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Efficacy of Ozone Gas against Mumps Virus under Experimental Environment Conditions

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Abstract

Microorganisms are present all around us, both in the surrounding air and on the surfaces. Some of them may cause infections and also contaminate food, drugs, medical equipment and other products. Today modern societies deal with this problem by filtrating and ventilating the indoor air, by disinfecting or sterilizing surfaces, but without total success. Ozonification has received increasing popularity in control of environmental pathogens. However, the knowledge of its effect against viral flora in indoor air and on internal surfaces is poor.

The objective of the present study was to develop a new method using commercially available ozone-generating apparatus "Hygienist" (Sani-Sport Inc., Montreal Canada) for neutralisation of mumps virus in indoor air and on internal surfaces of an experimentally contaminated room.

A significant anti-viral efficacy has been noticed after a period of 20 minutes of exposure to ozone gas concentration of 20-25 ppm and relative humidity > 90% in all samples of indoor air and internal surfaces, which showed that the virus was inactivated, ranging from at least 5 Log_{10} to undetectable.

These results showed that the present method offers possibilities for: a rapid and efficient viral decontamination of both highly contaminated indoor air and internal surfaces, as well as for very challenging application to health institutions, sport clubs, as well as other sectors where outbreaks of mumps virus occur frequently.

Keywords: *Mumps virus; Ozone; Neutralisation of virus*

Abbreviations: Abbreviations: PPM: Particles Per Million; PFU: Plaque Forming Units; Rh: Relative humidity

Introduction

Mumps is a single stranded RND virus of the genus *Rubula virus* known as a common cause of mumps infection which is an acute, highly contagious disease transmitted by droplets spread from the upper respiratory tract of humans. Mumps infection normally affecting children but can occur at any age [1]. Clinical manifestation of the disease starts with a non specific prodrome, which can include malaise, headache followed by inflammation and swelling of the parotid glands and sometimes of other salivary glands and occasionally by inflammation of the testis, ovary, pancreas or meningitis [2].

Outbreaks of mumps infection are frequently reported in the last decade in sport clubs and a variety of community of the modern societies, as consequences of inadequate vaccination programs [3,4]. There is currently no specific antiviral treatment available, but mumps vaccine has been available since 1960 [2]. Control measures for mumps consist of immunisation of susceptible populations and isolation of those symptomatic or potentially exposed.

Since mumps virus is spread into surrounding environment by droplets from the upper respiratory tract of infected people, numerous methods for environmental prevention have been developed and used with variable degrees of efficacy. One of them is ozonification.

Ozone, the triatomic form of oxygen (O_3) is a powerful oxidizing microbicidal agent, commonly used in the pharmaceutical and food industry, water disinfection, as well as environmental control of pathogenic flora due to its effectiveness against Gram-positive and Gram-negative bacteria and a variety of virus species [5-9]. Ozone does not promote microbial resistance and lives no residues. Ozone gas has a number of potential advantages over other decontaminating gases and liquid chemicals, and it can diffuse to completely fill the space of all areas within a room, including crevices, fixtures, fabrics and the under surfaces of furniture [9,10]. The only disadvantage of ozone use is the potential toxicity to humans at high concentrations. However, the health hazard can be overcome by insuring that the space to be decontaminated is temporarily closed to people during treatment in order to prevent gas leaks into the environment.

Materials and Methods

Ozone-generating apparatus

The ozone generating apparatus "Hygienist" (Figure 1) was a commercially available portable module (Sani Sport Inc., Montréal, Canada) containing both ozone generating and ozone neutralizing devices which was equipped with a controlled dial to determine the approximate ozone generation in ppm.

Figure 1: *Ozone generating apparatus "HYGIENIST" (Sani Sport, Montreal Canada).*

Ozonization test-chamber

The test chamber was a specifically prepared laboratory room in which ozone generating apparatus "Hygienist" and ozone sampler was installed. An ozone sampler has been connected to the exterior ozone measuring system BMT 964 RD (BMT Messtechnick GMBH, Germany) for accurately recording ozone concentration in experimental room. The probe of a hygrometer (VWR Scientific, Ontario) for measuring relative humidity (Rh) has also been placed inside the experimental room at an adequate place. A portable commercial humidifier (Humidifirst Inc., Florida) was used to provide a burst of water vapour in ambient, as required.

