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Susceptibility of monkeypox virus aerosol suspensions in a rotating chamber

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A B S T R A C T

Viral aerosols can have a major impact on public health and on the dynamics of infection. Once aerosolized, viruses are subjected to various stress factors and their integrity and potential of infectivity can be altered. Empirical characterization is needed in order to predict more accurately the fate of these bioaerosols both for short term and long term suspension in the air. Here the susceptibility to aerosolization of the monkeypox virus (MPXV), associated with emerging zoonotic diseases, was studied using a 10.7 L rotating chamber. This chamber was built to fit inside a Class three biological safety cabinet, specifically for studying airborne biosafety level three (BSL3) microorganisms. Airborne viruses were detected by culture and quantitative polymerase chain reaction (qPCR) after up to 90 h of aging. Viral concentrations detected dropped by two logs for culture analysis and by one log for qPCR analysis within the first 18 h of aging; viral concentrations were stable between 18 and 90 h, suggesting a potential for the MPXV to retain infectivity in aerosols for more than 90 h. The rotating chamber used in this study maintained viral particles airborne successfully for prolonged periods and could be used to study the susceptibility of other BSL3 microorganisms.

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1. Introduction

Viral members of the *Poxviridae* family such as variola have been eliminated from natural circulation in the late 1970s (Fenner et al., 1988; Henderson, 1980). However, other closely related viruses, such as the monkeypox virus (MPXV), are still a threat to human health as emerging zoonotic diseases. MPXV continues to be the causative agent of sporadic enzootic outbreaks on the African continent (Di Giulio and Eckburg, 2004; Reynolds and Damon, 2012; Rimoin et al., 2010). Naïve and unvaccinated populations are becoming more susceptible to poxviral infections, as seen in a recent outbreak that occurred in the upper Midwestern United States in 2003. This limited outbreak was a result of MPXV-infected Gambian giant rats and dormice imported from the African continent that were housed with prairie dogs (CDC, 2003). Cross infection of the prairie dogs by the imported animals resulted in 35 laboratory-confirmed cases and 36 suspected or probable cases of clinical MPX. The majority of the patients had been exposed to infected prairie dogs. The incidence of MPXV in a non-endemic area such as the US heightened awareness about the population's susceptibility to a poxviral disease such as MPX. Furthermore, this reemphasized the ongoing concern regarding the potential use of MPXV or other poxviral agents such as variola virus for nefarious purposes. Although not suspected in the importation outbreak or in naturally occurring cases, the aerosol route of exposure remains the most probable route of exposure in the case of a deliberate release of a poxvirus. To date, there is very little that is known about the susceptibility of these viruses in the aerosol environment. No empirically derived data which details the relative susceptibility of MPXV when airborne is available to predict disease communicability better in the event of an outbreak.

Disease transmission by the aerosol route depends on the deposition of the suspect pathogen in the respiratory system of the host and on the integrity and the infectious potential of the pathogen. Empirical characterization of the infectious bioaerosol, therefore, is required in order to better understand this route of exposure. It has been postulated that variola virus, for example, can initiate infection by the inhalation of about 10^2 infective particles (Tucker, 2011). However, disease induction could theoretically be caused by a single infectious viral particle as long as it reaches a susceptible area of the lung. (Fenner, 1988; Nicas et al., 2004; Westwood et al., 1966). The induction of the infectious process through aerosol exposure begins with the transport of the viral-laden particles to the susceptible host and depends on the effects of physiochemical factors on the integrity of the virus (Nicas et al., 2005).

The pathogenic viruses contained in the *Poxviridae* family are known to remain infectious for prolonged periods of time in the environment (Essbauer et al., 2007; Wolff and Croon, 1968), yet little is known about the long term stability of these viruses in

