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SURVIVAL AND INACTIVATION OF BACTERIOPHAGE Φ6 ON N95 RESPIRATOR MATERIAL

by

BETELHEM WAKA

B.S., BIOLOGY
GEORGIA STATE UNIVERSITY

A Thesis Submitted to the Graduate Faculty
of Georgia State University in Partial Fulfillment
of the Requirements for the Degree

MASTER OF PUBLIC HEALTH

GEORGIA STATE UNIVERSITY
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APPROVAL PAGE

Survival and Inactivation of Bacteriophage Φ6 on N95 Respirator Material

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ACKNOWLEDGEMENTS

I would like to express my deep gratitude to my Thesis Chair and mentor, Dr. Lisa Casanova. In addition to this thesis, her expertise and guidance have helped me throughout the Public Health program. I also want to thank Dr. Christine Stauber, my second committee member who meticulously edited my thesis draft and was incredibly supportive. I would also like to express my gratitude to my academic advisor Dr. Okosun for guiding me throughout the program. In addition, I want to thank the memorable faculty at Georgia State University that have introduced me to public health and encouraged my passion for it.

Finally, I want to thank my family, my fiancé, and my friends. They have been a constant source of unparalleled support throughout my educational process and especially while working on my thesis. Their patience, kindness, and unconditional support has impacted my life in many ways throughout the years. They have always believed in me, always been there for me and have always encouraged me to follow my dreams.

Thank you all for helping to shape me into the person I am today.

ABSTRACT

BETELHEM WAKA
Survival and Inactivation of Bacteriophage Φ6 on N95 Respirator Material

Introduction: Preventing healthcare professionals from acquiring occupational infectious diseases is very important in maintaining healthcare delivery systems. For protection in the work place, healthcare professionals use PPE which helps prevent exposure to pathogens during patient care. N95 respirators protect healthcare workers against airborne pathogens that are known to be associated with different respiratory diseases. Since previous studies have shown that viruses can survive on PPE surfaces, it is important to examine the survival of viruses on respirators to determine if reuse of the same N95 respirator is possible when PPE shortages occur.

Goal: The goal of this research is to determine the inactivation of bacteriophage Φ6 on the surface of N95 respirators at ambient temperature and two different relative humidity levels, 40 and 60%.

Result: The linear regression showed that rate of inactivation was much lower in 40% than 60% RH (40%: Slope= -0.046± 0.007040; 60%: Slope= -0.20± 0.006136). Over 24 hours, there was a ~1 Log_{10} reduction in virus at 20°C and 40% RH, while there was a ~4 Log_{10} reduction at 20°C and 60% RH. Within the timeframe of a single patient encounter, there was a <0.02 Log_{10} reduction in virus at 40% RH and a <0.1 Log_{10} reduction at 60% RH.

Conclusion: Bacteriophage Φ6 survives on N95 respirators for up to 24 hours at ambient temperature and 40 and 60% relative humidity levels. Inactivation rate was lower in 40% than 60% RH. The results showed that enveloped viruses survive on the surface of N95 respirators for longer than a single patient encounter. Therefore, this should be taken into consideration when doing a risk assessment of reusing N95 respirators when shortages occur.

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Chapter I
INTRODUCTION

1.1 Background

Preventing healthcare professionals from acquiring occupational infectious diseases is very important in maintaining healthcare delivery systems. For protection in the work place, healthcare professionals use personal protective equipment (PPE). PPE helps prevent exposure to pathogens during patient care. PPE includes gloves, gowns, masks, and respirators. Individually fitted N95 respirators, which are worn over the nose and mouth, are part of PPE worn by doctors and nurses. They are commonly used to protect the human respiratory system against airborne particles and pathogens that are known to be associated with different respiratory diseases. Examples of pathogens causing respiratory diseases are rhinovirus, respiratory syncytial virus (RSV), parainfluenza virus, severe acute respiratory syndrome-associated coronavirus (SARS-CoV), influenza virus, etc. (WHO, 2007). N95 masks are designed to reduce exposure to bacterial or viral particles (Balazy et al., 2006; Johnson, Druce, Birch, & Grayson, 2009). The Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) recommend N95 respirators as a means of protecting healthcare workers when dealing with respiratory diseases that have increased risk of transmission (CDC; WHO, 2007). The Institute of Medicine (IOM) recommends properly fitted N95 masks for giving the most protection against airborne infections, such as influenza (IOM, 2006).

