

Airborne virus sampling – Efficiencies of samplers and their detection limits for infectious bursal disease virus (IBDV)

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Abstract

Introduction. The airborne transmission of infectious diseases in livestock production is increasingly receiving research attention. Reliable techniques of air sampling are crucial to underpin the findings of such studies. This study evaluated the physical and biological efficiencies and detection limits of four samplers (Andersen 6-stage impactor, all-glass impinger “AGI-30”, OMNI-3000 and MD8 with gelatin filter) for collecting aerosols of infectious bursal disease virus (IBDV).

Materials and Method. IBDV aerosols mixed with a physical tracer (uranine) were generated in an isolator, and then collected by the bioaerosol samplers. Samplers’ physical and biological efficiencies were derived based on the tracer concentration and the virus/tracer ratio, respectively. Detection limits for the samplers were estimated with the obtained efficiency data.

Results. Physical efficiencies of the AGI-30 (96%) and the MD8 (100%) were significantly higher than that of the OMNI-3000 (60%). Biological efficiency of the OMNI-3000 (23%) was significantly lower than 100% ($P < 0.01$), indicating inactivation of airborne virus during sampling. The AGI-30, the Andersen impactor and the MD8 did not significantly inactivate virus during sampling. The 2-min detection limits of the samplers on airborne IBDV were $4.1 \log_{10} 50\% \text{ egg infective dose (EID}_{50}) \text{ m}^{-3}$ for the Andersen impactor, $3.3 \log_{10} \text{EID}_{50} \text{ m}^{-3}$ for the AGI-30, $2.5 \log_{10} \text{EID}_{50} \text{ m}^{-3}$ for the OMNI-3000, and $2.9 \log_{10} \text{EID}_{50} \text{ m}^{-3}$ for the MD8. The mean half-life of IBDV aerosolized at 20 °C and 70% was 11.9 min.

Conclusion. Efficiencies of different samplers vary. Despite its relatively low sampling efficiency, the OMNI-3000 is suitable for use in environments with low viral concentrations because its high flow rate gives a low detection limit. With the 4 samplers investigated, negative air samples cannot guarantee virus-free aerial environments, which means that transmission of infectious agents between farms may still occur even when no virus has been detected.

Key words

airborne transmission, sampling efficiency, bioaerosol, Gumboro virus

INTRODUCTION

Airborne transmission has been epidemiologically implicated as one possible route for disease transmission in the livestock production industry [1, 2, 3, 4, 5]. Although the importance of airborne transmission has not been well understood, the epidemics of infectious viral diseases have been dealt with by mass slaughter, not only of the animals on infected farms, but also of those on healthy farms in the vicinity which are potentially at risk of airborne infection. An impressive example is the foot-and-mouth epidemic in the United Kingdom in 2001 that applied a slaughter strategy based on epidemiological models that included airborne transmission mechanism. Although the epidemic was finally controlled, veterinarians have been sharply critical of what they perceived as over-slaughtering [6]. Epidemiologists and veterinarians clearly have very different perspectives on airborne transmission; these could be reconciled by pragmatic air sampling of infectious viral agents in the air in epidemics.

Adopting a neutral standpoint in the debate, we believe that airborne virus sampling may enable the disputing parties to converge, and can be helpful for making rational decisions during epidemics for at least two reasons. The first is that the air sampling results will show the dispersion patterns of the infectious virus outside the infected farms, thus improving the reliability of the epidemiological models. Secondly, the importance of airborne transmission between farms could be determined using veterinary knowledge (e.g. about initial infection route, minimum infectious dose of virus) along with the outcomes of air sampling. This can result in a more reasonable culling strategy that avoids unnecessary slaughtering. Two questions remain: how to achieve accurate air sampling and how to ensure a virus-free air environment? These questions can be addressed by investigating the sampling efficiency and limitations of bioaerosol samplers.

Sampler efficiency can be categorized into physical and biological efficiencies. Physical efficiency is defined as the ratio of the number of microorganisms collected by the sampler to the number present in the sampled air volume. This depends on the sampler design, the size of bioaerosols, and the aspiration, transportation and retention of bioaerosols in a sampling process. Biological efficiency is the ratio of the number of viable microorganisms recovered from the air

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sample to the number that the sampler is expected to collect. The biological efficiency depends on the microbial resistance to the sampling and analysis stress.

The efficiencies of bioaerosol samplers have been extensively investigated [7, 8, 9]. Some studies conducted measurements with different samplers side-by-side in isolators or on sites where the microbial concentrations were unknown [10, 11]. In these studies, relative efficiency (sampler vs. sampler) was obtained; this can be used as the criterion for selecting the optimal sampler for virus sampling. However, in airborne transmission models, the exact concentration of viable airborne virus is required, as it reflects the extent of virus dispersion and determines the infection probability of recipients at risk. Thus, absolute efficiency (sampler vs. air), which allows the amount of virus collected by a sampler to be related to the actual airborne virus concentration, must be studied. Such an efficiency can be investigated by conducting air sampling in environments with known viral concentrations (e.g. an air environment created by aerosolizing specified amounts of viral suspensions) [12, 13].