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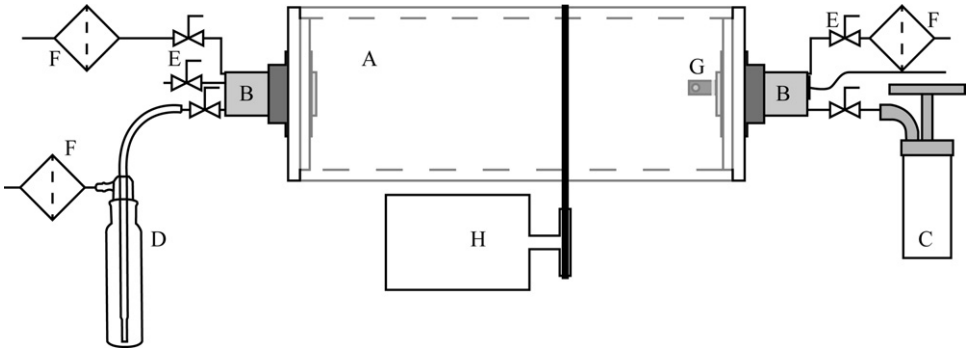

Fig. 1. Rotating chamber. (A) Aluminum cylinder has an interior diameter of 17.8 cm, an outer diameter of 20.3 cm and is 45.7 cm long, total volume is 10.7 L; (B) end caps are composed of three pieces; a 2.5 cm thick aluminum disc (white) mounted on the outer ring of a double sealed ball bearing (dark gray), the extended inner ring of the ball bearing holds a 5.1 cm diameter aluminum rod (light gray) with 3 holes giving access to the interior of the chamber, the chamber is supported by the aluminum rods which stay fixed in position during chamber rotation; (C) Collison three-jet nebulizer; (D) AGI-4 sampler; (E) ball valves controlling access to the interior of the chamber through the end caps; (F) capsule HEPA filters; (G) thermocouple probe with USB connection for recording temperature and relative humidity inside the rotating chamber; (H) electric motor with speed controller providing rotation with a V-belt strapped around the chamber.

aerosols. Considering the relatively low infective dose required for disease induction with many of the pathogenic members of this viral family, it is of uttermost importance to define the potential of a virus such as the MPXV to retain its infectivity over time when airborne. This virus was selected for this study for two major reasons. The first is its hardiness which makes it a good candidate for aerosol longevity studies. The second is the public health importance of this virus and other *Poxviridae*.

Most studies involving prolonged exposures of particles to the airborne state are performed in rotating chambers such as described by Goldberg et al. (1958), where the competing forces exerted on the airborne particles allow a prolonged suspension period by encouraging a spiral-like motion to the particles. Although some rotating chambers set up in BSL3 laboratories are available for aerosol aging studies, the size of these chambers (Adams et al., 1982; Cutler et al., 2011; Ijaz et al., 1994; Karim et al., 1985) would require specialized equipment for safety issues. The MPXV is handled only in biosafety level three (BSL-3) laboratory environments and is highly regulated as a select biological agent by the U.S. Centers for Disease Control and Prevention (CDC) and the U.S. Department of Agriculture (USDA). The small rotating chamber described here was used inside a Class three biological safety cabinet (Class III BSC) to study the viral efficiency of MPXV and to evaluate the potential usage of the chamber for other similar pathogenic agents.

2. Methods

2.1. Aerosol chamber

A custom-built rotating chamber with a volume of 10.7 L was used for this study. This chamber, illustrated in Fig. 1, placed within a standard (8'×4'×3') Class III BSC (GermFree Laboratories, Ormond Beach, FL) which was predominantly used for animal aerosol exposures. The rotating chamber consisted of a 45.7 cm long aluminum cylinder with an interior diameter of 17.8 cm. The cylinder is closed on both ends with aluminum caps mounted on 5 cm interior diameter double-sealed ball bearings. Aluminum rods with access ports were pressure fitted in the bearings and remained stationary during rotation, which was provided by a V-Belt strapped around the chamber. The apparatus was connected to a 1/10 hp motor with speed controls. The chamber rotated on a horizontal axis at a controlled speed set between 0.8 and 1.0 rpm. The speed of rotation was selected according to the capacity of the motor and on the optimum rate of rotation as determined using equations found in Gruel et al. (1987) and Asgharian and Moss (1992). A Collison three-jet nebulizer (BGI Inc., Waltham, MA) was connected to one side of the chamber and an AGI-4 sampler (model 7541, Ace Glass Inc., Vineland, NJ, USA) was connected to the other side. Capsule filters connected opposite to the sampler and to the nebulizer were used to regulate pressure in the chamber during samplings and nebulizations. All ports were controlled manually by ball valves.

