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Generation of avian influenza virus (AIV) contaminated fecal fine particulate matter ($PM_{2.5}$): genome and infectivity detection and calculation of immission

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Abstract

As a model for aerosol transmission, chicken feces was spiked with avian influenza virus (AIV) subtype H10N7 and used to generate a fine particulate matter aerosol. For this an innovative aerosol chamber was developed, that collected $PM_{2.5}$ on quartz microfiber filters. With AIV contaminated $PM_{2.5}$-dust coated filters different incubation times ranging from 0 to 4 days and storage mainly at +4 and +20 °C and at different relative humidity (RH) were performed. Embryonic death in inoculated hen's eggs with filter elute was the AIV infectivity read out. To determine viral genome presence quantitative real time RT-PCR was applied.

The filter elutes contained AIV genome as well as viable virus whereby +20 °C indicated a borderline temperature for infectious virus stability. In addition, high relative humidity was critical for AIV viability in $PM_{2.5}$. The results allowed a dispersion calculation of infectious AIV in aerosols assuming a worst case scenario

for an AIV outbreak in poultry farms. Thus exposure to AIV associated with $PM_{2.5}$ is possible near to infected farms and may be a serious risk for fatal influenza disease in both man and animals. Airborne transmission should be effectively preventable by dispersion of water combined with disinfection into the inside air as well as the exhaust air stream of AIV infected farms.

Keywords: Avian influenza virus; Chicken feces; Fine particulate matter ($PM_{2,5}$); Airborne transmission; Quartz microfiber filters

1. Introduction

Some of the most highly contagious viruses of human and veterinary concern are airborne pathogens, e. g. human and avian influenza virus (AIV) and foot-and-mouth disease virus (FMDV). The highly pathogenic avian influenza viruses (HPAI) have been a threat to the poultry industry for many years. However, when the Asian H5N1 subtype emerged, a trans-boundary spread became evident and a new dimension of zoonotic danger and fear for evolution of a pandemic virus was raised (Belshe, 2005; Peiris et al., 2007; WHO, 2007). After the first localized Asian H5N1 epidemic in Germany, the virus was spread rapidly and caused many small epidemics in wild birds. In 2007, the virus also was detected on duck raising farms (Rinder et al., 2007; Starik et al., 2007; Weber et al., 2007; Harder et al., 2008). The routes of transmission of Asian H5N1 and other HPAI subtypes among susceptible mammals and birds is still a matter of debate. Air borne transmission of infection cannot be excluded and might be an effective way of infection transmission when virus is inhaled directly in the deeper respiratory tract (Shinya et al., 2006; Tellier, 2006; Brankston et al., 2007; Nicholls et al., 2007). Fine particulate matter from AIV

contaminated fecal dust can play an important role in the transmission and onset of infection since particles as small as $PM_{2,5}$ (particle size < 2,5 µm) can directly invade the lower part of the respiratory tract (DFG, 2008). Rapid and direct transport of AIV into the lungs will be in favor for onset of infection (Shinya et al., 2006, van Riel et al., 2006). The indoor exposure to aerosolized HPAI-AIV is equally given for humans working in poultry stables and susceptible birds. As workers can prevent virus inhalation by using appropriate masks birds are most endangered by aerosolized AIV within the flocks and possibly in nearby holdings.

Recently the stability and transmission of aerosolized Newcastle Disease Virus has been tested under practical conditions with chickens (Li et al., 2009). The results indicated that virus shed from infected animals readily aerosolized and airborne transmission to susceptible chickens was very efficient. We therefore wanted to know, i) whether AIV in contaminated fecal dust can be found in the $PM_{2.5}$ fraction and, ii) if this is the case, how long viable virus is present under environmental conditions.

2. Materials and Methods

2.1. AIV stock solution

The low pathogenic avian influenza virus (LPAI) subtype H10N7 has been isolated and multiplied in 10 day old embryonated chicken eggs as described (Rinder et al., 2007). Allantoic fluid was collected at the time of embryonic death, clarified by centrifugation at 800 x g, the supernatants were pooled and the virus was pelleted by ultra centrifugation in a Beckman Coulter Optima L-90K ultra centrifuge. The pellets were pooled, re-suspended in phosphate buffer and stored at -70 °C. The concentrated AIV stock solution contained 10^9 EID_{50}/ml and showed a

hemagglutination (HA) titer of 1:2048 in allantoic fluid from infected eggs, the titer in MDCK-cells was 3 x 10^5/ml $TCID_{50}$.

2.2. Generation of a storable powder of chicken feces (PCF)

About 8 kg of fresh feces were sampled in a layer farm and analyzed by RT-qPCR to be free of AIV genomes. To avoid bacterial or fungal infection of embryos careful removal of these germs was necessary. Since filtration of feces suspensions can cause an incalculable loss of spiked AIV by adsorption to micro filter membranes , it was steam sterilized (20 min; +121 °C). Subsequently the material was dried at +70 °C, ground and sieved very fine to a storable powder of chicken feces (PCF) with a final particle size of < 63 µm. Approximately 51 % of PCF dry mass were water soluble.

2.3. Generation and sampling of AIV contaminated fecal $PM_{2,5}$

Aliquots of PCF (50 g/l) were re-suspended in distilled water and supplemented with 5 ml/l AIV stock solution corresponding 10^8 EID_{50}/g PCF. Comparable AIV concentrations were found in fresh feces of infected birds (WHO, 2007). The AIV/PCF-suspension and compressed air (flow of 5 l/min) were used in an aerosol generator (AGK 2000, Palas GmbH, Germany) for nebulization. Figure 1 demonstrates the aerosol generator and the generation of different air streams (A, B, C) leading to the collection of $PM_{2,5}$ (stream A) on quartz microfiber filters. The aerosol generator produced droplets with particle sizes < 15 µm diameter. In the new developed mixing and drying chamber these droplets shrank to $PM_{2,5}$. For this the aerosol was diluted and dried with compressed air in a ratio of 1/6.6 (v/v). The resulting air stream (38 l/min) was split in 3 parts in an isokinetic air stream splitter: Stream A (84 % or 32 l/min) for the collection of AIV contaminated fecal $PM_{2,5}$ on