As the overall efficiency of a sampler might be imperfect [14, 15], a negative air sample (virus free) does not necessarily mean that airborne virus is absent but could mean that the viral concentration is below the sampler's detection limit. Previous studies have proved that airborne transmission between animals may occur even though no infectious agents have been recovered from the air samples [16, 17]. Thus, care should be taken when interpreting the negative air samples. This is especially true when carrying out airborne impact studies by sampling airborne infectious agents downwind of animal houses, where the pathogen concentrations decline gradually until they are undetectable [18, 19]. Assuming that infection through an airway fits a single-hit model (i.e. one infectious bioaerosol may trigger an infection in a recipient) [20], a safety area is not at the spot where negative air samples are obtained, but instead is some distance away. That distance can be calculated from the sampler's detection limit, which has not been well established for most of the samplers and viral species.

OBJECTIVES

The objective of the presented study was to investigate the sampling efficiencies (both physical and biological) and detection limits of four bioaerosol samplers for collecting

aerosolized infectious bursal disease virus (IBDV) in a HEPA-filtered isolator. In addition, half-life of IBDV was examined at 20 °C temperature and 70% relative humidity (RH)

MATERIALS AND METHODS

General experimental set-up. To obtain sampling efficiency in an aerosolization experiment it is necessary to know the actual viral concentration in the airspace and the amounts of infectious virus collected by the samplers. In practice, these two concentrations cannot be directly measured, as the virus may potentially lose infectivity in some non-sampling processes, such as aerosol generation and air sample handling. Thus, in this study, infectivity losses in the non-sampling processes were investigated and excluded from the efficiency calculations. Details on infectivity loss during aerosol generation and during sample handling are given below under the headings 'Loss during aerosolization' and 'Loss in air sample handling procedure'.

Figure 1 shows the overview of the experimental set-up: Virus-containing aerosols were generated in the isolator from viral suspension with uranine (fluorescein sodium, CAS#518-47-8, Fisher Scientific, Landsmeer, the Netherlands). In the meantime, the aerosols gently settled on a Petri dish that was used for evaluating the infectivity loss during aerosolization. At two moments (0 min and 20 min) after aerosol generation, samplers started sampling for a 2-min period. The resulting air samples were then analyzed in the laboratory to obtain the numbers of virus. Whether or not the air samples were subjected to extra handling processes depended on the sample type.

Bioaerosol samplers. The four bioaerosol samplers investigated were the Andersen 6-stage impactor (TE-10-800, Pacwill Environmental Ltd., Canada), the all-glass impinger (7540, Ace Glass Inc., USA), the OMNI-3000 (Evogen Inc., USA), and the MD8 airscan (16746, Sartorius, Goettingen, Germany) with gelatin filter.

The Andersen impactor [21] collects bioaerosols and separates them in different stages according to their particle sizes. Operating at an air flow of 28.3 L min⁻¹, the Anderson impactor collects particles > 7.1 µm in stage 1, particles 4.7–7 µm in stage 2, particles 3.3–4.7 µm in stage 3, particles 2.1–3.3 µm in stage 4, particles 1.1–2.1 µm in stage 5 and particles 0.65–1.1 µm in stage 6. In this experiment, each

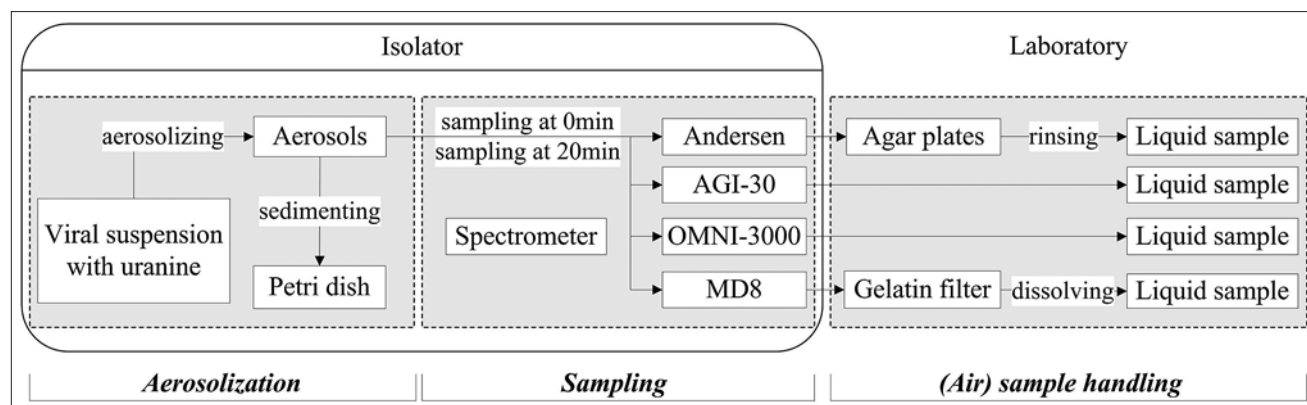


Figure 1. Overview of the experimental set-up, showing the three main processes involved (i.e. aerosolization, sampling and sample handling) and where they took place (HEPA isolator or laboratory)

glass petri plate of the Andersen impactor was filled with 27 mL Mycoplasma Experience (ME) agar (Mycoplasma Experience, Reigate, UK) as the collecting medium.