2.2. Viral strain

The MPXV (strain Zaire 79) was obtained from BEI Resources (ATCC, Manassas, VA) at a viral titer of 6.1E+08 PFU/ml. The stock virus was stored at −80 °C until used in the aerosol experiments.

2.3. Aerosol production, sampling and characterization

Viral suspensions were nebulized into the chamber at 20 psi with dry HEPA-filtered compressed air. Aerosol samples were taken with AGI-4 samplers filled with 10 ml of Eagle's Minimum Essential Media (EMEM) with penicillin and streptomycin, without fetal bovine serum (FBS) and supplemented with 25 μL of antifoam A and then operated at sonic velocity at 6 L/min. For every sample, a 5 min nebulization was followed by a 2 min aging period (for C_0 and G_0) with all ports of the chamber sealed and a 10 min air sampling period; this is the initial nebulization sample. The same aliquot of viral suspension was then used for a second 5 min nebulization followed by an aerosol aging period ranging from 2 min to 90 h (for C_{aged} and G_{aged}). Each time point was done in triplicate. The air from the chamber was flushed with 100 L of dry filtered air (10 L/min for 10 min) between each iteration of the nebulization. Negative control ($n = 2$) samples were collected after the 100 L air flush. All aerosol samples and remaining liquid samples from nebulizers were placed at −80 °C until analysis by plaque assay and quantitative PCR (qPCR). Relative humidity and temperature inside the rotating chamber were recorded every 5 min with a thermocouple probe (model RH-USB, Omega Engineering, Inc., Stamford, CT, USA). Particle size distribution was determined at zero and 18 h time points with aerosols of sterile media and during nebulization of MPXV with an aerodynamic particle sizer (APS; model 3321, TSI Inc., Shoreview, MN).

2.4. Plaque assays

Plaque assays were performed on Vero E6 cells (ATCC number CRL-1586) grown to 95% confluency in complete EMEM

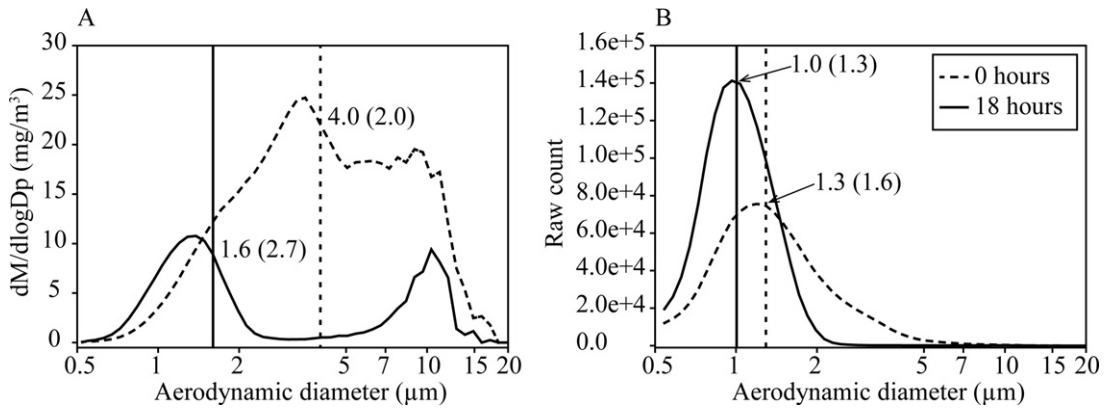

Fig. 2. Aerodynamic distribution of airborne particles inside the rotating chamber after nebulization of sterile media with a Collison three-jet nebulizer. Samples (20 s) were taken with the APS immediately after nebulization was stopped (dashed line) and after 18 h of aging in the rotating chamber (solid line). Graph (A) shows the total mass versus the particle size; the transecting lines indicate the mass median aerodynamic diameter (MMAD). Graph (B) shows the data from the same two samples with the total count versus the particle size, the count median aerodynamic diameter (CMAD) is indicated by the transecting lines. The geometric standard deviation is presented in both graphs within brackets.

