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Survival of aerosolized coronavirus in the ambient air

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ABSTRACT

An inactivation of airborne pathogenic Middle East Respiratory Syndrome (MERS-CoV) virus was investigated under controlled laboratory conditions. Two sets of climatic conditions were used in the experiments; (1) representing common office environment (25 °C and 79% RH) and (2) climatic conditions of the Middle Eastern region where the virus was originated from (38 °C and 24% RH). At the lower temperature, the virus demonstrated high robustness and strong capability to survive with about 63.5% of microorganisms remaining infectious 60 min after aerosolisation. Fortunately, virus decay was much stronger for hot and dry air scenario with only 4.7% survival over 60 min procedure.

1. Introduction

A variety of pathogenic microorganisms exists in the air and could potentially be transmittable over long distances. They include influenza virus, SARS virus, *Mycobacterium tuberculosis*, foot and mouth decease and many others (French, Kelly, Jones, & Clancy, 2002; Sergeev et al., 2013). First periodicals characterizing viral capability to survive in airborne form appeared in 1960s (Harper, 1961, 1963; Hemmes, Kool, & Winkler, 1962; Hemmes, Winkler, & Kool, 1960; Hood, 1963), where the authors reported very interesting outcomes on airborne virus behaviour and transmission efficiency. A comprehensive study on survival of various strains of airborne influenza virus was recently reported by Pyankov, Pyankova, and Agranovski (2012) showing presence of virulent virus even 90 min after aerosolization.

A number of SARS outbreaks in 2002–2003, with the first one reported in Guangdong Province, China in November 2002 and mainly affecting Asian region took 774 lives achieving the fatality rate of 9.6% (WHO, 2004, http://www.who.int/csr/sars/country/table 2004_04_21/en/). These outbreaks ignited strong research interest towards identification of virus transmission routs (Atkinson & Wein, 2008; Spekreijse, Bouma, Koch, & Stegeman, 2011; Tellier, 2006), exposure effects (Agranovski et al., 2010; Hermann, Muñoz-Zanzib, & Zimmerman, 2009; Kwon & Swayne, 2010) and transmission dynamics (Lipsitch et al., 2003). Also, a number of monitoring methods and techniques has been developed to meet the demand for conventional (Agranovski et al., 2004, 2005; Agranovski, Agranovski, Reponen, Willeke, & Grinshpun, 2002; Agranovski, Myojo, & Braddock, 2001; Agranovski, Safatov, Agafonov, Pyankov, & Sergeev, 2008; Hermann et al., 2006), and rapid detection of targeted pathogens in the ambient air (Agranovski et al., 2006; Pyankov et al., 2007; Usachev, Usacheva, & Agranovski, 2013). A comprehensive review by Weber and Stilianakis (2008) was produced at that stage describing corresponding research outcomes on virus inactivation and discussing models of the virus transmission. A number of important parameters of air influencing microorganism survival and delivery have been identified including air temperature and humidity, microbial resistance to external physical and biological stresses, solar intensity and others.

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In September 2012, a sixth new type of coronavirus was identified in Saudi Arabia and called Middle East Respiratory Syndrome Coronavirus (MERS-CoV) and has since spread to 1600 patients in 27 other countries (Gao, Yao, Yang, & Li, 2016), with the largest outbreaks in Saudi Arabia (according to WHO − 80% of cases), United Arab Emirates and South Korea (Choi, Kim, Cho, & Kim, 2015). Most people infected with MERS-CoV developed severe acute respiratory illness, including fever, cough, and shortness of breath; many of them died. Considering some possibility of respiratory transmission (Azhar et al., 2014), as well as transmission of viable virus settled on the surfaces (Otter et al., 2016), it is important to investigate survival of airborne virus aerosolized in the ambient air.

This paper presents results of investigation of time related inactivation of MERS-CoV in the ambient air at a range of climatic conditions, representing common office environment, and climatic conditions of the Middle Eastern region where the virus was originated from.