quartz microfiber filters (Type QF050-25, Pieper Filter GmbH, Germany; Average: 2 mg $PM_{2,5}$/Filter in 3 min). Stream B (13 % or 5 l/min) for monitoring particle size distribution and concentration inside the chamber. For this purpose, Stream B was diluted by a factor of 10 in a dilution chamber (VKL-10E, Palas GmbH, Germany) and analyzed with an aerosol spectrometer (welas 2000, Palas GmbH, Germany). Stream C (3 % or 1 l/min) for exhaust air stream which finally collected all other air streams. Exhaust air was heat treated (250 °C; > 10 s) and HEPA filtered before release to the atmosphere to assure complete disinfection. $PM_{2,5}$-dust coated filters were stored at -70 °C (control temperature) before elution.

2.4. Incubation of AIV contaminated fecal $PM_{2,5}$ deposited on filters

AIV contaminated fecal $PM_{2,5}$ deposited on filters was exposed to various combinations of incubation periods (0, 15 h, 48 h, 96 h), temperatures (-70, -20, +4, +20, +37 and +50 °C) and humidity ("water-saturated", "normal atmosphere", and "exsiccated dry"). "Water-saturated" means: dust-coated filters were exposed to water-saturated air ($RH_{4°}$ 90 %, $RH_{20°}$ 60 %); "normal atmosphere" means: dust-coated filters were exposed to unchanged indoor air ($RH_{22°}$ 30%) and "exsiccated dry" means: dust coated filters were exposed in silica gel dried air in an airtight jar ($RH_{4°}$ 20 %, $RH_{20°}$ 19 %). At the end of the different incubation procedures, filters were stored at -70 °C.

2.5. Inoculation of embryonated eggs and quantitative AIV detection

Ten or eleven day old embryonated hen's eggs (n = 2 per experiment) were inoculated with 200 µl of filter elutes into the allantoic cavity. With selected filter elutes (3 ml) titration in embryonated eggs (n = 4 per dilution) was performed to determine the content of viable virus. Allantoic fluid was pooled from the 4 eggs for

real time RT-PCR analysis and hemagglutination. From each egg, at least 4 ml of allantoic fluid was harvested either when the embryo was found dead or at the end of the incubation period after 4 days. According to end point dilutions indicating productive AIV replication in the eggs and the inoculation volume (0.2 ml) utilized, the virus content in the filter elutes was determined and calculated as infectious AIV present in the fine particulate matter eluted from the filters.

2.6. Hemagglutination test

Filter elutes as well as allantoic fluids were analyzed for hemagglutinating activity. Hemagglutination assays were carried out in V-bottomed microtiter plates using 50 µl of 2.5 % suspensions of chicken red blood cells in PBS as described (O.I.E. Terrestrial Manual, 2008). Fresh chicken blood was supplemented with 1.6 % sodium citrate in sterile H_2O and was centrifuged at 800 x g for 10 min at room temperature to separate red blood cells. Thereafter, the cells were washed 3 times with PBS before addition to the assay.

2.7. Reverse transcription quantitative real-time PCR (RT-qPCR)

Filters coated with AIV contaminated fecal $PM_{2,5}$ were eluted with 3 ml sterile PBS solution (8.0 g/l NaCl; 0.2 g/l KCl; 1.44 g/l Na_2HPO_4; 0.24 g/l KH_2PO_4; pH 7.4) and placed in a shaker for 30 min at 4 °C. Isolation of total RNA from elutes or allantoic fluid was performed with 200 µl using the TriZol isolation method (Invitrogen, de Shelp, Netherlands). The resulting 50 µl RNA extracts were tested by real time RT-qPCR protocol for influenza A viruses (Spackman et al., 2002) as recommended by the German National Reference Laboratory. Triplicates of 10 µl of every RNA extract were analyzed in a Mx3000P instrument (Stratagene, La Jolla, CA). Dilutions of AIV positive control standards were used for the quantification of PCR results. RNA

157 extracts from elutes of AIV negative fecal $PM_{2,5}$ as well as allantoic fluid of non
158 inoculated eggs served as negative controls. In accordance to the findings of
159 Spackman et al. (2002) the RT-qPCR was able to detect 10^{-1} EID_{50} of the virus.
160
161 *2.8. Dispersion calculation*
162 Dust from poultry farms is mainly composed of the abrasion from feces and litter
163 material. As measured in the exhaust air stream of a 120,000 animal broiler farm and
164 of a 40,000 animal layer farm in Bavaria (TÜV, 2000) the total dust emissions were
165 1.3 kg/h and 0.5 kg/h, respectively. About 10 % of the broilers total dust emissions
166 were particulate matter $PM_{2.5}$ and about 60 % PM_{10} while the layers total dust
167 emission contained only 3 % $PM_{2.5}$ and 33 % PM_{10}. Thus the main part of inhalable
168 fine particulate matter has been detected in the particle size range from 2.5 to 10 µm.
169 Based on these $PM_{2.5}$ and PM_{10} emissions a dispersion calculation was performed
170 according to the "Lagrangian dispersion model – LASAT" following conventional
171 assumptions (very stable boundary layers, very low and constant wind velocity as 1
172 m/s over 24 hours and a wind direction straight towards the next adjacent
173 settlement). In order not to underestimate the inhalable amount of viable AIV the
174 dispersion calculation was performed only for the PM_{10} particulate matter fraction.
175
176 **3. Results**
177
178 *3.1. Generation of filter samples coated with AIV contaminated fecal $PM_{2,5}$*
179 Particle size distributions of resulting aerosols showed - with high conformity -
180 maxima of particle numbers at 0.4 to 0.6 µm particle diameter (Fig. 2a) and maxima
181 of particle volumes (or masses) at 2 to 4 µm particle diameter (Fig. 2b). More than 90
182 % of the generated particles had sizes < 1.5 µm, and therefore represent particulate

matter with a size range less than 2.5 µm ($PM_{2.5}$) which can directly enter the alveolar part of the lungs (DFG, 2008).