(EMEM/NEAA; Sigma) supplemented with 2% FBS, penicillin (4 units/mL), streptomycin (4 μg/mL), gentamicin (20 μg/mL) and fungizone (0.1 μg/mL). Six-well plates were inoculated in triplicate as described by Nalca et al. (2010) with 100 μL of serially diluted samples and incubated with two ml of complete media for 96 h at 37 °C with 5% CO_2. After incubation, media was removed and two ml of 0.1% crystal violet solution were added to each well. Stain solution was removed after 1 h at room temperature and rinsed with tap water. Plates were placed upside down overnight for drying and plaques were counted the next day. Tissue culture infective viral concentrations were expressed as plaque forming units per liter of air assuming the entire 10.7 L of air from the chamber was collected into the AGI media (pfu/L of air). The efficiency of the virus in aerosol was determined by dividing the value obtained after aging (C_{aged}) by the value obtained after the initial nebulization (C_0). This resulted in a fraction of remaining infective viruses in the aerosol which can account for day to day variations and allows better comparisons of samples.

2.5. Quantitative polymerase chain reaction

Viral DNA was purified from 200 μL of undiluted aerosol with the High Pure Viral Nucleic Acid kit (Roche Applied Science, Indianapolis, IN) according to the manufacturer's protocol. MPXV genome concentrations were then quantified in duplicates by qPCR using the primers, probe and standard curve described previously (Verreault et al., 2012). Viral concentrations were expressed as number of MPXV genomes per liter of air. The aerosol viral efficiency by qPCR was calculated in the same way (G_{aged}/G_0) as for culture results.

2.6. Statistical analysis

Statistical analysis was performed with the SigmaPlot software (Systat Software, Inc., Version 11.2, San Jose, CA). Viral concentrations and fractions of remaining virus expressed as PFU or genomes were compared by one way analysis of variance (ANOVA). All pairwise multiple comparison procedures were done with the Holm–Sidak method. Differences between the proportions of infective viruses (PFU/genomes) were compared by *t*-test between time points. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Environmental conditions and aerosol characteristics

Chamber temperature remained stable at 20–22 °C across all aging assays. Relative humidity (RH) inside the chamber reached 97% and decreased by about 0.4% per hour during the aging periods. The RH remained high (60–97%) during all assays. APS data, illustrated in Fig. 2, indicated a mass median aerodynamic diameter (MMAD; Fig. 2A) of 4.0 μm with a geometric standard deviation (σ_g) of 2.0 immediately after nebulization of sterile media, and a MMAD of 1.6 μm with a σ_g of 2.7 after 18 h of aging; MPXV aerosols had a MMAD of 4.1 μm during nebulization. The count median aerodynamic diameter (CMAD) illustrated in Fig. 2B shows the distribution of the same samples of airborne particles by count rather than by total weight; the CMAD was 1.3 μm initially and 1.0 after 18 h of aging.

3.2. Quantitative PCR and plaque assay analysis

Quantitative PCR and plaque assay analysis revealed detectable concentrations of viral genomes and infective viruses throughout the experiment. As seen in Fig. 3, mean genome concentrations in the chamber were 1.2E+07 ± 7.9E+06 genomes per liter of air after 2 min and 2.6E+05 ± 1.5E+05 genomes/L air after 90 h of aging. Viral concentrations dropped rapidly (1 log) within the first 18 h of aging and stabilized between 18 and 90 h. Plaque assay analysis of the aerosol samples revealed a 2 log decrease within the first 18 h followed by a stabilization of the concentration (Fig. 3; empty circles). The initial loss of larger particles was confirmed by the APS data (Fig. 2).

The portion of viral genomes associated with PFU was calculated by the ratio of PFU to genomes in each aerosol sample (Fig. 4). No significant difference was observed between 2 and 30 min of aging. A ten-fold decrease in the proportion of infective particles was observed after 3 h. This decrease was significant ($P < 0.05$) after three, 18, 48 and 90 h when compared to the initial portion of infective viruses at 2 min. There was no significant difference between 30 min, 3 h, 18, 48 and 90 h.