There is a risk involved for healthcare workers when taking care of patients with many different infectious diseases. Respiratory infections can spread rapidly in healthcare settings, exposing both patients and caregivers. Previous experiences with the severe acute respiratory syndrome (SARS) outbreak in 2003 and swine flu outbreak in 2009 have shown that patient-to-healthcare worker transmission can occur (Lai, Cheng, & Lim, 2005; Wise et al., 2011).

Healthcare workers accounted for more than 20% of those infected with SARS (Lai et al., 2005). A study done in Hong Kong during the outbreak found that healthcare workers that used N95 respirators were significantly less likely to be infected (Seto *et al.*, 2003). This was not the case for those who used paper masks (2003). In the spring of 2009, pandemic influenza virus (pH1N1) was first identified. A study by Wise et al. (2011) looked at transmission of pandemic (H1N1) 2009 influenza to healthcare professionals, and the results suggested that healthcare workers may be at risk for being exposed to pH1N1 infection at work. In the event of a pandemic, the Occupational Safety and Health Administration (OSHA) considers healthcare workers to be at high risk for exposure to new influenza viruses (Wise et al., 2011).

A previous study has shown that viruses can survive on PPE materials and may transmit disease if a virus is transferred during handling of the materials (Lai et al., 2005). In addition to autoinoculation, it might also result in subsequent transmission of viruses to other patients and staff. A similar study looked at survival of coronavirus on PPE surfaces and found that viruses may survive on the respirator for hours (Casanova et al., 2010b) potentially posing a continued risk to the person handling the mask after wearing. To assess the risk posed by contaminated PPE, it is important to gather data on the survival of viruses on PPE surfaces.

A bacteriophage (a virus that infects bacteria) Φ6 was used for this study. This model virus is similar in structure to human respiratory viruses, such as influenza. Working with infectious viruses requires higher level biosafety facilities. It can also be expensive and labor intensive. More importantly it can be risky for researchers to work with human pathogens. Thus using phi6 as a surrogate for pathogenic enveloped viruses has many advantages especially when it comes to safety and cost effectiveness, and has been used in previous studies to understand the survival dynamics of influenza in the environment (Adcock et al., 2009).

Environmental factors such as relative humidity (RH), temperature and the type of surface influence the survival of viruses in the environment. A study done by Abad et al., (1994) on survival and persistence of enteric viruses on environmental surfaces has shown that they are able to survive for prolonged periods on different types of surfaces commonly found in healthcare environments. The study also found that RH is a factor influencing virus survival (Abad, Pinto, & Bosch, 1994). A study by McDevitt et al. (1984) looked at the role of absolute humidity in the inactivation of influenza viruses and found that that absolute humidity (a function of RH and temperature) and exposure times are strong predictors of virus inactivation.

1.2 Purpose of study

The suggestions proposed as a means to alleviate the burden posed by shortages of PPE are reusing by the N95 respirators or switching to alternative masks. However, N95 respirators are intended to be disposable (single-use) and alternative masks are potentially less protective. Although decontamination of N95 respirators is another alternative, the need for respirator decontamination is not well characterized because the data on virus survival on respirators is limited (Fisher & Schaffer, 2010). Thus, examining the survival of viruses on respirators is essential for determining if reuse of the same N95 respirator during a pandemic or an outbreak where PPE shortages might occur poses a risk to healthcare professionals, while adding to the data on virus survival on respirators. Therefore, the goal of this research is to determine the inactivation of bacteriophage Φ6 on the surface of N95 respirators at ambient temperature (20°C) and two different relative humidity levels, 40 and 60%, over a period of 24 hours.

Chapter II
LITERATURE REVIEW

2.1. Background

In 2003 there was a worldwide SARS outbreak (Lai et al., 2005; Seto *et al.*, 2003). The outbreak brought to attention the importance of PPE in preventing transmission of infection (Lai et al., 2005). PPE is very important in interrupting transmission of infectious agents from patients to healthcare workers. On the other hand, PPE itself can be an agent of transmission as it can become contaminated by pathogens while healthcare tasks are being performed (Casanova, Alfano-Sobsey, Rutala, Weber, & Sobsey, 2008a). Viruses can survive on PPE materials and may transmit disease if a virus is transferred during handling of the PPE materials (Lai et al., 2005). The authors stated that, "PPE items are potential fomites" (Casanova et al., 2008a).