The all-glass impinger (AGI-30) impinges airborne microorganisms into 20 mL liquid medium at an air flow of 12.5 L min⁻¹. In this experiment, the medium used to collect the IBDV was Hanks' balanced salt solution (HBSS, GIBCO, Breda, The Netherlands) with 0.005% Silicone Antifoam (85390, Sigma-Aldrich Inc., The Netherlands).

During sampling, the OMNI-3000 operates at a high airflow rate of 300 L min⁻¹. When sampling, the collection fluid, 10 mL Phosphate Buffered Saline (PBS), is drawn from a cartridge into a contactor where PBS rotates and comes into contact with the incoming air. The microorganisms in the incoming air are collected in the PBS. The PBS containing microorganisms drains back to the cartridge automatically after sampling.

The MD8 collects microorganisms on a gelatin filter (17528–80-ACD, Sartorius, Germany) by air filtration at air flow rate of 30 L min⁻¹. After sampling in this experiment, the virus-loaded filter was dissolved in 20 mL HBSS medium at 37 °C for further virus quantification.

Viral suspension, viral titration, and uranine analysis.

IBDV (a non-enveloped double-strand RNA virus) is the agent of infectious bursal disease in poultry. It can lead to immunosuppression and mortality in young chickens. In this study, IBDV was chosen as a model virus for airborne transmission because it had been detected in the exhaust air of a broiler room [22], and therefore a potential infection threat to healthy animals through airborne transmission. For safety considerations, a vaccine strain was used instead of a verogenic strain.

IBDV (virion diameter = app. 60–90 nm, Gallivac IBD, L258577) was provided by Merial BV, Velsbroek in The Netherlands. One vial contained 7 log₁₀ 50% egg infective dose (EID₅₀) of virus. The unit of EID₅₀ is the amount of infectious virus that will cause the death of 50% of inoculated embryonated eggs. The virus suspension for an aerosolization was made by suspending eight vials of IBDV in 20 mL HBSS with the addition of 0.1% uranine, which was shown in our previous experiment to have no influence on the IBDV viability.

The concentration of infective virus in a suspension was determined with an egg embryonic death test. Possible bacterial contamination, if any, was excluded by addition of antibiotics in a suspension. The suspension was serially diluted (10⁻¹, 10⁻², 10⁻³, 10⁻⁴, 10⁻⁵, 10⁻⁶). A volume of 0.5 mL of each serial dilution was injected into the allantoic cavity of five 9-day-old specific pathogen-free (SPF) embryonated eggs. The inoculated eggs were incubated at 37 °C for 7 days, and the viral concentration was calculated based on the death of the embryos and specific abnormalities of the living embryos, using the Spearman–Karber method [23], which obtains an estimate of a weighted average of the mid-points between consecutive log viral concentrations [24]. The principal variability of this estimation is from 0.03–0.1 log₁₀ EID₅₀ mL⁻¹ [24]. The final viral concentrations in the viral suspensions for aerosolization were 6–7 log₁₀ EID₅₀ mL⁻¹. The viral concentrations in the air samples were determined in the same way; each sample was analyzed twice to reduce the variation. The airborne viral concentrations, in EID₅₀ m⁻³ or log₁₀ EID₅₀ m⁻³, were obtained by dividing the amounts of

collected virus by the air volumes that were drawn through the bioaerosol samplers

The uranine concentrations were analyzed with a fluorescence detector (HP 1046 A, HP, US) with a detection limit of 0.002 µg mL⁻¹. The excitation and emission wavelengths for uranine were 494 nm and 521 nm, respectively.

Isolator. Aerosolization was performed in a 1.38 m³ stainless steel isolator (1.94×0.75×0.95 m, Beyer and Eggelaar, Utrecht, The Netherlands) with HEPA filters installed at both inlet and outlet. A temperature and humidity sensor (HygroClip2, ACIN Instrumenten BV, Rijswijk, The Netherlands) was installed in the middle of the isolator.

Aerosolization and air sampling. Wet aerosols were used in the presented study because this model best represents the mass vaccination of animals, especially in the poultry industry [25, 26], and also represents the aerosols originating from coughing, sneezing and urine splashing in human beings and animals.

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

The size distribution of virus-containing aerosols was monitored during air sampling by a spectrometer (Model 1.109, Grimm Aerosol Technik GmbH & Co., KG, Airing, Germany). The spectrometer measures aerosols in size range of 0.25–32 µm.