The remaining fractions of MPXV were determined by comparing the initial viral concentration after 2 min of aging to the concentration observed after the aging period of 2 min to 90 h. The results show a significant decrease in MPXV concentration after 3 h

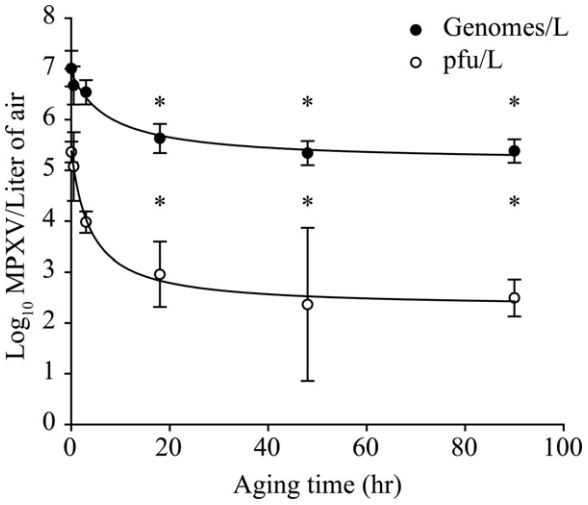

Fig. 3. Aerosol sample analysis by qPCR (full circles) and by plaque assay (white circles). Decay rate is rapid in the first hours and slows down with time. Error bars are standard deviations. Significant differences (one way ANOVA, Holm–Sidak method; $P < 0.05$) are indicated by asterisk (*). Differences are significant when compared to the first time point at 18, 48 and 90 h for qPCR and culture analyses.

by qPCR analysis (Fig. 5A) and after 18 h by plaque assay analysis (Fig. 5B).

4. Discussion

Although the physical decay rate of a polydispersed aerosol in a static chamber is relatively well understood and can be estimated using known constants such as terminal settling velocity (Hinds, 1999), actual suspension time in uncontrolled settings is less predictable due to highly variable environmental conditions. Biological aerosols are known to be generally hygroscopic (Ariya et al., 2009), rendering a higher susceptibility to variations in relative humidity and temperature. This can dramatically affect the physical dynamics of the aerosol, as well as the integrity of airborne viral particles and their potential to initiate infection once introduced in the host respiratory system. Modeling transport in a chamber that overcomes terminal settling velocity allows experimental observation of the effects of the constellation of physical and biological stressors when in aerosol. Rotating chambers have been used before to

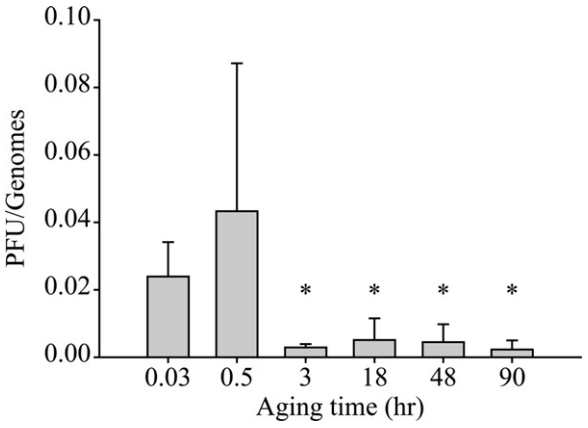

Fig. 4. Proportion of genomes detected associated with infective viral particles enumerated by plaque assay. Asterisk (*) indicates a significant difference as opposed to the samples taken at 0.03 h (t-test; $P < 0.05$). There is no significant difference in the proportion of infective viruses taken at time points 0.03 and 0.5 h. There are also no significant differences observed when comparing time points 0.5, 3, 18, 48 and 90 h among each other.