The aerosol generator produced very high concentrations of $PM_{2.5}$: a flow of 38 l/min aerosol with particle concentrations up to 30 mg/m³ could be maintained for several hours. Up to 84 % of the aerosol particles could be collected on quartz microfiber filters. Therefore sampling times of 3 min were sufficient to collect 2.1 mg (median matter) of $PM_{2.5}$ dust on filters. Comparable loads of fine particulate matter were obtained in routine atmosphere air quality monitoring programs within sampling times of at least 24 hours or more. The filter loads showed good reproducibility within every dust generation series and from one experiment to the next.

3.2. Influence of temperature and humidity on stability of AIV genome in fecal $PM_{2,5}$

Filters coated with AIV contaminated fecal $PM_{2,5}$ were incubated up to 4 days under conditions typical of European summer and winter temperatures at normal and extreme humidity. Even extreme incubation temperatures ranging from -20 °C to +50 °C and an incubation time up to 4 days did not cause any significant change of the number of AIV genome copies detected by quantitative real time RT-PCR, when filters were incubated in a normal indoor atmosphere. The number of the AIV genome fragment copies also remained unchanged when the dust-coated filters were incubated in a water-saturated atmosphere or in exsiccated dry air (data not shown). However, only the infectious stability of AIV particles in fine particulate matter forms the basis for a risk assessment under environmental conditions. We therefore determined the lethal effect of AIV genome containing $PM_{2,5}$ in embryonated eggs.

3.3. Infectivity of AIV in fecal $PM_{2,5}$ after incubation at different temperature and atmosphere conditions

Immediate testing (0 h) and after the 15 h incubation period of the filters always produced positive results (data not shown). Table 1a, b shows the results of the egg inoculation experiments after a 2 and 4 day incubation period of quartz microfiber filters coated with AIV genome positive $PM_{2.5}$. Hemagglutination titers were only obtained from eggs containing dead embryos from which the majority died on day 2 after inoculation. Hemagglutination correlated well with a dramatic raise of AIV genome copy numbers represented by low ct values ranging from 11 to 20 in allantoic fluid from dead embryos indicating a productive virus replication in the eggs. In contrast, before egg inoculation, there were relatively high real time RT-qPCR ct values in the filter elutes ranging from ct 25 to 36 which in no case resulted in a positive hemagglutination (not shown). This demonstrates that a lack of hemagglutination activity is not decisive for the absence of infectious AIV particles. It became evident that normal atmosphere and exsiccated dry air each supported long term viral infectivity in contrast to a water-saturated atmosphere. A 4 day incubation at +4 °C under exsiccated air conditions did not destroy AIV infectivity (Table 1b grey shaded). In one experiment, even after a 4 day incubation at +20 °C, the filter elute still contained infective AIV that killed the embryos and their allantoic fluid showed a low ct in real time RT-qPCR as well as hemagglutinating activity (Table 1b upper section). The same result was obtained after filter incubation under normal atmosphere conditions in two independent experiments (Table 1a middle section). In all experiments filter incubation at a temperature of +37 °C did not result in infective elutes after 2 days (Table 1a, b). Since filter incubation in normal atmosphere revealed long term survival (2 and 4 days) of infectious AIV we performed titration experiments with filter elutes using 4 embryonated eggs per dilution. The embryonic death as well as hemagglutination titers of pooled allantoic fluid from eggs containing dead embryos were taken as an indicator of infectious AIV. In addition in all allantoic

fluid samples from embryos that had died before day 4 post inoculation, a significant decline of real time RT-qPCR ct values was measured compared to the ct values in filter elutes before egg inoculation. After a 2 day incubation, the 1/100 dilution marked the limit of infectivity in parallel to the storage control temperature of -70 °C (Table 1a). At an elute dilution of 1/10, three embryos from 4 eggs had died from the infection with elutes stored at +4 °C as well as from the infection with -70 °C storage control samples. The egg inoculation with filter elutes after an incubation time of 4 days caused the death of 1 out of 4 embryos and reached the detection limit in hemagglutination at the dilution of 1/50 of the allantoic fluid pool. As demonstrated in five independent experiments (Table 1a), filter elutes which were incubated for 4 days at +20 °C in normal atmosphere indicated AIV infectivity in 2 experiments. Another 3 experiments did not result in embryonic death and hemagglutination of the allantoic fluid. The same conditions were chosen in a titration experiment that did not result in embryonic death nor in hemagglutination and AIV genome detection by real time RT-qPCR.

As determined from filter elute titration results in embryonated eggs, the content of infectious virus particles per mg $PM_{2.5}$ was calculated to be 3,950 viable AIV/mg $PM_{2.5}$ in the -70 °C storage control sample. For the +4 °C incubation sample 3,750 and 710 viable AIV/mg $PM_{2.5}$ were calculated after a 2 day and 4 day incubation at normal atmosphere, respectively. Thus, related to the virus content of the -70 °C storage control sample, the number of viable AIV/mg $PM_{2.5}$ decreased within 4 days at +4 °C in normal atmosphere by more than 80 %. Compared to the content of viable AIV in the PCF/AIV suspension before nebulization (10^8 EID_{50}/g PCF) the deposited $PM_{2,5}$ of the -70 °C storage control sample contained viable AIV in an amount of 4 x 10^6 EID_{50}/g $PM_{2.5}$.

261 *3.4. Dispersion calculation*

262 Figure 4 a and b shows the calculation results of PM_{10} as maximum immission
263 concentration values per hour. The PM_{10} immission concentration at the next
264 adjacent settlement at a distance of 350 m to the broiler farm and 150 m to the layer
265 farm was about 140 µg/m³ and 95 µg/m³, respectively. Using the virus titration results
266 of the samples stored at +4 °C for 2 days (3,750 viable AIV/mg $PM_{2.5}$, fictively taken
267 as PM_{10}), the PM_{10} immission concentrations at the next adjacent settlements would
268 correspond to 530 viable AIV/m³ at the broiler farm and 360 viable AIV/m³ at the layer
269 farm.