2. Materials and methods

All experiments with pathogenic microorganisms were performed in a high containment facility at the biosafety level of BSL3 + .

2.1. Virus and cells

Human betacoronavirus EMC (HCoV-EMC/2012) was kindly provided by Erasmus Medical Center (Rotterdam, Netherlands). MERS-CoV isolate HCoV-EMC/2012 was propagated in VeroE6 cells in Dulbecco's Modified Eagle Medium (DMEM; Gibco) supplemented with 2% fetal calf serum (Gibco), 1 mM L-glutamine (Lonza), 100 U/ml penicillin and 100 μg/ml streptomycin (Gibco). The HCoV-EMC/2012 virus stock was titrated by the end-point titration in VeroE6 cells. VeroE6 cells were inoculated with tenfold serial dilutions of virus stock in DMEM supplemented with 2% fetal calf serum, 1 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin. Five days after inoculation, cytopathic effect (CPE) was scored and 50% Tissue Culture Infective Dose ($TCID_{50}$) was calculated from 10 replicates by the Spearman-Karber method (Karber, 1931; Spearman, 1908).

2.2. Experimental setup

A laboratory setup used for experiments is presented in Fig. 1. A virus containing suspension was prepared and aerosolized to the experimental aerosol chamber by a 3-jet Collison nebulizer (BGI, Inc., USA) at the flowrate of 6 L/min of HEPA-filtered compressed air over 2 min time. Then the nebulizer was switched off. The experiments were performed for two sets of parameters of the air; (1) representing common office environment (25 °C and 79% RH), and (2) climatic conditions of the Middle Eastern region where the virus was originated from (38 °C and 24% RH). To minimise gravitational aerosol settlement during experiments, our previously developed rotational aerosol chamber (RAC) (Pyankov et al., 2012) was used in this project. It consists of a drum secured on a stationary axis with air-sealed ball bearings. The design of the axis enables to host pipelines used for aerosol charging and monitoring. The RAC was placed in the biosafety compartment to eliminate contamination of the laboratory air with viruses. The second reason to use the compartment was based on a fact that, as required by the experimental program, the virus survival ought to be investigated at elevated temperatures representing Middle Eastern climatic conditions – place of origin and maximum spread of the investigated microorganism. To achieve constant elevated temperature in the RAC over the entire experimental runs, the air in the compartment was heated up to a required value and then used to make up air drawn from the chamber by the samplers, as well as insulating the RAC from colder ambient air in the laboratory minimizing temperature fluctuations inside the RAC. The constant air humidity was achieved by adding required amount of steam to the compartment by the air humidifier (Model 7146, Air-O-Swiss, Switzerland). The air temperature and relative humidity were monitored in the RAC with the accuracy of ± 0.5 °C and ± 2% respectively over the

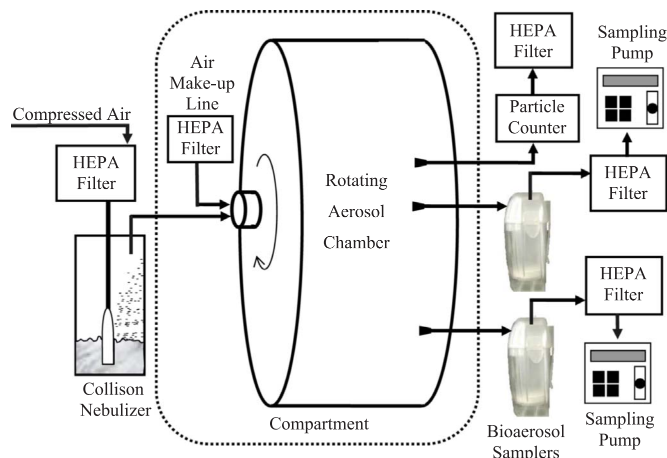

Fig. 1. Experimental setup.

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