Two sets of 4 samplers were pre-installed in the isolator and tested at a time. Each set comprised one of the four bioaerosol samplers. The four samplers in the first set were installed close to each other in an area of 0.4 m × 0.4 m in the middle of the isolator, and took samples at 0 min after aerosolization. After the sampling, they were replaced by the second set of four samplers, which took air samples at 20 min after aerosolization. The sampling duration was 2 min for all samplers and for each of the two measurement moments. A previous study had revealed that with such a setup, the samplers hardly interfered with each other [27].

The inlet of the isolator was kept open during sampling to allow air to enter (70 L min⁻¹) to compensate for the negative pressure in the isolator induced by the samplers. The isolator was ventilated (70 m³ h⁻¹) for 120 min between two aerosolization events to remove traces of airborne virus.

Aerosolization was repeated 5 times at an average temperature of 20 °C and RH of 70%.

Nomenclature		
L_a	loss of viral infectivity during aerosolization	%
$C_{\text{petridish,virus}}$	viral concentration in sample on Petri dish	EID ₅₀ mL ⁻¹
$C_{\text{suspension,virus}}$	viral concentration in original suspension for aerosolization	EID ₅₀ mL ⁻¹
$C_{\text{petridish,tracer}}$	uranine concentration in sample of Petri dish	µg mL ⁻¹
$C_{\text{suspension,tracer}}$	uranine concentration in original suspension for aerosolization	µg mL ⁻¹
L_h	loss of viral infectivity in air sampling handling procedure	%
$C_{\text{liquid,virus}}$	viral concentration in liquid sample transferred from agar plate (Andersen impactor) or gelatin filter (MD8)	EID ₅₀ mL ⁻¹



Nomenclature		
$V_{liquid,virus}$	volume of liquid sample transferred from agar plate (Andersen impactor, app. 5–6 mL) or gelatin filter (MD8, 20 mL)	mL
$V_{suspension,virus}$	volume of original suspension added to an agar plate (0.1 mL) or a gelatin filter (0.5 mL)	mL
E_p	physical efficiency of sampler	%
E_b	biological efficiency of sampler	%
$C_{sampler,tracer}$	uranine concentration in air measured with a sampler	$\mu\text{g m}^{-3}$
$C_{MD8,tracer}$	uranine concentration in air measured with MD8	$\mu\text{g m}^{-3}$
$C_{sampler,virus}$	viral concentration in air measured with a sampler	$\text{EID}_{50} \text{ m}^{-3}$
DL	detection limit of sampler	$\text{EID}_{50} \text{ m}^{-3}$
B	viral concentration calculated by assuming that an undiluted sample infects 3 eggs (out of 5), ($\beta = 2.5$)	$\text{EID}_{50} \text{ mL}^{-1}$
V_{liquid}	total volume of liquid sample from bioaerosol samplers (e.g. 20 mL for MD8)	mL
V_{air}	air volume drawn through bioaerosol samplers in 2-min sampling	m^3
$t_{1/2}$	Half-life of IBDV	min
$C_{sampler,virus}^0$	viral concentration in air measured with a sampler at 0 min after aerosolization	$\text{EID}_{50} \text{ m}^{-3}$
$C_{sampler,virus}^{20}$	viral concentration in air measured with a sampler at 20 min after aerosolization	$\text{EID}_{50} \text{ m}^{-3}$
T	Time span between 2 sampling moments in each aerosolization ($T=20$)	min
A	correction factor for dilution due to ventilation and deposition ($\alpha = \Sigma(C0_{MD8,tracer} / C20_{MD8,tracer})/5$)	
$C_{MD8,tracer}^0$	uranine concentration in air measured with MD8 at 0 min after aerosolization	$\mu\text{g m}^{-3}$
$C_{MD8,tracer}^{20}$	uranine concentration in air measured with MD8 at 20 min after aerosolization	$\mu\text{g m}^{-3}$

Loss during aerosolization. A Petri dish containing 20 mL of HBSS was placed on the floor of the isolator and exposed to the air to collect the virus and uranine during the 1.2 min of aerosolization. The viral aerosols settled gently and became immersed in the HBSS. It was assumed that this sampling procedure did not cause any stress to the virus infectivity. Immediately after aerosolization, the liquid sample in the Petri dish was vortexed and sent for viral and uranine analysis. The loss of viral infectivity during aerosolization (L_a) was calculated with Eq. 1.

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

Loss in air sample handling procedures. The AGI-30 and the OMNI-3000 collect microorganisms in liquid media which can be immediately diluted and analyzed. However, the microorganisms collected from the samples of the Andersen impactor and the MD8 have to be transferred into liquid media before analysis. Loss of viral infectivity in such a transferring procedure was tested as described below.