There is a risk involved for healthcare workers when taking care of patients with infectious diseases. SARS cases were reported in healthcare facilities in patients, healthcare workers, and even visitors (Casanova, Rutala, Weber, & Sobsey, 2008b; Casanova, Rutala, Weber, & Sobsey, 2010a; Lai et al., 2005). Healthcare workers accounted for more than 20% of those infected with SARS (Seto et al., 2003). Coronaviruses have been known to cause respiratory diseases like the common cold. But, it was not until the SARS outbreak resulted in serious and even fatal infections that the severity of coronavirus infection was understood (Casanova, Rutala, Weber, & Sobsey, 2009).

2.2. N95 respirators

According to the National Institute for Occupational Safety and Health (NIOSH) respiratory protection approval regulation (42 CFR 84), N95 refers to a filter class not a respirator. However, they have come to be known as N95 respirators because many filtering face piece respirators have an N95 class filter. This respirator is a type of particulate filtering face piece that filters at least 95% of airborne particles (CDC, 2012). N95 filtering face piece respirators are commonly used to protect the human respiratory system against airborne particles that are known to be associated with different respiratory diseases. N95 masks are designed to reduce exposure to bacterial or viral particles (Balazy et al., 2006; Johnson et al., 2009).

A study done by Casanova et al. (2010b) examined the survival and inactivation of on PPE of transmissible gastroenteritis virus (TGEV), a surrogate for SARS coronavirus. This study found that, on an N95 respirator, only a small amount of infectious virus was lost in the first 2 hours and the virus was still detectable for up to 24 hours. This survival experiment suggests that, "coronaviruses can survive on PPE items for the duration of a single patient encounter" (2010b). This study has shown that viruses may survive on the respirator for hours and pose a continued risk to the person wearing and handling the mask (Casanova, Jeon, Rutala, Weber, & Sobsey, 2010b).

A study done by Seto et al. (2003) during the SARS outbreak found that healthcare staff who used N95 masks were significantly less likely to have been infected. Since droplets are generated at face level, it is very important to use the N95 respirator to prevent droplet transmission (Seto et al., 2003).

2.3. Bacteriophage Φ6

Bacteriophage Φ6 is used as a model for respiratory viruses such as influenza virus (Adcock et al., 2009). Working with infectious viruses requires higher level biosafety facilities. It can also be expensive and labor intensive. More importantly it can be risky for researchers to work with human pathogenic viruses. Thus using a surrogate virus has many advantages especially when it comes to safety and cost. Because working with infectious viruses like SARS requires specially trained researchers working in BSL-3 laboratory containment, there are significant challenges involved in studying the survival of this virus effectiveness (Adcock et al., 2009; Casanova et al., 2009; Casanova et al., 2010a). A study by Casanova et al. (2009) focused on the use of surrogate viruses to overcome these challenges and expand the available data on coronavirus survival. In addition, the results of a study by Adcock et al. (2009) indicate that these enveloped bacteriophages can serve as surrogates for inactivation experiments of influenza like viruses.

Environmental factors such as RH, temperature and the type of surface influence stability of the virus.

2.4. Environmental Surfaces

Ansari et al. (1991) stated that, "the potential of a vehicle to spread a given infectious agent is directly related to the capacity of the agent to survive in or on that vehicle." Viruses such as influenza and SARS coronavirus can survive for hours on surfaces (Casanova et al., 2008b). This can result in the transmission of the viruses when they come in contact with hands (2008b).

Hands have been implicated in the spread of infectious diseases because they come in contact with contaminated surfaces or fomites and result in self-inoculation or spread to others

(Ansari, Springthorpe, Sattar, Rivard, & Rahman, 1991). This is especially the case when caring for sick patients. Studies looking at the potential role of hands in the spread of respiratory viral infections found that hands may be a more important vehicle for the spread of rhinoviruses (Ansari et al., 1991; Mbithi, Springthorpe, Boulet, & Sattar, 1992). Rhinovirus has been shown to survive on human hands after being picked up from environmental surfaces and may result in self-inoculation and transmission (Mbithi et al., 1992; Sizun et al., 2000). In addition, Respiratory syncytial virus (RSV) has also been shown to survive for extended periods on surfaces and result in transmission (Mbithi et al., 1992; Sizun et al., 2000).

The results from the study by Casanova et al., (2010a) suggested that enveloped viruses can remain infectious on surfaces for a period of time in which people could come in contact with them. This poses a risk for exposure that could result in infection and transmission. Surfaces may act as vehicles for the spread of infection (Abad, Pinto, & Bosch, 1994; Casanova et al., 2010a).