Mumps virus

Mumps virus (ATCC VR-106) was cultured according to ATCC protocol which was used for production of experimental virus suspension, as well as for the evaluation of deactivation of the virus by ozonification [11]. The virus experimental suspension was prepared as clarified cell-free supernatant, with concentration of 1.3×10^9 PFU (plaque forming units) per ml [12]. Then, the experimental suspension has been spread in ambient air and on internal surfaces of experimental room by means of commercially available spraying device, Electric Hot Fogger (Chem-Tex, Houston, TX, USA).

Determination of the titre of the virus

To determine the titre of the virus in experimental viral suspensions, control and experimental surfaces samples, serial dilutions of viral suspensions were applied to a confluent cell layer of SL-29 cells (ATCC CLR1590). After a 1 hour infection the virus was removed and the cells were kept in culture for additional 16 hours. The PFU were determined as described above [12].

Test procedure

Experimental trial was conducted in a specifically prepared laboratory room, having volume of 60 m^3. The ozone generator "Hygienist" was installed on the floor in the center of the space. Ozone sampler and Rh probe have been installed on the walls of the room. Then, the experimental virus suspension has been spread in the ambient air and on internal surfaces by commercially available spraying device (Electric Hot Fogger).

Three samples of indoor air and 3 samples from per internal surfaces (floor, ceiling) and four wall samples have been taken prior to the experimental decontamination and used as negative control [13,14]. The experiment conditions involved increasing the ozone level over period of 15 min to 25 ppm, maintaining this level for 10 min at which point the humidity was produced by rapid burst of water vapour. This resulted in Rh increase to > 90% within 5 min. Following this, the ozone generator was on until the end of ozone exposure time of 20 minutes.

After period of ozonification of the experimental room, ethanol spraying system of "Hygienist" was switched on; this resulted in a decrease of ozone to < 1 ppm within 15 min. Then the samples of ambient air and internal surfaces were taken, as previously described, and kept with control samples (taken before ozonification) in the Bio safety cabinet for the duration of the test.

Results

Viability of the virus in control samples, taken from indoor air and internal surfaces of experimental room before ozonification is shown in Table 1.

Samples	*PFU	Log_{10} of reduction**
Ambient air	$2.1 \times 10^8/m^3$	0.73
Floor (***n = 3)	$2.07 \times 10^8/F^2$	0.53
Ceiling (***n = 3)	$4.33 \times 10^7/F^2$	0.76
Wall (***n = 4)	$1.24 \times 10^9/F^2$	0.72

Table 1: *Viability of the virus in control samples taken from indoor air and internal surfaces of experimental room before ozonification.*
**PFU = Plaque formation units.*
***When compared to virus concentration in 2 ml of experimental virus suspension.*
****n = Statistical analysis was performed by ordinary one way analysis of variance.*

In ambient air the virus showed decrease in infectivity up to 0.73 Log_{10}, probably as a result of the drying process [11], as shown in Figure 2 and 3.

Viability of the virus in experimental samples, taken from indoor air and internal surfaces of experimentally contaminated room after ozonification is shown in Table 2.

Samples	*PFU	Log_{10} of reduction**
Ambient air	Non-detect/m^3	8.07
Floor (***n = 3)	6.56 x 10^2/ F^2	5.50
Ceiling (***n = 3)	8.13 x 10^1/F^2	5.73
Wall (***n = 4)	2.74 x 10^2/F^2	5.56

Table 2: *Viability of the virus in samples taken from indoor air and internal surfaces of experimentally contaminated room after ozonification. *PFU = Plaque formation units.*
***When compared to control samples.*
****n = Statistical analysis was performed by ordinary one way analysis of variance.*

The virus showed decrease in infectivity going from undetectable in the ambient air up to 5.73 Log_{10} as a result of the ozonification process, as shown in the Figure 2 and 3.