Six Andersen impactor plates with ME agar were prepared and each received an addition of 0.1 mL of viral suspension with uranine. At 0 min after adding the suspensions, 3 of the agar plates were rinsed with 2 mL HBSS 3 times using a plastic spreader (TS30C, bioTRADING Benelux BV, Mijdrecht, The Netherlands). The rinsing-off liquid (in total about 5 to 6 mL) was mixed and the viral concentrations analyzed. The other

3 agar plates were rinsed in the same way, but after 60 min exposure to the ambient air at room temperature.

Eight MD8 gelatin filters placed in Petri dishes each received an addition of 0.5 mL of viral suspension with uranine. At 0 min after adding the suspensions, 4 of the filters were dissolved in 20 mL 37°C HBSS, and the other 4 were dissolved after 60 min exposure to the ambient air at room temperature.

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

$$L_h = \left(1 - \frac{C_{liquid,virus} \times V_{liquid,virus}}{C_{suspension,virus} \times V_{suspension,virus}}\right) \times 100\% \quad (2)$$

The same procedures were used to transfer Andersen impactor and MD8 samples, which collected in the isolator in liquid form.

Sampling efficiency. The absolute physical efficiency of the gelatin filter has been reported to exceed 96%, even for virions < 80 nm [28]. As the sizes of aerosols in the presented study were larger than those of virions, the gelatin filter can be expected to have an even higher efficiency. MD8 with gelatin filter was selected as the reference sampler (100% physical efficiency) in this study, and the physical efficiencies (E_p , %) of other samplers were determined by comparing the amount of uranine collected by these samplers, with results from the reference (Eq. 3). By combining the data in this study with data collected in a previous study [27], a larger dataset was obtained for calculating the physical efficiency. E_p values at 0 min and 20 min were pooled because they were not significantly affected by the sampling moment (Tab. 2).

$$E_p = \frac{C_{sampler,tracer}}{C_{MD8,tracer}} \times 100\% \quad (3)$$

The biological efficiency (E_b , %) was determined by excluding the loss of viral infectivity during aerosolization and in air sample handling procedures, as expressed in Eq. 4.

$$E_b = \frac{C_{sampler,virus} / C_{suspension,virus}}{C_{sampler,tracer} / C_{suspension,tracer}} \times \frac{1}{1-L_a} \times \frac{1}{1-L_h} \times 100\% \quad (4)$$

Detection limit. With the egg embryonic death test, the lowest detectable viral concentration in a liquid sample is 2.5 $\text{EID}_{50} \text{ mL}^{-1}$ [23], assuming that 3 out of the 5 eggs inoculated with undiluted sample become infected. Therefore, Eq. 5 was used to calculate the detection limit (DL , $\log_{10} \text{EID}_{50} \text{ m}^{-3}$) of the bioaerosol samplers for sampling the airborne IBDV.

$$DL = \log \left[\frac{\beta \cdot V_{liquid}}{(1-L_h) \cdot V_{air} \cdot E_p \cdot E_b} \right] \quad (5)$$

Half-life. The half-life ($t_{1/2}$) expresses the survivability of microorganisms in the air. In this study, it was defined as the time taken for the concentration of infective virus to decrease by half. The half-life was calculated with Eq. 6. Because the viral aerosols were also diluted during sampling and deposited during the 20 min between the first and second air samplings, the concentrations of the infective virus were corrected using the tracer (α).

$$t_{1/2} = \frac{(\log_{10} 2) \times T}{\log_{10} (C_{sampler,virus}^0 / \alpha C_{sampler,virus}^{20})} \quad (6)$$



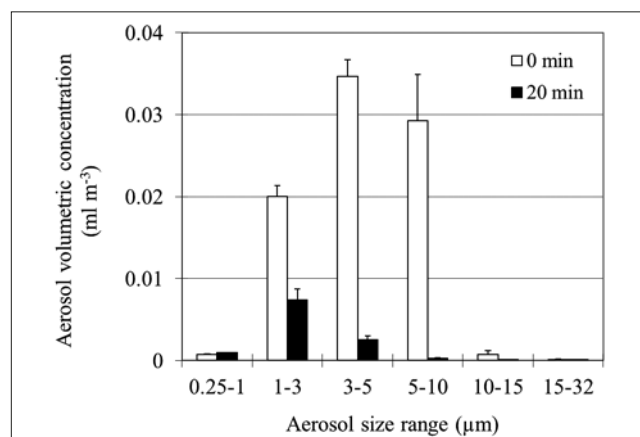
Table 1. Responses and factors tested with GLM procedure

Description	Re-sponse	Factor	Level
Loss in air sample handling procedures	L_h	Interval before handling	0 min, 60 min
		Sampler type	Andersen, AGI, OMNI, MD8
Physical efficiency	E_p	Sampling moment	0 min, 20 min
		Interaction	–
Biological efficiency	E_b	Sampler type	Andersen, AGI, OMNI, MD8
Half-life	$t_{1/2}$	Sampler type	Andersen, AGI, OMNI, MD8

Data analysis. All data were analyzed with the SAS program (SAS 9.1.3 Service Pack 4, SAS Institute Inc., Cary, NC, USA). The effects of the factors of interest on the responses were calculated with a general linear model (GLM) (Tab. 1). The losses of viral infectivity during aerosolization and in air sample handling procedures were compared to ‘no loss’ (0%) with a one-sample T-test. The physical and biological efficiencies were also compared with 100% by means of a one-sample T-test. The one-sample T-test compares the mean of a sample to a known value. If the known value is within the 95% confidence interval of the presented sample values, the mean of the sample values is not significantly different from the known value.