A study by Brady et al. (1990) looked at the survival of parainfluenza virus on different commonly contaminated environmental surfaces in a hospital. The results of the virus survival experiment showed that parainfluenza viruses can persist on non-absorptive surfaces (if the surface remains moist) for as long as 10 hours. The virus survived for up to 2 hours when the surface is allowed to dry (1990). The survival of the virus was prolonged when the initial concentration of virus was increased (Brady, Evans, & Cuartas, 1990; Casanova et al., 2010a; Lai et al., 2005).

A study done by Abad et al. (1994) on survival and persistence of enteric viruses on environmental surfaces has shown that relative humidity (RH) is also a factor influencing virus survival. The results of this study showed that human enteric viruses survived for extended

periods on fomites. Based on the environmental surfaces the virus showed different patterns behavior. The results of this study showed that enteric viruses are able to survive for prolonged periods on different types of surfaces commonly found in healthcare environments (Abad, Pinto, & Bosch, 1994).

2.5. Humidity and temperature

A study was done by Lowen et al. (2007) using the guinea pig model to evaluate the effects of temperature and relative humidity on influenza virus spread. They performed transmission experiments under controlled conditions. They found that transmission was highly efficient at low RH of 20%–35%. On the other hand, transmission was completely blocked at a high relative humidity of 80% (100% transmission at 20-35% RH, 25% transmission at 50% RH, 75% transmission at 65% RH, and 0% transmission at 80% RH). Similarly, another report has shown viral stability is maximal at low RH (20%–40%), minimal at intermediate RH (50%), and high at elevated RH (60%–80%). Some laboratory studies have shown that, viruses survive better in an environment with high RH and low temperatures. While another study reported that when RH levels are below 50%, there is higher survival of enveloped viruses on inanimate surfaces (Abad, Pinto, & Bosch, 1994; Lowen et al., 2007; Mbithi, Springthorpe, & Sattar, 1991). Low RH is most favorable for enveloped viruses (in aerosol form), such as measles and influenza (Sobsey & Meschke, 2003).

A study by Casanova et al., (2010a) looked at survival of this virus on environmental surfaces and on how survival is affected by environmental variables, such as air temperature and relative humidity (RH). The results of this study showed that survival of the virus was greater at low RH, which the author stated was also the case for previous studies of coronaviruses and

other enveloped viruses in aerosols. Previous studies have also observed greater survival at low RH in other enveloped viruses such as influenza virus (Casanova et al., 2010a). In addition, the results also showed that when high numbers of viruses are deposited, the viruses survive longer at ambient temperature and humidity levels ranging from 20% to 60%. The results from this study suggest that there are interactions between temperature and RH, but RH has a greater effect on viral inactivation than temperature (2010a).

Temperature is a factor that influences virus survival (Casanova et al., 2009). When looking at temperature, the experiment by Lowen et al. (2007) found that transmission occurred with greater frequency at 5°C than at 20°C, while no transmission was detected at 30°C. Similarly, the study by Ijaz et al. (1985) found that at ~20°C, aerosolized human coronavirus 229E (HCV/229E) was found to survive best at medium (~50%) RH while at low (~30%) RH the virus survival decreased. On the other hand, the survival of aerosolized virus decreased the most in high (~80%) RH. At low (~6°C) temperature, virus survival was high at medium and low RH. In addition, at low temperature and high RH the survival remained high unlike at ~20°C. Looking at temperature, under conditions of high RH, the results of the study suggested that the fluidity of the lipid-containing envelope is stabilized at low temperature (Ijaz, Brunner, Sattar, Nair, & Johnson-Lussenburg, 1985). The relationship between each variable and inactivation of the virus may vary depending on the type of virus (Casanova et al., 2010a).

A study looking at the effect of temperature and RH on survival of Hepatitis A Virus on environmental surfaces found that the ability of viruses, like HAV, to survive for long periods on the surfaces suggests that hard environmental surfaces and different types of fomites could act as potential vehicles for extended periods after contamination (Mbithi et al., 1991). This is especially true in healthcare settings.

Despite knowing all of this, our knowledge of survival of respiratory viruses on PPE surfaces is still very limited. The actual survival (inactivation rate) rate of a virus on the N95 respirator mask is unknown. Therefore, it is important to study the inactivation of viral particles on the respirator. It will help to quantify the risk of exposure and possible transmission associated with surfaces.

Chapter III
MATERIALS AND METHODS

The purpose of this experiment was to measure the survival rate of bacteriophage Φ6 on N95 respirator material at 40% and 60% RH over a period of 24 hours.