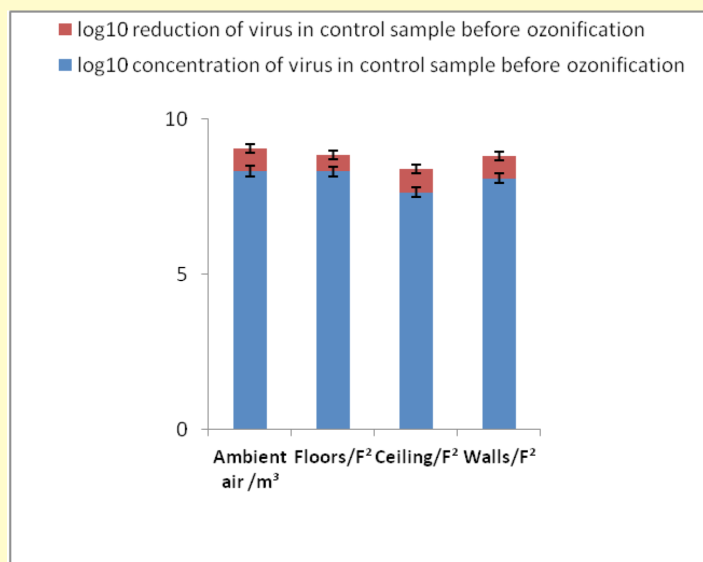

Figure 2: *Log_{10} concentration of the virus in control samples with Log_{10} reduction.*

Discussion

Microorganisms are ubiquitous and can be found all around us, in our daily environment. Some of them may cause respiratory infections and other diseases. As potential contaminants they represent a concern mainly in the pharmaceutical industry, healthcare facilities and food industry. Developed countries deal with this problem by applying a variety of disinfection or sterilization procedures on surfaces, and by ventilating and filtrating the indoor air, but without adequate success.

Ozone is a potent germicide that has been used extensively for water purification [15]. The understanding of its biocidal capability against the microorganisms primarily responsible for indoor air quality is still relatively limited. Ozone gas has several advantages as a practical anti-viral agent. It can effectively penetrate every part of a room, including sights that might prove difficult to gain access to with conventional liquid by normal disinfection procedures [16,17].

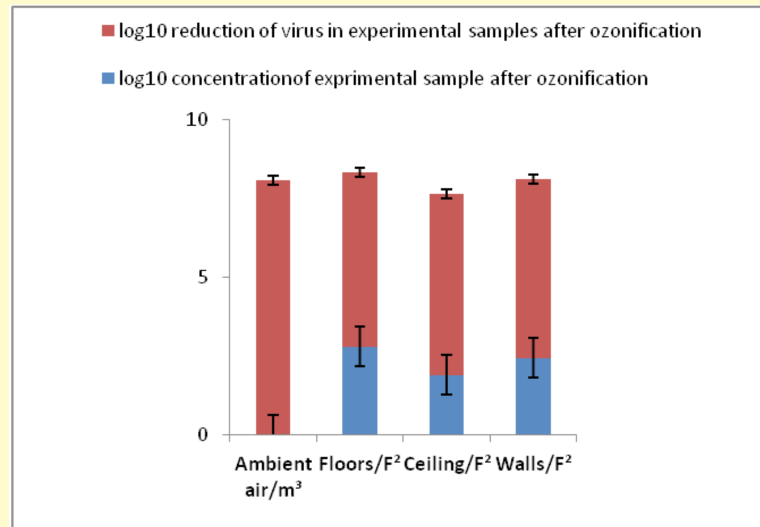

Figure 3: *Log$_{10}$ concentration of the virus in experimental samples with Log$_{10}$ reduction.*

The gas is easy and economical to produce, and it is natural compound which decays quickly back to oxygen with a half-life of about 20 min. The use of spraying of ethanol in confined room considerably speeds up the reverse of the gas to oxygen as shown in the present study. In addition, in the event of possible malfunction during application, the gas is rapidly detected by smelling, and hence can be avoided.

The major disadvantage of ozone is its potential toxicity at high concentration, which precludes its use in populated areas, and its ability to corrode certain materials, such as natural rubber on prolonged exposure. This means it can only be used in rooms that have sealed off or quarantined for the duration of the treatment. Sensitive materials can be temporally covered or removed if necessary during the ozonification protocol. However, since the standard protocol requires less than an hour to perform, thanks to very efficient decay of ozone to oxygen, this is not generally a barrier to utilisation.