270

271 ***4. Discussion***

272

273 The most common methods for viral aerosol sampling are liquid impactors, solid
274 impactors combined with petri dishes and different filter types such as cellulose,
275 polycarbonate, gelatine and PTFE (Verreault et al., 2008). So far, influenza virus in
276 bioaerosol has most frequently been collected by liquid impactors. Recently, Pyankov
277 et al. (2007) also performed the collection of airborne AIV (LPAI H11N9) in an open
278 channel type aerosol chamber using liquid impactors in combination with real-time
279 PCR and the hemagglutination assay. Recently, Fabian et al. (2008) collected human
280 influenza virus from exhaled breath on Teflon filters and detected viral genome using
281 real-time PCR and in addition performed viral particle count. They found that 87 % of
282 the exhaled particles were under 1 µm in diameter, and therefore suggested that fine
283 particle aerosols may play a role in human influenza transmission.
284 In this study, we collected avian influenza A virus (LPAI H10N7) from the air stream
285 in a new aerosol chamber by deposition of the fine particulate matter generated from
286 aerosolized spiked chicken feces on quartz microfiber filters. For the first time, a

287 particle size of $PM_{2.5}$ from fractionated AIV spiked chicken feces aerosol has been
288 used in infection experiments with embryonated eggs as a highly sensitive system for
289 viable AIV. For inoculation of living chicken embryos it was necessary to avoid non-
290 specific toxic effects due to viable bacterial content of feces and filtration was not an
291 option due to incalculable losses of spiked virus. Therefore, a sterilized storable
292 powder of chicken feces (PCF) with a final particle size of < 63 µm was produced. In
293 contrast to other detection methods for AIV in untreated bioaerosol, e.g.
294 hemagglutination or genome tracing by real time PCR (Pyankov et al., 2007), virus
295 replication could be determined in vivo. Since in virus contaminated aerosols larger
296 particles are less hazardous than smaller ones (Verreault et al., 2008), the intention
297 was to exclusively analyze the < 2.5 µm bioaerosol fraction (fine particulate matter –
298 $PM_{2.5}$) trapped on quartz microfiber filters which are used in routine atmosphere air
299 quality monitoring programs. It became evident that the 3 ml elute of the filters
300 contained viral genome as well as infectious AIV when 10^8 EID_{50}/g feces were
301 present in chicken feces powder before nebulization. Compared to this the -70 °C
302 storage control sample contained viable AIV in $PM_{2.5}$ dust deposited on filters in an
303 amount of 4 x 10^6 EID_{50}/g $PM_{2.5}$. The lack of hemagglutinating activity in filter elutes
304 could be a result of the relatively large elute volume (3 ml). Severe damage of the
305 hemagglutinin protein can be excluded since infectivity of AIV in filter elutes was not
306 affected. A homogenous size distribution of particle numbers and particle volumes
307 was achieved in fine particulate matter with the aerosol generator (Fig. 2 a and b). It
308 was obvious that quartz microfiber filters had no destructive effect neither on AIV
309 genome stability nor on infectivity within the calculated number of genome copies
310 and viable AIV/mg $PM_{2.5}$ settled on the filters. However, it should be noted that the
311 RT-qPCR assay detects only a small fragment of the entire AIV genome which might
312 be much more robust than the entire genome. In addition, the amount of viable

spiked virus was relatively high and the recovery rate of the system has not been tested for a low virus dose spiked in feces.

Relative humidity (RH) has a great impact on droplet size and structural integrity of particles in aerosol (Lowen et al., 2007; Verreault et al., 2008). We therefore tested three different RH conditions – normal indoor atmosphere, exsiccated dry air and water-saturated atmosphere. The relative higher stability of AIV in exsiccated dry air and normal indoor atmosphere was confirmed as described for *orthomyxo*- and *paramyxoviridae* (Schaffer et al., 1976; Ijaz et al., 1985; Lowen et al., 2007). Temperatures above +4 °C are well known to have a negative impact on virus infectivity (Ijaz et al., 1985; 1987). Temperatures of +37 °C and +50 °C completely abolished AIV infectivity in fine particulate matter after a 2 day filter incubation. However, the AIV subtype H10N7 used in this study remained remarkably stable for a period of even 4 days at +20 °C in exsiccated dry air and under normal indoor humidity conditions. Differences in stability of aerosolized influenza A virus have been reported indicating higher decay rates for human and swine strains compared to avian viruses (Mitchell et al., 1968; 1972). The presence of infectious AIV in fine particulate matter originating from contaminated feces and its relative stability at +20 °C might explain the H5N1 infection of people in subtropical and tropical regions. Short-term exposure of feces dust to temperatures up to +37 °C has not been proven to immediately destroy infectivity of contaminating AIV if the virus protective effect of protein and lipid is considered (EFSA, 2008; Verreault, 2008). Close contact with virus shedding animals or aerosol from dried animal feces and low relative humidity would be the most important conditions for effective virus transmission (Brankston et al., 2007; Lowen et al., 2007).

To estimate the possibility of a relevant exposure of humans and animals near to infected farms we performed a dispersion calculation. For this it was assumed that all

animals in the farm have been infected simultaneously, and therefore, the complete PM_{10} emission of each farm would be AIV contaminated without a decay of virus infectivity during aerosol transmission, for example by UV radiation. As described by De Benedictis et al. (2007) UV radiation light is not able to inactivate AIV in a timely manner, as data have shown that 45 min exposure to a UV source is not sufficient for complete inactivation of HPAI strain A/chicken/Pakistan/94 (H7N3). Another example for long term AIV stability in feces (more than 20 days) is that of subtype H7N2 (EFSA, 2008). However, AIV stability under environmental conditions may be greatly influenced by subtype peculiarities. According to our worst case scenario, a virus immission concentration of 530 viable AIV/m³ means that an adult person with an average respiratory volume of 20 m³/24 h (AGS, 2008) could inhale up to 10^4 infectious virus particles per day at a distance of 350 m to an infected farm. Since chicken have an average respiratory volume of 1.2 m³/24 h (Flindt, 2000), they could inhale up to 300 infectious virus particles per day by breathing an 70 µg/m³ PM_{10} aerosol containing 260 viable AIV/m³ coming from a neighbouring farm at a distance of 550 m.