3.1. Virus

Bacteriophage and host were kindly provided by Dr. Leonard Mindich, University of Medicine and Dentistry New Jersey, and used throughout this experiment. Bacteriophage Φ6 was propagated in the host bacterium *Pseudomonas syringae* using the soft agar coliphage propagation method. Briefly, 30 mL of host bacterial culture was grown for 24 hours on a rotating shaker (100 rpm, 25°C). At 24 hours, 2 mL of virus stock was added and incubated with shaking for an additional 24 hours. "Soft" agar was prepared by adding agar to tryptic soy broth at a final concentration of 0.7%, and bottom agar plates were prepared using full strength tryptic soy agar in 150 mm petri dishes. Fresh virus stock (0.5 mL) and log phase host culture (0.5 mL) were added to 30 mL of soft agar and dispensed into bottom agar plates. Plates were incubated at 25°C for 24 hours. The top soft agar layer was then harvested, and soft agar from all plates was pooled, purified by centrifugation (5900×*g*, 30 minutes, 4°C)., and stored as stock in 20% glycerol-tryptic soy broth at -80°C.

3.2. Survival experiments

Virus stock was diluted in Phosphate buffered saline (PBS) to target a concentration of 10^5 PFU in 10μL. 10μL of virus stock dilution was placed onto six 1 cm^2 coupons of N95 respirator material (3M, St. Paul, MN) in a petri dish (total virus per carrier 10^5 PFU).

For the zero time point, the carriers were then immediately transferred into tubes. Two ml of 1.5% beef extract, pH 7.5, was added into the tubes. The tubes were then agitated on a shaker at 60 rpm for 20 minutes. Samples were assayed using the double agar layer (DAL) plaque assay on tryptic soy agar (TSA). Plates were then incubated at 25°C for 24 hours. After incubation, plaques were counted and recorded.

For the other time points (2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 hours), after the virus was placed onto the carriers, they are placed into controlled humidity environments at either 40% (±2%) or 60% (±2%) RH, created by placing saturated salt solutions in sealed glass tanks. Temperature and RH were monitored daily.

Virus survival at each time point was expressed as $\log_{10}(N_t/N_0)$, where N_t is the virus concentration in PFU/mL at time t, and N_0 is the initial virus concentration in PFU/mL in the control sample at time 0.

3.3. Statistical Analysis

Analysis was done using Excel 2007 (Microsoft Corp.) and GraphPad Prism 5 (GraphPad, San Diego, CA). The parameter $\log_{10}(N_t/N_0)$ vs. time was used to perform regression analysis for both humidity settings (40% and 60% RH).

Chapter IV
RESULTS

The survival of bacteriophage Φ6 over 24 hours at 20°C and 40 and 60% RH is shown in figures 1 and 2 respectively. The slope of the regression shows that the rate of virus inactivation is much lower in 40% than 60% RH. The differences between the two slopes are statistically significant ($p < 0.0001$).

