

SWINE HEALTH

Title: Effect of temperature and relative humidity on UV₂₅₄ inactivation of airborne viral pathogens – NPB #09-112

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INDUSTRY SUMMARY

The proposed study was Part Two of a 2-year project to test whether ultraviolet light could serve as a practical and cost-effective method to inactivate aerosolized pathogens in commercial swine facilities. Work performed in Year One showed that the swine pathogens we tested were highly susceptible to inactivation by UV₂₅₄. The issue that drove the research in Year Two was the fact that the UV inactivation of pathogens is known to be affected by relative humidity and temperature. In general, higher relative humidity decreases the k-value (requires more UV for inactivation) and higher temperature increases the k-value (takes less UV for inactivation) (Tseng and Li, 2005; Walter and Ko, 2007). Understanding the interaction of relative humidity and temperature on the rate of inactivation of swine pathogens under a range of temperature and relative humidity is a critical step moving this technology to the field. Therefore, our objective was to determine the effects of temperature x relative humidity on the rate of inactivation (k-value) of SIV, BVDV, and PRRSV.

KEYWORDS

Aerosol, ultraviolet, UV₂₅₄, virus inactivation, influenza, PRRSV

SCIENTIFIC ABSTRACT

The proposed study was Part Two of a 2-year project to test whether ultraviolet light could serve as a practical and cost-effective method to inactivate aerosolized pathogens in commercial swine facilities. Work performed in Year One showed that the swine pathogens we tested were highly susceptible to inactivation by UV₂₅₄. The issue that drove the research in Year Two was the fact that the UV inactivation of pathogens is known to be affected by relative humidity and temperature. In general, higher relative humidity decreases the k-value (requires more UV for the same effect) and higher temperature increases the k-value (Tseng and Li, 2005; Walter and Ko, 2007). Understanding the interaction of relative humidity and temperature on the rate of inactivation of swine pathogens under a range of temperature and relative humidity is a critical step

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INTRODUCTION

This research addressed the National Pork Board 2009 RPF, Category B (*Swine Health*) Number 3 (*Biosecurity*). Specifically, the long-term goal of this project was to develop cost-effective technology for the inactivation of airborne swine pathogens. Control of “area spread” is considered a pre-requisite for regional control of both endemic and foreign animal pathogens of swine.

The fundamental question of this research was, “can ultraviolet play a role in developing a cost-effective technology for the inactivation of airborne swine pathogens”? UV inactivation of viral pathogens has been a proven method of disinfection since the 1930s (Jensen, 1964). In theory, all airborne viruses are susceptible to UV inactivation (Tseng and Li, 2005). This is the basis of studies to control the transmission of human pathogens by engineering UV into upper room ventilation systems (Walker and Ko, 2007; Beggs and Sleight, 2002). For example, a study published in 2008, showed $\geq 85\%$ reduction in viable, airborne vaccinia virus using upper-room UV fixtures (McDevitt et al., 2008). UV photons damage genetic material (DNA and RNA) by causing the formation of "dimers," i.e., linkages between adjacent thymidine (DNA) or uracil (RNA) nucleotides. Pathogens vary in their sensitivity to UV inactivation with DNA viruses being more resistant than RNA viruses (Tseng and Li, 2005). Recently, UV inactivation has been engineered into areas where people congregate (Beggs and Sleight, 2002), generally by placing UV₂₅₄ light tube grids into existing ventilation ductwork. Compared to filtration, ultraviolet lights and other hardware are inexpensive and easily adapted to existing ventilation systems. Although data is available on the UV inactivation of several human viral pathogens (Tseng and Li, 2005; Thurston-Enriquez, 2003), little data is available on the UV susceptibility of viral pathogens of swine.

OBJECTIVES

Estimate the k-value (inactivation rate) of airborne influenza, BVDV (surrogate for hog cholera virus), and PRRSV under a range of (temperature X relative humidity) conditions.

Work performed in Year One showed that influenza A virus, BVDV, and PRRSV were susceptible to inactivation by UV₂₅₄. The issue addressed in Year Two was whether UV inactivation of pathogens would be affected by relative humidity and temperature. That is, to determine whether this technology will be of value to pork producers, we need to establish that UV inactivation will function under a wide range of temperatures and relative humidity. Therefore, our objective was to determine the effects of temperature x relative humidity on the rate of viral inactivation (k-value).

