling (Zhao et al. 2011b).

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

	E. faecalis	*E. coli*	*C. jejuni*	*M. synoviae*	Gumboro
Sampling efficiency (%)[1]					
Andersen[2]	100	100	100	100	100
AGI-30	100	100	100	100	100
OMNI-3000	62	62	0.6	62	14
MD8	100	38	2	100	100
Detection limit[3]					
Andersen[2]	3.9	3.9	3.7	3.8	4.1[4]
AGI-30	3.9	4.2	3.8	4.0	3.3
OMNI-3000	2.5	2.5	4.5	2.7	2.5
MD8	4.1	4.4	5.4	4.3	2.9

[1] 100% means that in our study the measured efficiency was not significantly different from 100%.
[2] Physical and biological efficiencies of the Andersen impactor were set to 100% because it collected similar amounts of viable microorganisms as the AGI-30.
[3] Detection limit was calculated based on a 2 min sampling duration. The unit of DL is $\log_{10}$ CFU m^{-3} for bacteria, and $\log_{10}$ egg infective dose 50% (EID_{50}) m^{-3} for virus.
[4] Detection limit was calculated by assuming agar plates of Andersen impactor were rinsed 1 h after sampling.

CONCLUSIONS

EU reference $PM_{2.5}$ IPS cannot be used for dust sampling in livestock production systems because of overloading. PM_{10} and $PM_{2.5}$ CPSs are equivalent to IPS when used in environments with low dust concentrations, and are more resistant to dusty environments. Therefore, CPSs are promising devices for dust sampling in livestock production systems.

The physical and biological efficiencies of the bioaerosol samplers vary. In order to perform accurate measurement of airborne microorganisms, the efficiency of a sampler should be investigated beforehand. In this study, we found OMNI-3000 was not suitable for sampling *C. jejuni* and MD8 was not suitable for *C. jejuni* and *E. coli*.