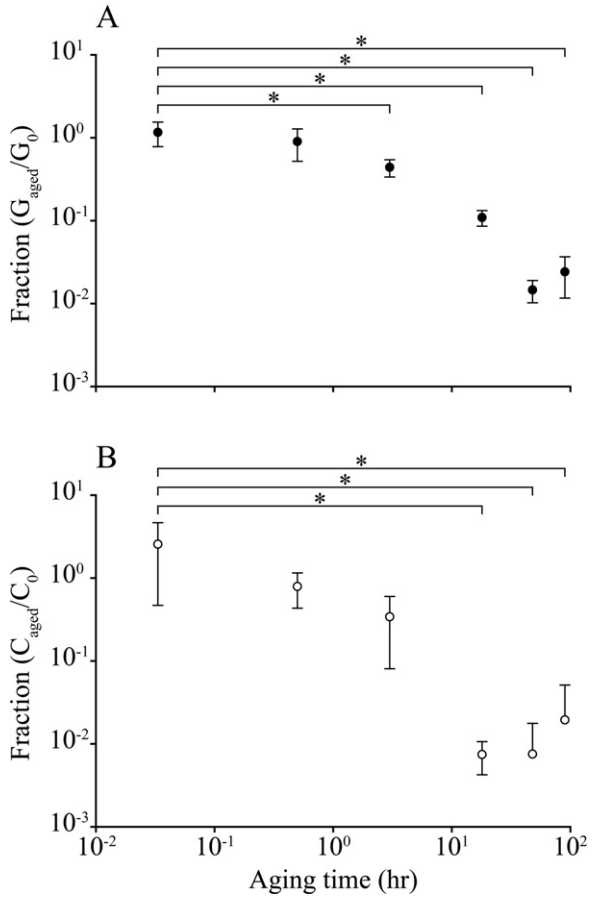

Fig. 5. Aerosol sample analysis by qPCR (A; full circles) and plaque assay (B; white circles). The concentration of the aged aerosol in genomes per liter of air (G_{aged}), or in pfu/L of air (C_{aged}), was divided by the concentrations detected after the initial aerosolization ($t = 0.03$ h; G_0 for qPCR and C_0 for culture) to determine the remaining fractions. Error bars are standard deviations, and each data point is the mean of three repeats; time points 0.5 and 18 h include four repeats. Significant differences (One way ANOVA, Holm–Sidak method; $P < 0.05$) are indicated by asterisk (*).

study aging viral aerosols (Adams et al., 1982; Akers et al., 1973; Elazhary and Derbyshire, 1979a, 1979b, 1979c; Harper, 1961; Ijaz et al., 1985, 1994; Karim et al., 1985; Sattar et al., 1984; Songer, 1967; Webb et al., 1963); however, the large size of the chambers used in these studies render their use in standard biosafety cabinets impractical or impossible. The results from this study show that a small 10.7 L rotating chamber which can easily be set up in standard Class three biosafety cabinets can be used to study the long term efficiency of airborne viral pathogens.

Infective viruses were recovered from suspended aerosols after 90 h of aging in the rotating chamber. Fig. 3 illustrates how the concentrations of total and infective MPXV tended to stabilize between 18 and 90 h; this suggests that viral particles could have been detected beyond 90 h of aging.

The rapid initial loss of viral genomes could be explained by the deposition of larger particles on the chamber's wall. Although the airborne particles in the rotating chamber are submitted to both centrifugal and gravitational forces (Asgharian and Moss, 1992), the larger particles remain more affected by gravity and settle more rapidly than the smaller particles. Another possible explanation for the initial loss, in regards to infectivity, could be the harsh conditions to which the viral suspension is subjected to during nebulization. The strong shearing forces exerted on the nebulization liquid in the Collison are known to affect the cultivability of bacteria (Stone and Johnson, 2002). These forces could also affect viral integrity thus lowering the number of infective viruses and

lowering the preservation of infectivity in the airborne state. However, these same conditions could also increase the number of PFU detected by breaking up clumps of aggregated viruses. This phenomenon could explain the high fractions of PFU detected after 2 min of aging as illustrated in Fig. 5B.

The damaged viral particles would also become more sensitive to the sampling process. Although aerosol sampling by liquid impingement is relatively gentle when compared to other sampling methods (Verreault et al., 2008), it is likely that some viral particles were inactivated during sampling. The aerosol stability of MPXV infectivity could thus have been underestimated. Less aggressive methods of aerosolization may minimize this effect by reducing the damages to the viral particles. The aerosol efficiency of viruses should ideally be studied from aerosols produced naturally by the shedding of infected hosts or from secondary aerosolization produced from infected matter. The high variability associated with viral production from these sources unfortunately makes the studying of naturally generated viral bioaerosols exceedingly difficult.

Another problem encountered when studying the aerosol stability of viruses is the rapid loss of the larger particles due to the effect of gravity; this effect can also be observed when using rotating chambers. In order to minimize this loss over time, it is possible to mimic aerosol suspension of viruses and to study their stability in various environmental conditions by using a physical support, such as spider webs (Smither et al., 2011), to retain the viral-laden particles in conditions very similar to the aerosol environment. This would prevent the physical loss of larger particles due to gravity. However, the viral particles still need to be nebulized and are thus subjected to potentially destructive physical stress. Although no single method is ideal, the tools and techniques available for studying airborne microorganisms, when used conjointly, could help answer important questions regarding the stability and potential hazards associated to these microorganisms.