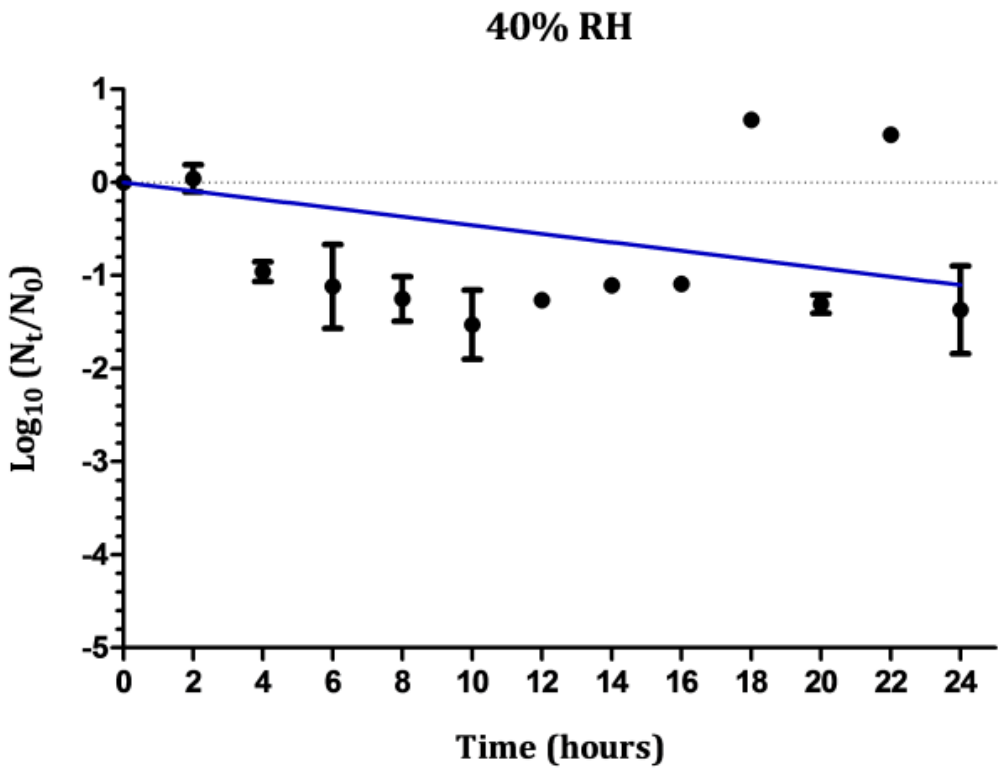

Figure 1. Survival of bacteriophage Φ6 over 24 hours at 20°C and 40% RH.
6 trials per point; observed data=points; bars=95% CI; linear regression analysis=line.

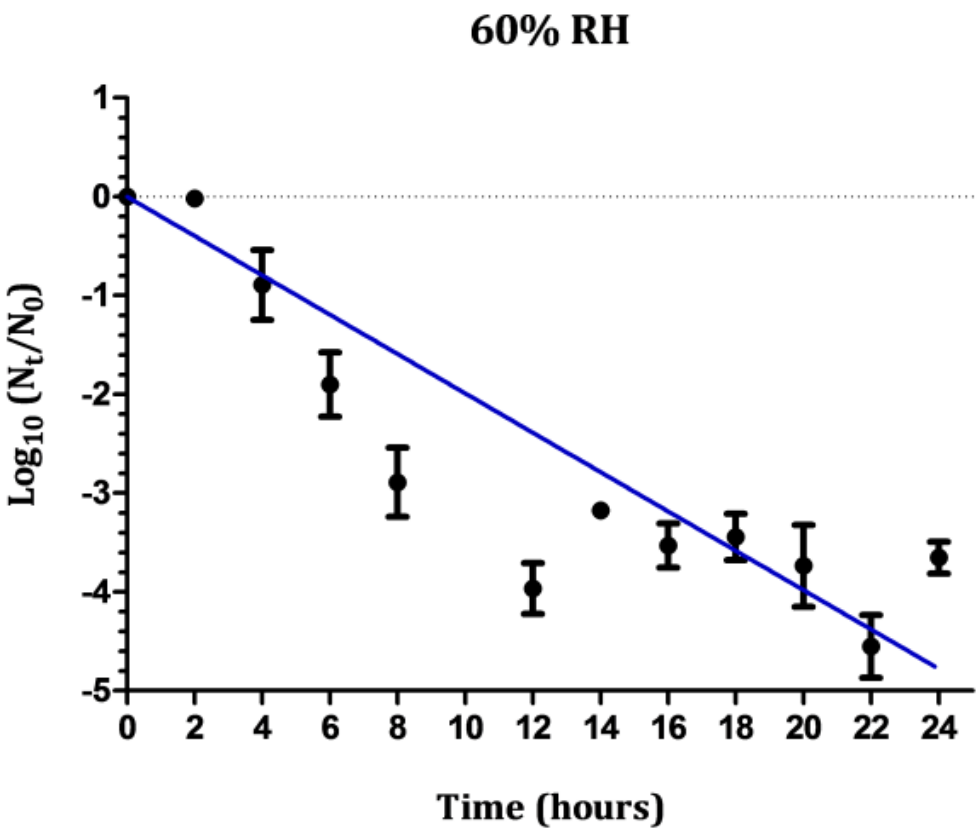

Figure 2. Survival of bacteriophage Φ6 over 24 hours at 20°C and 60% RH.
6 trials per point; observed data=points; bars=95% CI; linear regression analysis=line.

Table 1. Slopes of regression lines for virus inactivation at 20°C and 40% and 60% RH

RH	Slope	95%CI	P value
40%	-0.046	± 0.007040	< 0.0001
60%	-0.20	± 0.006136	< 0.0001

Table 1 shows the slopes of the regression lines with 95% CI at 20°C for both 40% and 60% RH.

At 40% RH, the slope was -0.046 ± 0.007040, while at 60% RH the slope was -0.20 ± 0.006136.

Both slopes are significantly non zero, $p < 0.0001$ for both 40% and 60% RH.