RESULTS

Aerosol size distribution. Figure 2 shows the volumetric concentrations of aerosols in different size ranges measured by a spectrometer during sampling in the isolator. At 0 min after aerosolization the aerosols were mostly in the size range 1–10 μm . At 20 min, aerosols in the range of 1–5 μm dominated. The reduced volumetric concentration of aerosols in the range of 3–10 μm at 20 min was probably the result of settling and evaporation.

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

Losses of viral infectivity in non-sampling processes. During aerosol generation, 95% (L_a , with a standard error of 2%) of the virus lost infectivity. Statistical analysis showed that this loss was significantly different from 0% (null hypothesis of no loss; $p < 0.01$).

Rinsing the agar plates of the Andersen impactor immediately after addition of virus reduced the infectivity

of the added virus by only 1%. The loss increased to 89% when the agar plates were rinsed 60 min after the viral suspension had been added. Statistical analysis showed that the interval before rinsing significantly affected the loss ($p = 0.02$).

The viral infectivity was not significantly affected by the handling procedure for MD8 samples. The effect was the same regardless of whether the gelatin filter was dissolved immediately after adding 0.5 mL viral suspension, or the filter was dissolved 60 min after this addition. The length of time interval before dissolving the filter had no effect on the loss of viral infectivity ($p = 0.91$).

Sampling efficiency and detection limit. Table 2 shows the physical efficiencies of the four bioaerosol samplers. Sampling moment had no effect on the physical efficiency ($p = 0.50$), the efficiency data at 0 and 20 min were pooled. Statistical analysis revealed that the samplers differed significantly in their physical efficiencies ($p < 0.01$). Multiple comparisons showed that the physical efficiencies of the MD8 and the AGI-30 were 36% – 77% higher than the other two samplers. The sampler with the lowest physical efficiency (23%) was the Andersen impactor. The interaction effect of sampler type and sampling moment was not significant ($p = 0.92$).

Table 2. Physical efficiencies of the four bioaerosol samplers

Bioaerosol sampler	Physical efficiency (\pm SE ¹ , %)	n	One-sample T-test	GLM analysis	
			p ²	Factor	p ³
Andersen	23 ^c \pm 4 ^a	30	< 0.01	Sampler type	< 0.01
AGI-30	96 ^a \pm 7	26 ^b	0.58	Moment	0.50
OMNI-3000	60 ^b \pm 7	30	< 0.01	Interaction	0.92
MD8	100 ^a \pm 8	26 ^b	1.00		

¹ SE: standard error

² Probability that the physical efficiency did not differ from 100%

³ Probability that the factor has no effect on physical efficiency

⁴ The low physical efficiency obtained for the Andersen impactor probably results from difficulty of rinsing uranine off the agar plates

⁵ Four outliers resulting from malfunction of the fluorescent meter were excluded from the calculation

^{a,b,c} Means with a common superscript letter are not significantly different ($p > 0.05$)

Table 3 shows the biological efficiencies of the samplers for collecting IBDV. The biological efficiency did not differ significantly among the samplers ($p = 0.35$). The efficiencies of the Andersen impactor (61%) and the AGI-30 (68%) were not significantly different from 100%, indicating that airborne IBDV was not inactivated during sampling. The biological efficiency of the MD8 was calculated to be higher than 100%. The efficiency of the OMNI-3000 (23%) was significantly lower than 100% ($p < 0.01$).

Table 3. Biological efficiencies of the four bioaerosol samplers

Bioaerosol sampler	Biological efficiency (\pm SE ¹ , %)	n	One-sample T-test	GLM analysis	
			p ²	Factor	p ³
Andersen	61 ^a \pm 30	5	0.27	Sampler type	0.35
AGI-30	68 ^a \pm 25	5	0.25		
OMNI-3000	23 ^b \pm 10	5	< 0.01		
MD8	100 ^{a,c}	5	0.41		

¹ SE: standard error

² Probability that biological efficiency did not differ from 100%

³ Probability that there is no effect of the factor on the biological efficiency of Andersen, AGI-30 and OMNI-3000

⁴ Mean biological efficiency of MD8 was slightly (not significantly) higher than 100% due to the inherent variation in virus analysis. Its biological efficiency was set at 100% because a virus cannot multiply in the collection medium without a host

^{a,b,c} Means with a common superscript letter are not significantly different ($p > 0.05$)

The samplers' estimated 2-min detection limits for airborne IBDV were $4.1 \log_{10} \text{EID}_{50} \text{ m}^{-3}$ for the Andersen impactor, $3.3 \log_{10} \text{EID}_{50} \text{ m}^{-3}$ for the AGI-30, $2.5 \log_{10} \text{EID}_{50} \text{ m}^{-3}$ for the OMNI-3000, and $2.9 \log_{10} \text{EID}_{50} \text{ m}^{-3}$ for the MD8.