The longevity of the MPXV in a laboratory controlled aerosol state suggests that this virus could potentially remain infectious for long periods of time in the environment. The most uncharacterized route of exposure during epidemics remains to be the aerosol route of transmission. Respiratory protection in conjunction with prophylactic medical intervention such as ring vaccination is part of the precautions taken when facing such an outbreak. A better understanding of the fate of airborne viruses or other infectious agents greatly improves our response to such events by extension of the informational database of susceptibility for viruses that can be transmitted by the aerosol route and are important to human health.

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References

Adams, D.J., Spendlove, J.C., Spendlove, R.S., Barnett, B.B., 1982. Aerosol stability of infectious and potentially infectious reovirus particles. Appl. Environ. Microbiol. 44, 903–908.

Akers, T.G., Prato, C.M., Dubovi, E.J., 1973. Airborne stability of simian virus 40. Appl. Microbiol. 26, 146–148.

Ariya, P.A., Sun, J., Eltouny, N.A., Hudson, E.D., Hayes, C.T., Kos, G., 2009. Physical and chemical characterization of bioaerosols – implications for nucleation processes. Int. Rev. Phys. Chem. 28, 1–32.

Asgharian, B., Moss, O.R., 1992. Particle suspension in a rotating drum chamber when the influence of gravity and rotation are both significant. Aerosol Sci. Tech. 17, 263–277.

Centers for Disease Control Prevention (CDC), 2003. Update: multistate outbreak of monkeypox – Illinois, Indiana, Kansas, Missouri, Ohio, and Wisconsin, 2003. Morb. Mortal. Wkly Rep. 52, 642–646.

Cutler, T.D., Wang, C., Hoff, S.J., Kittawornrat, A., Zimmerman, J.J., 2011. Median infectious dose (ID(50)) of porcine reproductive and respiratory syndrome virus isolate MN-184 via aerosol exposure. Vet. Microbiol. 151, 229–237.

Di Giulio, D.B., Eckburg, P.B., 2004. Human monkeypox: an emerging zoonosis. Lancet Infect. Dis. 4, 15–25.

Elazhary, M.A., Derbyshire, J.B., 1979a. Aerosol stability of bovine adenovirus type 3. Can. J. Comp. Med. 43, 305–312.

Elazhary, M.A., Derbyshire, J.B., 1979b. Aerosol stability of bovine parainfluenza type 3 virus. Can. J. Comp. Med. 43, 295–304.

Elazhary, M.A., Derbyshire, J.B., 1979c. Effect of temperature, relative humidity and medium on the aerosol stability of infectious bovine rhinotracheitis virus. Can. J. Comp. Med. 43, 158–167.

Essbauer, S., Meyer, H., Porsch-Ozcurumez, M., Pfeffer, M., 2007. Long-lasting stability of vaccinia virus (orthopoxvirus) in food and environmental samples. Zoonoses Public Health 54, 118–124.

Fenner, F., 1988. Smallpox and its Eradication. WHO, Geneva.

Fenner, F., Henderson, D.A., Arita, I., Jezek, Z., Ladnyi, I.D., 1988. Smallpox and its Eradication. WHO, Geneva.

Goldberg, L.J., Watkins, H.M., Boerke, E.E., Chatigny, M.A., 1958. The use of a rotating drum for the study of aerosols over extended periods of time. Am. J. Hyg. 68, 85–93.

Gruel, R.L., Reid, C.R., Allemann, R.T., 1987. The optimum rate of drum rotation for aerosol aging. J. Aerosol Sci. 18, 17–22.

Harper, G.J., 1961. Airborne microorganisms: survival tests with four viruses. J. Hyg. (Lond.) 59, 479–486.

Henderson, D.A., 1980. Smallpox eradication. Public Health Rep. 95, 422–426.

Hinds, W.C., 1999. Aerosol Technology, 2nd ed. Wiley-Interscience.

Ijaz, M.K., Sattar, S.A., Alkarmi, T., Dar, F.K., Bhatti, A.R., Elhag, K.M., 1994. Studies on the survival of aerosolized bovine rotavirus (UK) and a murine rotavirus. Comp. Immunol. Microbiol. Infect. Dis. 17, 91–98.