5. Conclusion

As poultry farms are known to emit fine particulate matter ($PM_{2.5}$ and PM_{10}) which is composed of the abrasions from feces and litter material, they are a potential risk for airborne neighborhood infection. According to the results from a dispersion calculation for a 120,000 animal broiler farm, assuming a worst case scenario for an adult person, she/he could inhale up to 10^4 infectious AIV per day living in a nearby settlement. The 50 % human infective dose by aerosol inoculation of influenza virus A2/Bethesda/10/63 has been determined to be only 0.6 – 3 $TCID_{50}$ (Tellier, 2006). So

365 far the minimal infective dose of H5N1 for man has not been determined, however, it
366 is clear that inhalation of AIV contaminated dust aerosols should be avoided. It also
367 can be concluded that animals in a nearby poultry farm can become exposed and
368 potentially infected by inhalation of about 300 infectious AIV per day. This risk
369 assessment relies on the aerosol 50 % infective dose for hens which has been
370 estimated to be only 2 $TCID_{50}$ (Savatov et al., 2007).
371 As recently demonstrated in the guinea pig model the formation of droplet nuclei is
372 important for virus transmission, and that high RH (80%) leads to an increase in size
373 of exhaled small droplets (< 5 µm) and also a rapid settling of large droplets limits the
374 spread of virus (Lowen et al., 2007). In AIV affected poultry farms, culling actions and
375 subsequent cleaning of the stables can cause very high dust production and the
376 generation of bioaerosol including fine particulate matter. Thus airborne AIV
377 transmission might be effectively prevented by fine dispersion of water combined with
378 environmentally tolerable disinfection (Heckert et al., 1997) into the inside air of
379 stables as well as the exhaust air stream of AIV infected farms.

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Table 1

Infectivity of AIV in fine particulate matter ($PM_{2,5}$) generated from spiked avian feces after storage for two and four days at different temperatures and humidity conditions

a) Incubation under “normal atmosphere” conditions

b) Incubation under “exsiccated dry air” and “water saturated air” conditions

Figure 1

Experimental set up and flowchart of air streams A, B, C

Stream A leads to the deposit of fine particulate matter

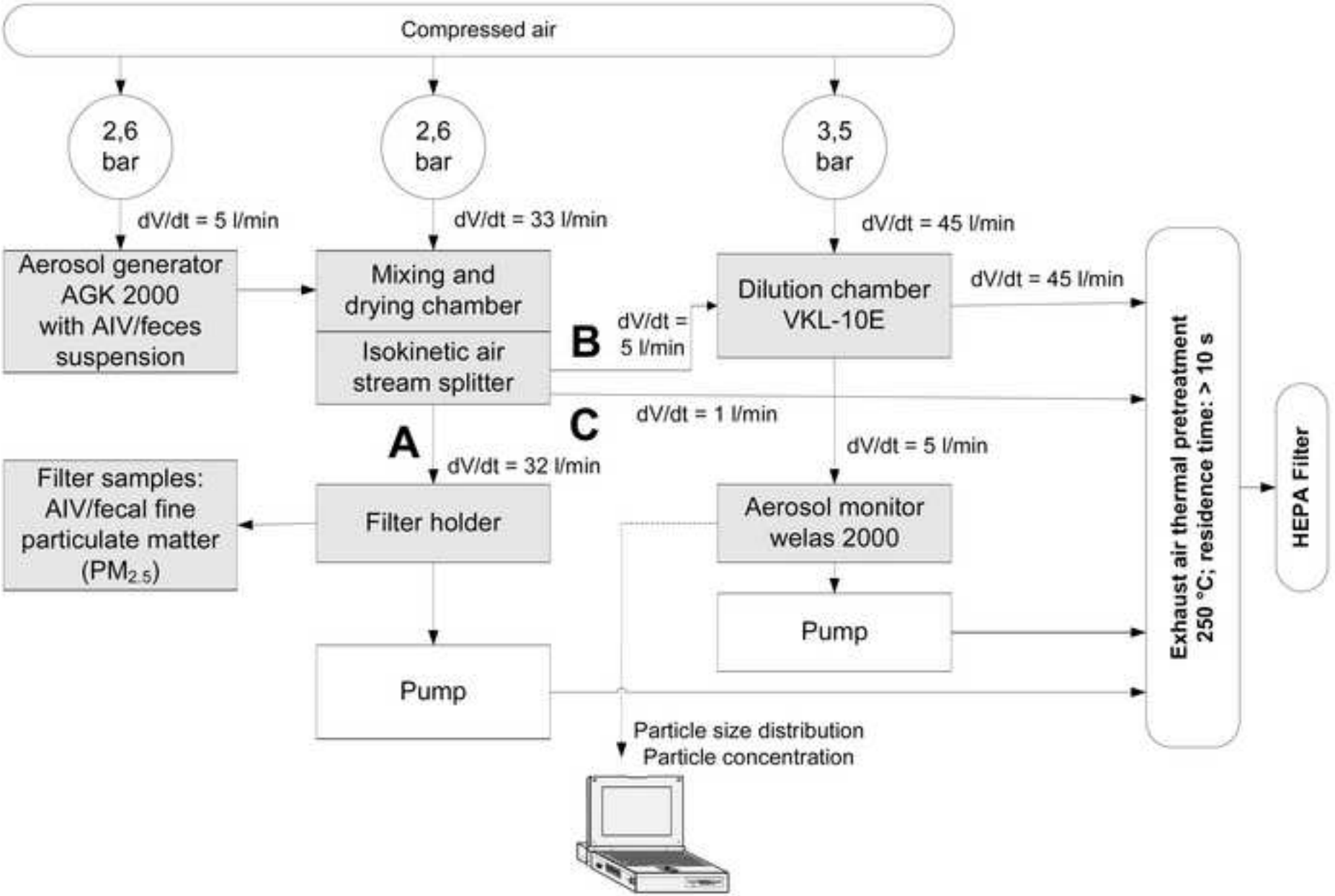
Stream B was diluted by a factor of 10 and analyzed with an aerosol spectrometer