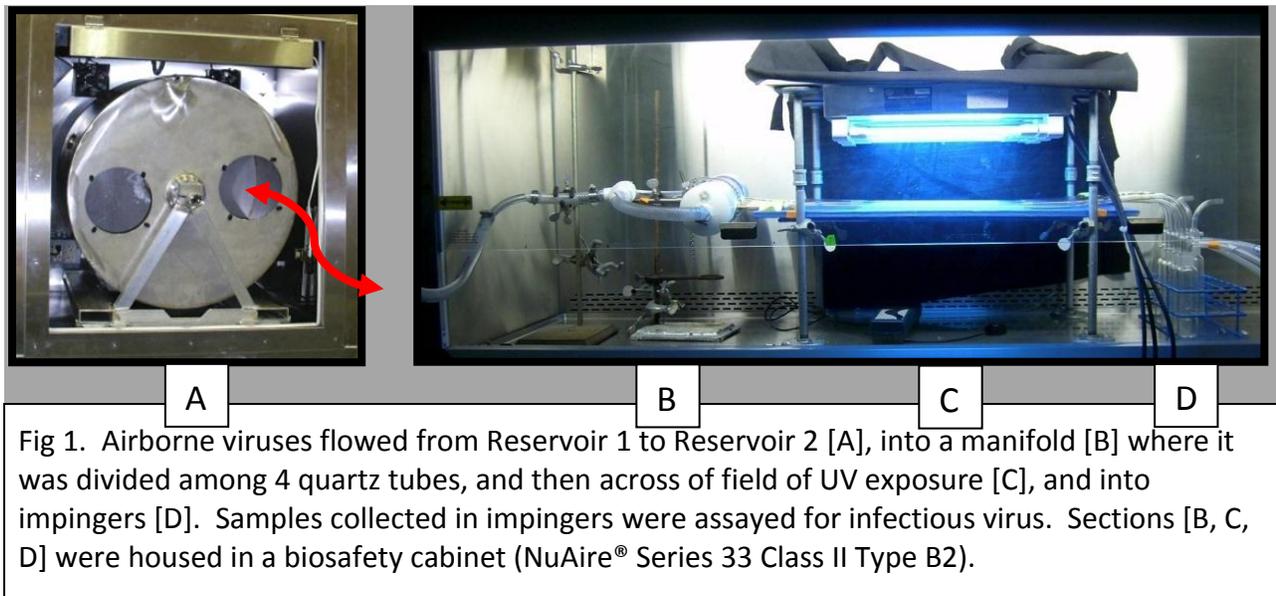
MATERIALS AND METHODS

Experimental design

To conduct the experiment, a system was constructed such that, throughout the 45 min experiment, aerosolized virus continuously flowed from Reservoir One to Reservoir Two and then across a UV₂₅₄ exposure field (Figure 1). Airflow through the system was impelled by negative pressure generated by four AGI-30 glass impingers (Ace Glass, Vineland, NJ, USA), each operating at a flow rate of 12.5 L per min, i.e., air flow through the system totaled 50 L per min.

- The temperatures of Reservoir One and Reservoir Two were manipulated to achieve the targeted temperature and relative humidity at the UV₂₅₄ irradiation field.

- As shown in Figure 1, a manifold adjoining Reservoir Two functioned to equally distribute aerosolized virus into four quartz tubes placed parallel to each other in the field of ultraviolet irradiation. Each quartz tube represented a different level of UV₂₅₄ treatment (one quartz tube was completely covered = no treatment).
- Commercially-available ultraviolet (UV₂₅₄) lamps (American Ultraviolet Co., Lebanon, IN) were mounted in Biological Safety Cabinet, (NuAire, Series 33 Class II Type B2). The dose of UV₂₅₄ to which the samples were exposed was measured using UV₂₅₄ radiometer sensors (Technika, Co., Scottsdale, AZ).
- Air samples collected by the impingers downstream of the UV₂₅₄ exposure field were titrated for infectious virus. Titration results were analyzed in the context of UV₂₅₄ exposure dose.



Viruses

Influenza A/Swine/Iowa/73 (H1N1) (National Veterinary Service Laboratories, Ames, IA), was propagated on Madin-Darby canine kidney (MDCK) cells (American Type Culture Collection (ATCC), Manassas, VA)

Singer strain bovine viral diarrhea virus (BVDV) type 2 (provided by Dr. J. Ridpath, USDA:NADC), was propagated on bovine turbinate cell line T7 (provided by Dr. J. Ridpath, USDA:NADC).

Porcine Respiratory and Reproductive Syndrome virus (kindly provided by Dr. Scott Dee, University of Minnesota) was propagated on MARC-145 cells.

Procedures

Environmental conditions For each replicate, the actual temperature and relative humidity of the aerosol was measured (Vaisala, HMI41 indicator and HMP46 temperature probe, Helsinki, Finland) inside the manifold located immediately upstream of the UV₂₅₄ exposure field. Temperature and relative humidity measurements were taken prior to starting the replicate and then at the beginning, midpoint and end of each replicate to confirm that the system operated at targeted parameters.

Aerosolization of virus The virus solution to be aerosolized consisted of 25 ml of stock virus and 25 ml of sterile 1X PBS (Thermo Fisher, Rockford, IL) and 0.1% (v/v) antifoam A emulsion (Sigma Chemical Co.).