Table 2. Predicted times (in hours) for decimal reductions for virus inactivation at 20°C and 40% and 60% RH

RH	Reduction [$\log_{10}$ (N_t/N_0)]	Time* (hours)
40%	-1 (90%)	22
	-2 (99%)	44
	-3 (99.9%)	66
	-4 (99.99%)	87
60%	-1 (90%)	5
	-2 (99%)	10
	-3 (99.9%)	15
	-4 (99.99%)	20

** Time rounded up to whole number*

Table 2 shows the predicted values calculated from the regression lines to achieve 90%, 99%, 99.9%, and 99.99% reduction of the virus for both 40 and 60% RH. Over 24 hours, there was a ~1 Log_{10} reduction in virus at 20°C and 40% RH, while there was a ~4 Log_{10} reduction at 20°C and 60% RH. Within the timeframe of a single patient encounter (assuming it is ~30 minutes), there was a <0.02 Log_{10} reduction in virus at 20°C and 40% RH, while there was a <0.1 Log_{10} reduction at 20°C and 60% RH. Time required for 99.99% reduction was ~87 hours at 40% RH and ~20 hours at 60% RH.

Chapter V
DISCUSSION

5.1. Discussion

Respiratory infections can spread rapidly in healthcare settings (Seto et al., 2003; Wise et al., 2011). PPE is very important in interrupting transmission of infectious agents from patients to healthcare workers (Casanova et al., 2008a). Since healthcare workers are the frontline of defense during an outbreak or pandemic, it is very important to prevent them from acquiring infections, especially in healthcare settings. Previous studies have been done on the survival of both enveloped and non-enveloped viruses on PPE and other environmental surfaces at different temperature and humidity settings (Abad, Pinto, & Bosch, 1994; Ijaz et al., 1985; Lowen et al., 2007; Mbithi et al., 1991; Sobsey & Meschke, 2003). This study was done to determine the inactivation of bacteriophage Φ6, model virus similar to influenza and other important human viruses, on the surface of N95 respirators at ambient temperature (20°C) and two different relative humidity levels (40% and 60%) that simulate the environmental conditions of healthcare facilities.

The results of this study showed that, when applied at high titer (10^5 pfu), bacteriophage Φ6 survived on N95 respirators for up to 24 hours at ambient temperature and 40% and 60% relative humidity levels. Inactivation rate was lower in 40% than 60% RH. The differences between the two slopes are highly significant ($p < 0.0001$). This coincides with previous studies that have shown that viral survival is maximal at low RH, 20%-40% (Abad, Pinto, & Bosch, 1994; Lowen et al., 2007; Mbithi et al., 1991; Sobsey & Meschke, 2003). Lowen et al. (2007)

stated that, “At low RH, evaporation of water from exhaled bio aerosols would occur rapidly, leading to the formation of droplet nuclei; conversely, at high RH, small respiratory droplets would take on water, increase in size and settle more quickly out of the air” to describe the mechanism that could potentially explain the observed influence of RH on the virus particle. Droplet nuclei are very small so they stay in the air for a long period. As a result, they increase the chance for transmission of pathogens (2007).

The results from the study by Casanova et al. (2010a) looking at how survival of a virus on environmental surfaces is affected by factors such as air temperature and relative humidity (RH) suggested that there are interactions between temperature and RH, but RH has a greater effect on viral inactivation than temperature. There are different mechanisms that result in viral inactivation on surfaces. Viral inactivation could be caused by structural damage due to accumulation of viral capsid at the air-water interface (Casanova et al., 2010a). Another mechanism could be desiccation, “loss of water molecules triggers lipid membrane phase changes, cross-linking, Maillard reactions, and peroxide formation” (2010a). Both mechanisms may be involved in the inactivation of a virus but their contribution depends on RH.

McDevitt et al. (2010) stated that, “enveloped viruses, such as influenza virus, are thought to be less stable in the environment than non-enveloped viruses and more sensitive to higher relative humidity.” However, this experiment has shown that they are stable for a prolonged period of time, especially in low RH (40%). The results of our study showed that enveloped viruses survive on the surface of N95 respirators for longer than a single patient encounter. Within the timeframe of a single patient encounter (~30 minutes), there was a <0.02 Log_{10} reduction in virus at 20°C and 40% RH, while there was a <0.1 Log_{10} reduction at 20°C and 60% RH. These results are similar to a study done by Casanova et al. (2010b) examining the

survival and inactivation of coronaviruses on PPE found that, on an N95 respirator, only a small amount of infectious virus was lost within the first 2 hours and the virus was still detectable for up to 24 hours. The results of this survival experiment suggested that viruses can survive on PPE surfaces for the duration of a single patient encounter. Over 24 hours, there was a ~1 Log_{10} reduction in virus at 20°C and 40% RH, while there was a ~4 Log_{10} reduction at 20°C and 60% RH. Time required for 99.99% reduction was ~87 hours at 40% RH and ~20 hours at 60% RH. Therefore, at 40% RH the virus survives for a few days, while at 60% RH the virus survives for at least a day, until there is 99.99% reduction.