Stream C was directly drained off in an exhaust air stream

Figure 2

Comparison of size distribution of particle numbers (a) and particle volumes (b) of fine particulate matter aerosols ($PM_{2.5}$) generated from suspensions of AIV spiked chicken feces samples (data of four filter generation runs)

Figure 1


Compressed air
2,6 bar
2,6 bar
3,5 bar
dV/dt = 5 l/min
dV/dt = 33 l/min
dV/dt = 45 l/min
Aerosol generator AGK 2000 with AIV/feces suspension
Mixing and drying chamber
Isokinetic air stream splitter
B
dV/dt = 5 l/min
Dilution chamber VKL-10E
dV/dt = 45 l/min
C
dV/dt = 1 l/min
A
dV/dt = 32 l/min
dV/dt = 5 l/min
Filter samples: AIV/fecal fine particulate matter ($PM_{2.5}$)
Filter holder
Aerosol monitor welas 2000
Pump
Pump
Particle size distribution
Particle concentration
Exhaust air thermal pretreatment 250 °C; residence time: > 10 s
HEPA Filter

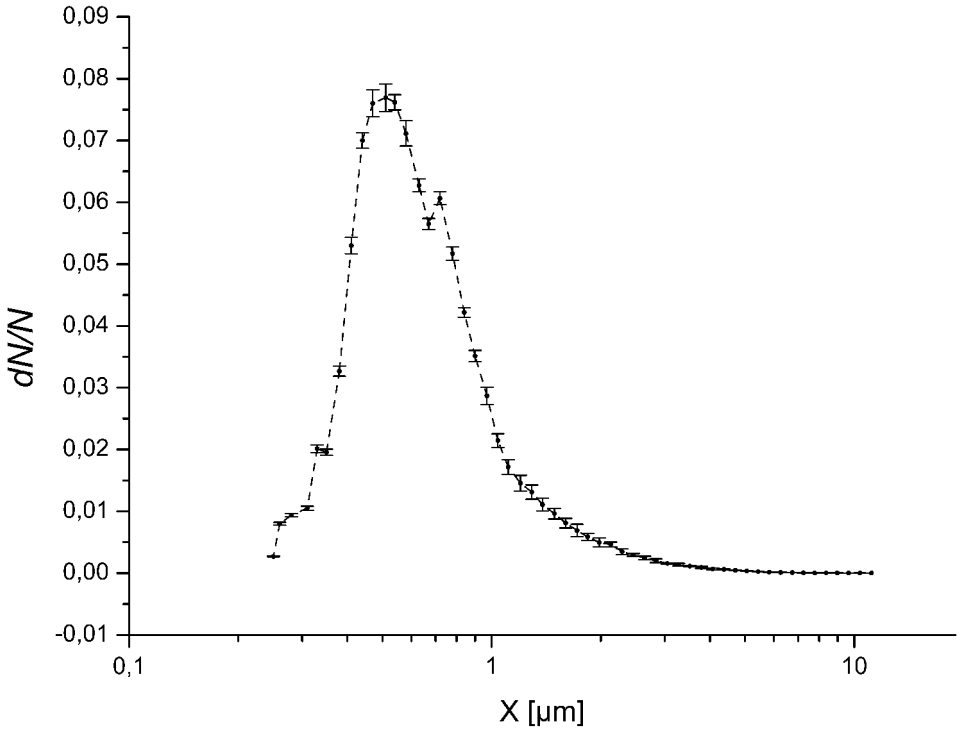


Figure 2a: Average of 4 filter samples and standard deviation

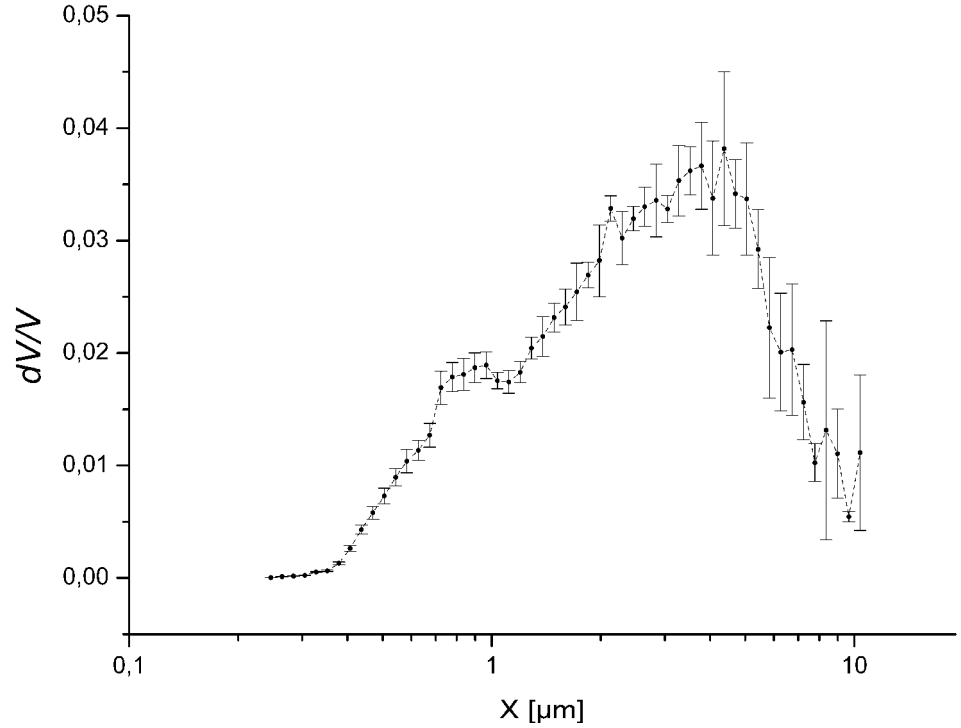


Figure 2b: Average of 4 filter samples plus standard

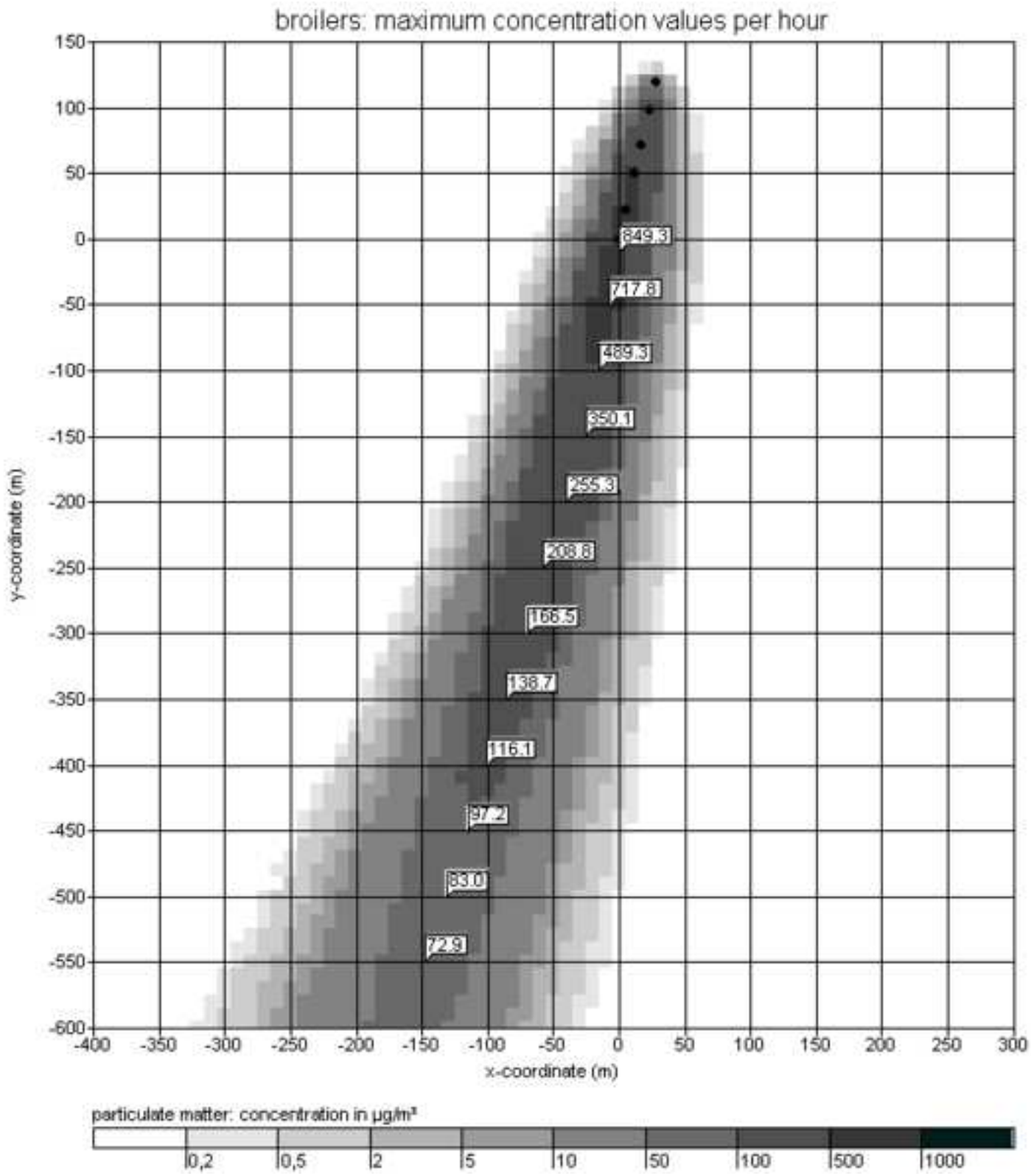


Figure 3B

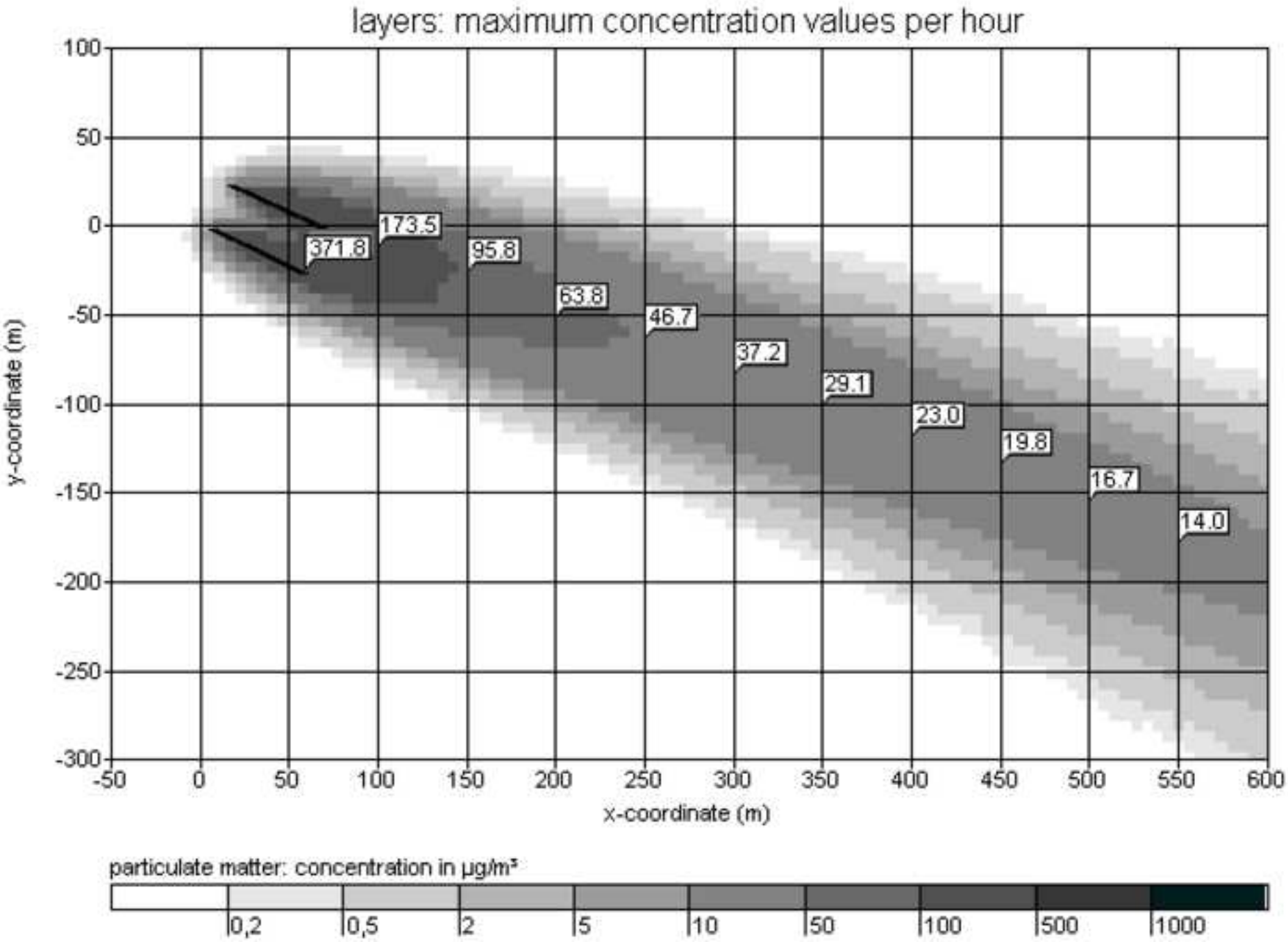

layers: maximum concentration values per hour
y-coordinate (m)