Ijaz, M.K., Sattar, S.A., Johnson-Lussenburg, C.M., Springthorpe, V.S., Nair, R.C., 1985. Effect of relative humidity, atmospheric temperature, and suspending medium on the airborne survival of human rotavirus. Can. J. Microbiol. 31, 681–685.

Karim, Y.G., Ijaz, M.K., Sattar, S.A., Johnson-Lussenburg, C.M., 1985. Effect of relative humidity on the airborne survival of rhinovirus-14. Can. J. Microbiol. 31, 1058–1061.

Nalca, A., Livingston, V.A., Garza, N.L., Zumbrun, E.E., Frick, O.M., Chapman, J.L., Hartings, J.M., 2010. Experimental infection of cynomolgus macaques (*Macaca fascicularis*) with aerosolized monkeypox virus. PLoS ONE 5, e12880.

Nicas, M., Hubbard, A.E., Jones, R.M., Reingold, A.L., 2004. The infectious dose of variola (smallpox) virus. Appl. Biosaf. 9, 118–127.

Nicas, M., Nazaroff, W.W., Hubbard, A., 2005. Toward understanding the risk of secondary airborne infection: emission of respirable pathogens. J. Occup. Environ. Hyg. 2, 143–154.

Reynolds, M.G., Damon, I.K., 2012. Outbreaks of human monkeypox after cessation of smallpox vaccination. Trends Microbiol. 20, 80–87.

Rimoin, A.W., Mulembakani, P.M., Johnston, S.C., Lloyd Smith, J.O., Kisalu, N.K., Kinkela, T.L., Blumberg, S., Thomassen, H.A., Pike, B.L., Fair, J.N., Wolfe, N.D., Shongo, R.L., Graham, B.S., Formenty, P., Okitolonda, E., Hensley, L.E., Meyer, H., Wright, L.L., Muyembe, J.J., 2010. Major increase in human monkeypox incidence 30 years after smallpox vaccination campaigns cease in the Democratic Republic of Congo. Proc. Natl. Acad. Sci. U.S.A. 107, 16262–16267.

Sattar, S.A., Ijaz, M.K., Johnson-Lussenburg, C.M., Springthorpe, V.S., 1984. Effect of relative humidity on the airborne survival of rotavirus SA11. Appl. Environ. Microbiol. 47, 879–881.

Smither, S.J., Piercy, T.J., Eastaugh, L., Steward, J.A., Lever, M.S., 2011. An alternative method of measuring aerosol survival using spiders' webs and its use for the filoviruses. J. Virol. Methods 177, 123–127.

Songer, J.R., 1967. Influence of relative humidity on the survival of some airborne viruses. Appl. Microbiol. 15, 35–42.

Stone, R.C., Johnson, D.L., 2002. A note on the effect of nebulization time and pressure on the culturability of *Bacillus subtilis* and *Pseudomonas fluorescens*. Aerosol Sci. Tech. 36, 536–539.

Tucker, J.B., 2011. Breaking the deadlock over destruction of the smallpox virus stocks. Biosecur. Bioterror. 9, 56–67.

Verreault, D., Moineau, S., Duchaine, C., 2008. Methods for sampling of airborne viruses. Microbiol. Mol. Biol. Rev. 72, 413–444.

Verreault, D., Sivasubramani, S.K., Talton, J.D., Doyle, L.A., Reddy, J.D., Killeen, S.Z., Didier, P.J., Marx, P.A., Roy, C.J., 2012. Evaluation of inhaled cidofovir as postexposure prophylactic in an aerosol rabbitpox model. Antiviral Res. 93, 204–208.

Webb, S.J., Bather, R., Hodges, R.W., 1963. The effect of relative humidity and inositol on air-borne viruses. Can. J. Microbiol. 9, 87–92.

Westwood, J.C., Boulter, E.A., Bowen, E.T., Maber, H.B., 1966. Experimental respiratory infection with poxviruses. I. Clinical virological and epidemiological studies. Br. J. Exp. Pathol. 47, 453–465.

Wolff, H.L., Croon, J.J., 1968. The survival of smallpox virus (variola minor) in natural circumstances. Bull. World Health Organ. 38, 492–493.