Half-life. The half-life of airborne Gumboro vaccine virus at 20°C temperature and 70% RH determined by sampling with the different bioaerosol samplers are shown in Table 4. There was no significant difference in the half-life determined by the 4 bioaerosol samplers. The mean half-life was 11.9 ± 2.0 min.

Table 4. Half-life of infectious bursal disease virus aerosolized at 20°C temperature and 70% relative humidity.

Bioaerosol sampler	Half-life (\pm SE ¹ , min)	n	GLM analysis	
			Factor	P ²
Andersen	15.5 ± 7.0	5		
AGI-30	11.3 ± 2.1	5		
OMNI-3000	14.6 ± 5.7	5	Bioaerosol sampler	0.57
MD8	8.2 ± 1.9	5		
Mean	11.9 ± 2.0	20		

¹ SE: standard error.

² Probability that there is no effect of the factor on half-life.

DISCUSSION

A striking finding was the great loss (95%) in the infectivity of the IBDV that occurred during aerosolization by the spray-head. The loss of infective virus was probably due to the sudden change in microenvironment immediately after aerosolization, which caused the virus to undergo dehydration and oxidization stresses [29]. The initial infectivity loss in this process can be much greater than the loss in the subsequent atmospheric equilibrium state [30]. Other studies have reported that during aerolization, bacteria were subjected to shear force which deagglomerates the suspensions into small aerosols and causes bacteria to fragment [8, 31]. However, this does not seem to be the main reason for the inactivation of virus in the presented study, because the aerosol size produced by the spray-head was much larger than the virus itself. The infectivity loss of the vaccine virus found in this study also suggests that current vaccination method in the poultry industry by wet aerosolization is not as effective as originally thought. In practice, the final vaccine dosage received by hens should be estimated by correcting the initial vaccine loss during aerosolization

Whereas collected bacteria can be directly cultured on the agar plates of the Andersen sampler [32], a virus needs hosts to replicate and to be quantified. In the presented study, airborne virus collected by the Andersen impactor was transferred from the agar plates into liquid media for further quantification with the egg embryo host. To date, two methods have been used for this transfer: homogenizing the microbial-loaded agar into liquid [33] and rinsing the microorganisms off the agar surface [34]. Previously, when using the latter method, it was found that 4 bacterial species were fully-transferred to liquid media without significant losses [34]. This method was therefore used to transfer the virus in the current study. The result reported above shows IBDV was recovered without loss from agar plates by rinsing

immediately after adding the viral suspension, but that about 89% of infective virus was lost when the agar plates were rinsed after storage for 60 min in ambient air at room temperature. Statistical analysis showed storage time had a significant effect on the loss of virus ($p=0.02$). The result indicates that IBDV was either firmly bound on the agar surface or penetrated deeper into the agar layers with the flows of liquid, thus hampering the rinsing efficiency.

In the presented study, the MD8 with gelatin filter had the highest physical efficiency. This finding was in agreement with others in which gelatin filters were extremely efficient (>93%) when used for collecting uranine [27], bacillus atrophaeus (0.9 μm), polystyrene latex (0.35 μm), and MS2 virion (<0.08 μm) [28].

The efficiency of the AGI-30 (96%) was similar to that of the reference sampler, which is consistent with the finding of the study by Terzieva *et al.* [35] using 0.57–0.8 μm *Pseudomonas fluorescens* aerosols. The cut-off diameter of the AGI-30 is actually as low as 0.31 μm [36], and in the presented study most aerosols (in volume) were larger than this (Fig. 2). It is therefore not surprising that in this study the AGI-30 had high physical efficiency.

The OMNI-3000 was less efficient than the MD8 and the AGI-30. Its physical efficiency (60%) was within the range reported by Kesavan and Schepers [37], who found that its efficiency was 22–92% for aerosols (either solid or liquid) of 0.5–8 μm . The cut-off diameter of the sixth stage of the Andersen impactor is 0.65 μm . Although it was expected that this sampler would be effective for collecting the aerosols, its efficiency was only 23%. As the efficiency was calculated from the amounts of uranine collected but the uranine could be firmly attached to the agar [34], it seems likely that the low efficiency found for the Andersen impactor was actually due to the low recovery of uranine from the agar plates by the rinsing method.