Thus, the objective if the present study was to develop a particular and efficient method for decontamination of confined space, experimentally contaminated by the virus. High Rh concentration has been applied to achieve major impact of the virucidal effect of ozone as shown in a similar study previously [13,18].

Previous similar studies have been carried out on experimentally contaminated size limited hard surfaces [7,8,12,14,16]. The Present method is based, for the first time, on mumps virus inactivation in indoor air and on internal surfaces of an experimentally contaminated room, which represents a condition closer to environmental decontamination of indoor space. In spite of the fact that present method has been used for the first time for inactivation of the virus in indoor space, the developed procedure could be useful in various community, hospitals, health care facilities, dental offices, sport clubs, cruise lines and other locations where outbreaks of mumps or other infectious diseases are relatively common.

Conclusion

Present method can be helpful for microbial decontamination of contaminated space in hospitals, health-care institutions, dental offices, sport clubs, hotels and transportation sector, as well as all other spaces where outbreaks of pathogenic flora occurs.

Bibliography

1. Haviid A., *et al*. "Mumps". *Lancet* 371.9616 (2008): 932-944.
2. Plotkin SA and Rubin S. "Mumps vaccine". Plotkin SA., *et al*. (Eds) Vaccines, 5th edn. Philadelphia. PA: WB Sanders (2008): 436-465.
3. Tan KE., *et al*. "Mumps virus detection during outbreak in highly unvaccinated population in British Columbia". *Journal of Public Health* 102.1 (2011): 47-50.
4. Dayan GH., *et al*. "Recent resurgence of Mumps virus in the United States". *The New England Journal of Medicine* 358.15 (2008): 1580-1589.
5. Broadwater WT., *et al*. "Sensitivity of three selected bacterial species to ozone". *Applied Microbiology* 26.3 (1973): 391-393.
6. Kim JG., *et al*. "Use of ozone to inactivate microorganisms on lettuce". *Journal of Food Safety* 19.1 (1999): 17-33.
7. Wells, K.H., *et al*. "Inactivation of Human Immunodeficiency Virus Type 1 by Ozone *in Vitro*". *Blood* 78.7 (1991): 1882-1890.
8. Khandre MA and AE Yousef., "Susceptibility if Human Rotavirus to Ozone, High Pressure, and Pulsed Electric Field". *Journal of Food Protection* 65.9 (2002): 1441-1446.
9. Barker J., *et al*. "Effects of Cleaning and Disinfection in Reduction the Spread of Noravirus Contamination via Environmental Surfaces". *Journal of Hospital Infection* 58.1 (2004): 42-49.
10. Malik YS., *et al*. "Disinfection of Fabric and Carpets Artificially Contaminated with Calicivirus: Relevance in Institutional and Health Care Centers". *Journal of Hospital Infection* 63.2 (2006): 205-210.
11. Mumps virus (AATCC VR-106).
12. Hudson JB., *et al*. "Development of a particular method for using ozone gas as a virus decontamination agent". *Ozone: Science & Engineering* 31.3 (2009): 216-223.
13. Verreault D., *et al*. "Method for sampling of airborne viruses". *Microbiology and Molecular Biology Reviews* 72.3 (2008): 413-444.
14. Julian TR., *et al*. "Comparison of surface sampling methods for virus recovery from fomites". *Applied and Environmental Microbiology* 77.19 (2011): 6918-6925.
15. USEPA (Environmental Protection Agency). "Wastewater Technology Fact Sheet: Ozone Disinfection". Report # EPA 832-F-99-063 prepared by the Office of Water Washington DC.
16. Burleson GR., *et al*. "Inactivation of viruses and bacteria by ozone with and without sonification". *Applied Microbiology* 29.3 (1975): 340-344.
17. Aydogan A and Gurol MD. "Application of gaseous ozone for inactivation of *Bacillus Subtilis* spores". *Air & Waste Management Association* 56.2 (2006): 179-185.
18. Dyas A., *et al.* "Ozone killing action against bacterial and fungal species; Microbial testing of a domestic ozone generator". *Journal of Clinical Pathology* 36.10 (1983): 1102-1104.

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