x-coordinate (m)
371.8
173.5
95.8
63.8
46.7
37.2
29.1
23.0
19.8
16.7
14.0
particulate matter: concentration in µg/m³
0,2
0,5
2
5
10
50
100
500
1000

Table 1a Incubation under “normal atmosphere” conditions

no.	incu-bation **temp. °C**, dilution	incubation time: **2 days** RT-qPCR ct filter elute	RT-qPCR ct all. fluid	HA titer after egg passage	incubation time: **4 days** RT-qPCR ct filter elute	RT-qPCR ct all. fluid	HA titer after egg passage
Storage control sample – 70 °C			not exposed				
	undiluted	26,0	18,5	1:16			
	1:2	27,0	12,5	1:16			
	1:10	34,2	11,8	1:32			
	1:50	32,8	12,9	1:8			
	1:100	34,9	-	-			
1	-20	26.5	20.6	1:8	27.0	19.0	1:8
2	-20	26.5	11.9	1:16	26.9	11.2	1:16
1	4	27.9	19.7	1:8	33.5	19.2	1:8
2	4	27.7	11.9	1:8	34.8	19.4	1:8
3	4	-	12.5	1:4	36.0	16.3	1:4
4	4	34.4	13.5	1:16	35.2	12.9	1:16
5	4 undiluted	29.4	12.7	1:16	26.1	12.1	1:64
	1:2	26,6	16,5	1:16	37,1	-	-
	1:10	33,2	12,5	1:16	34,0	18,7	1:32
	1:50	33,2	12,2	1:16	34,5	-	-
	1:100	33,3	38,9	-	35,5	-	-
1	20	33.8	17.0	1:16	-	19.7	1:8
2	20	27.8	19.5	1:16	26.7	13.2	1:8
3	20	34.1	-	-	35.1	-	-
4	20	25.6	30.6	-	25.2	36.1	-
5	20 undiluted	26.0	40.8	-	25.9	-	-
	1:2	26,9	36,3	-	27,8	-	-
	1:10	34,0	-	-	29,4	-	-
	1:50	34,9	-	-	34,6	-	-
	1:100	34,3	-	-	35,8	-	-