Casanova et al. (2010b) stated that, "the potential long-term survival of viruses on contaminated PPE is an important factor when formulating recommendations for removal and handling of used PPE and reuse of PPE in the pandemic setting." The results of this study suggest that reuse of N95 respirators has an increased risk because the virus survives for more than a single patient encounter. In fact, the virus survives for much longer, potentially lasting throughout multiple patient encounters. In addition, each patient encounter potentially adds more viral load onto the respirator, increasing the amount of time required to complete inactivation. Therefore, this should be taken into consideration when doing a risk assessment of reusing N95 respirators when shortages occur.

According to the IOM, despite the concerns of reuse, if reuse is necessary, they suggest protecting the respirator from external surface contamination when there is a high risk of exposure to influenza and using the respirator in such a way that the physical integrity and efficacy of the respirator will be preserved. They also emphasize the need for hand hygiene before and after removal of the respirator (IOM). Proper and regular hand washing before and after contact with patients plays an important role in minimizing spread of infection, especially

in institutional settings (Ansari et al., 1991; Brady et al., 1990; Casanova et al., 2008b; Mbithi et al., 1992).

However, for reuse to be more practical, infectious agents must be removed from the surface of the respirator by disinfection. The Institute of Occupational Safety and Health (IOSH) in Taiwan did a study and looked at how disinfection and storage affects masks. They subjected N95 masks to five sterilization methods: dry heat (in a dryer), wet heat (in a steamer), high temperature/high pressure, ultraviolet rays, and a 75% alcohol solution spray. Their results showed that all the methods killed the bacteria (they used E. coli), under certain conditions. Some of the methods were more effective when used in combination, while others decreased the effectiveness of the mask. In the end, they recommended that reuse should only be considered when there is "an extreme shortage of masks in times of dangerous epidemics" (IOSH). However, the FDA has not cleared any mask or respirators that incorporate antimicrobial agents, only two surgical gowns that were cleared many years ago (Mechcatie, 2007).

When considering using other masks, the study by Seto et al. (2003) found that healthcare workers that used N95 respirators were significantly less likely to be infected. However, this was not the case for those who used paper masks (Seto *et al.*, 2003). On the other hand, the study by Johnson et al. (2009) found that both masks (N95 and surgical masks) are equally effective when used for short periods to prevent the spread of infection. But, they do state that "surgical masks are not designed to prevent inhalation of airborne particles" (Johnson et al., 2009). The IOM report discussing the reusability of masks during a pandemic sated that, "respirators provide better protection against airborne transmission of infection than do medical masks" (IOM, 2006). Consequently, this alternative of using a different mask also shows to be risky.

5.2. Recommendation

The reemergence of SARS or pandemic influenza virus could pose serious risks for nosocomial disease spread via contaminated surfaces. Given these gaps in our knowledge, the magnitude of the risk due to virally contaminated surfaces should be examined further. Future studies should look at the transfer of viruses to hands while handling the N95 respirators. It will provide data that will help in the assessment of risks posed by handling of contaminated PPE after patient care activities. This work will further help healthcare facilities do an overall risk assessment and choose the alternative with the lowest risk. In addition, healthcare institutions should provide appropriate PPE training for their staff and check on their consistent use. In addition, they should also periodically review their infection control practices to address any problems that may arise.

We need to consider all these possibilities and their potential risks for formulating future recommendations. There is higher risk of using alternative masks may not provide the same degree of protection as N95 respirators. In addition, our study has shown that viruses survive for an extended period on N95 respirator surfaces. Therefore, we suggest more research should be done in the product development field to come up with effective methods of disinfection of N95 masks that can sustain the integrity and efficacy of the respirators, or making the surface of the N95 mask antimicrobial so microbes will not be able to survive on the surface. These methods will potentially reduce the risk and allow for reuse of the N95 respirators. This will be especially helpful during an outbreak or pandemic because it will potentially prevent shortages. The results of this study should be taken into consideration when doing a risk assessment and developing protocols for reusing N95 respirators in emergency situations.

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