Different samplers vary in their ability to preserve microbial viability during sampling; this was also observed in this study for airborne IBDV. The Andersen impactor and the AGI-30, which had previously been reported not to inactivate the culturability of the 4 aerosolized bacteria species [27], did not stress the IBDV during sampling. However, the biological efficiency of the OMNI-3000 (23%) was significantly less than 100%, indicating the IBDV was inactivated by sampling stress. The stress was probably caused by the high flow rate of the OMNI 3000 during sampling, which exposes the virus to high shear forces. This sampler has a slit inlet 3 cm in length and approximate 0.2 cm wide. When it is sampling at an airflow rate of 300 L min^{-1} , the microorganisms are drawn into the sampler and impinge into the rotating collection liquid at a very high speed. The OMNI-3000 has also been reported to reduce bacterial viability during sampling [27].

The MD8 with gelatin filter was expected to be the least successful sampler for preserving microbial viability, due to its severe dehydration stress [13, 38]. However, there was no noticeable dehydration effect when the MD8 was used to collect airborne IBDV. This is probably because IBDV is resistant to dehydration stress. The virus seems to be more vulnerable to the high speed at which it was impinged into the collection medium of the OMNI-3000 (83 m s^{-1}) than to dehydration stress.

The detection limits of the bioaerosol samplers for collecting IBDV were calculated from their estimated physical and biological efficiencies. The detection limits of these samplers

ranged from 2.5–4.1 \log_{10} EID₅₀ m⁻³. Although the sampling efficiency of the OMNI-3000 is relatively low, its detection limit is the lowest of the 4 samplers because of its high air flow rate. For this reason, the OMNI-3000 can be best used in environments where the viral concentrations are low. Lower detection limits can be achieved by increasing the sampling duration of the samplers (Eq. 5). However, sampling durations for the Andersen sampler, the AGI-30, and the MD8 have seldom been extended for longer than 1 hour because of the decrease in physical efficiency (e.g. evaporation of collection medium of the AGI-30 compromises its physical efficiency) or in biological efficiency [39, 40].

The result obtained for the detection limit in the presented study clearly shows that a negative air sample is not proof that the air environment is safe. Healthy recipients can still be infected through the airborne transmission route, even though no infectious agents have been detected by the bioaerosol samplers [16, 17]. This means that the impact of airborne transmission is beyond that which can be measured with current sampling strategy. This is an important consideration when performing practical sampling for any biosecurity purpose. The epidemiologic model used as a basis for decision-making on culling strategies during epidemics should combine the limitation of samplers as a parameter, otherwise the epidemic situation may be underestimated.

The half-lives of many airborne viruses have been studied at different temperatures and RHs. The results showed a big variation in the viral half-life, ranging from several minutes to days [41, 42, 43]. In this study, the half-life of airborne IBDV at 20°C and 70% was found to be 11.9 min. Although the bioaerosol samplers differed in their efficiencies, the half-lives determined by these samplers did not differ significantly ($p = 0.57$). This is probably because the efficiency of each sampler was identical (or changed at a similar rate) at 0 min and 20 min.

The method of determining viral quantity with eggs has been used in several studies [44–46]. The principal variability of the method is from 0.03–0.1 \log EID₅₀ mL⁻¹ [24], which is lower than the viral concentrations in the samples in the presented study (1.6–7.7 \log_{10} EID₅₀ mL⁻¹). However, in practice, the variability is always higher than this because of the uncertainty in sample preparation, incubation condition, identification of egg abnormalities, etc. When the variability within treatments is combined, the standard error of the presented data seemed to be high for the biological efficiencies. The high variability could be reduced by increasing the replicates of aerosolization or increasing the number of sample analyses, but in both cases one should be aware that reducing variability by a factor of 2 entails increasing the replicates or samples by a factor of 4, which could be costly in experiments with viruses. Furthermore, we are not particularly interested in tiny differences, for instance, despite its relatively low efficiency, the OMNI-3000 is still a suitable sampler because of its low detection limit.

CONCLUSIONS

Research on the airborne transmission of pathogens frequently requires air to be sampled with efficient and sensitive bioaerosol samplers. In this study, the sampling efficiency and detection limit of four bioaerosol samplers for

collecting a vaccine strain of IBDV were evaluated. The AGI-30 (96%) and MD8 (100%) showed higher physical efficiencies than those of the Andersen impactor (23%) and the OMNI-3000 (60%). Only the OMNI-3000 inactivated viral infectivity during sampling, thereby resulting in a significantly low biological efficiency (23%). The 2-min detection limit of the OMNI-3000 (2.5 \log_{10} EID₅₀ m⁻³) was the lowest. This made it the most suitable sampler and thus suitable for detecting low concentrations of virus. In all four samplers investigated, a negative air sample (after 2-min sampling) does not guarantee that the atmospheric environment is safe: transmission of infectious agents between farms may still occur, even if no virus has been detected. Most of the virus lost infectivity during aerosol generation. From this, it can be concluded that the wet aerosolization vaccination method currently used in poultry industry is not as efficient as originally thought.

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