1	37	28.2	-	-	27.4	-	-
2	37	33.7	-	-	26.9	-	-
3	37	27.8	-	-	29.6	-	-

all. = allantoic; HA = hemagglutination; no. = number of independent experiments;
“-“ = not detectable;
grey shaded: viability of AIV after a four day incubation period

Table 1b Incubation under “exsiccated dry air” and “water saturated air” conditions

no.	incu-bation **temp. °C**	**RH**	incubation time: **2 days** **RT-qPCR ct** filter elute	all. fluid	**HA titer** after egg passage	incubation time: **4 days** **RT-qPCR ct** filter elute	all. fluid	**HA titer** after egg passage
1	4	**exs.**	26.6	12.3	1:16	26.3	20.3	1:8
2	4	**dry**	29.4	14.3	1:8	30.8	11.8	1:8
3	4	**air**	36.8	12.5	1:16	25.8	12.7	1:16
1	20		28.4	14.2	1:4	35.0	-	-
2	20		33.5	12.8	1:16	26.0	13.8	1:8
1	37		28.0	27.9	-	28.0	-	-
2	37		30.8	-	-	29.0	-	-
1	4	**wat.**	28.2	-	-	-	-	-
2	4	**sat.**	-	-	-	-	-	-
3	4		26.0	36.3	-	34.3	34.6	-
1	20		26.4	-	-	29.9	-	-
2	20		35.4	37.2	-	26.2	38.0	-
1	37		36.2	37.0	-	-	-	-
2	37		-	-	-	-	-	-

all. = allantoic; exs. = exsiccated; HA = hemagglutination;
no. = number of independent experiments;
RH = relative humidity; wat. sat. = water-saturated;
“-“ = not detectable;
grey shaded: viability of AIV after a four day incubation period