Previous research showed that antifoam A emulsion innocuous for cultured cells, influenza, BVDV, and PRRSV (Hermann et al., 2005). A fluorescent physical tracer (Rhodamine B) was included in the viral preparations as a quality control measure to ensure even distribution of the aerosolized virus across the 4 quartz tubes. The virus solution was maintained on ice and shielded from light until nebulization. The solution was aerosolized into Reservoir One using a 24-jet Collision nebulizer (BGI Inc., CN60, Waltham, MA, USA) operating on compressed air at 2.8 kg per cm (40 pounds per square inch). Under these parameters approximately 1.1 ml of virus solution was nebulized each minute into particles of approximately 1.9 μm diameter (Hermann et al., 2007).

Ultraviolet (UV₂₅₄) exposure The UV₂₅₄ emitter consisted of six low-pressure, mercury-vapor discharge lamps 28.8 cm in length (American UV Company, Lebanon, IN). Each of three 2-lamp, reflective, surface-mounted, 110 volt, fixtures were fitted with UV₂₅₄ bulbs (American UV Company). To avoid fluctuation in UV₂₅₄ intensity, lamps were operated at their maximum UV₂₅₄ emission capacity for 10 min before the start of each experiment. UV₂₅₄ emission was measured using three calibrated radiometers (Model 1700, International Light Inc. Newburyport, MA, USA; VLX3W Technika, Phoenix, AZ).

Exposure of airborne virus to UV₂₅₄ was done by passing the airborne virus through four (4) quartz tubes connected to a manifold. Quartz tubes were placed 25 cm from, and parallel to, the UV₂₅₄ emitters. Between each replicate, quartz tubes were cleaned with commercial quartz cleaner following the manufacturer's recommendations (Hellmanex® II, Hellma GmbH & Co., Mülheim, Germany). The UV₂₅₄ exposure dose measured directly below the quartz tubing. Each of the four quartz tubes was covered to deliver different UV₂₅₄ treatments. This was achieved by shielding all but a specific length of each tube, i.e., 1.3 cm (residence time of 0.07 sec); 3.2 cm (residence time of 0.14 sec); and 5.2 cm (residence time of 0.25 sec). A completely shielded tube served as a non-exposed (positive) control. Treatment was randomized to quartz tubes before each replicate. UV₂₅₄ irradiance was measured at the unshielded area of each quartz tube at the start, midpoint, and end of each replicate and averaged. For each treatment, the UV₂₅₄ dose delivered to airborne PRRSV was calculated as:

$$D = I \times T$$

Where:

D = Ultraviolet dose (mJ per cm²)

I = Irradiance (mW per cm²). Average of irradiance measured at start, midpoint, and end of each replicate.

T = Residence time (sec)

Data Analysis Rhodamine B levels were compared among samples to verify even flow rate for each of the UV₂₅₄ treatments. To analyze the main effects (UV₂₅₄ dose, temperature, and relative humidity) and their interactions, the TCID₅₀ data were log₁₀-transformed and analyzed using a random-coefficient ANCOVA (analysis of covariance) model with the quantitative explanatory variable "UV₂₅₄ dose" and categorical explanatory variables of "temperature" and "relative humidity" in SAS® Version 9.2 (SAS® Institute Inc., Cary, NC, USA). Inactivation constants (k), defined as the absolute value of the slope describing the linear relationship between the survival fraction of the microbial population and the UV₂₅₄ exposure dose (Goldberg et al., 1958), were estimated using the random coefficient model. Essentially, the inactivation constant (k) is the absolute value of the slope of the virus survival fraction by UV₂₅₄ exposure dose. Larger k values indicate more rapid virus inactivation.

RESULTS

Bovine viral diarrhea virus (surrogate for classical swine fever virus) All attempts to generate and recover infectious BVDV in the system were unsuccessful in the system.

Influenza A virus Recovery of influenza A virus was only successful at low temperature (7 C) and high relative humidity ($\geq 90\%$). The inactivation constant calculated at this combination of temperature and relative humidity was, $k = 0.0897$.

Porcine reproductive and respiratory syndrome virus PRRSV isolate MN-184 was recovered at all combinations of temperature and relative humidity attempted. Inactivation constants by temperature and relative humidity are listed in Table 1.

Table 1. PRRSV inactivation constants by temperature and relative humidity

| | | Relative Humidity | | | |
|-------------|--|-------------------|------------|-------------|------|
| | | $\leq 24\%$ | 25% to 79% | $\geq 80\%$ | |
| Temperature | | Main effects | 4.25 | 5.87 | 3.41 |
| | $\leq 15^{\circ}\text{C}$ | 5.68 | 5.42 | 7.04 | 4.58 |
| | 16 $^{\circ}\text{C}$ to 29 $^{\circ}\text{C}$ | 4.25 | 3.99 | 5.61 | 3.15 |
| | $\geq 30^{\circ}\text{C}$ | 3.59 | 3.33 | 4.96 | 2.49 |

DISCUSSION

Aerosols of PRRSV were successfully generated and recovered across a range of temperatures and relative humidities. These results are compatible with prior reports of airborne spread of PRRSV (Dee et al., 2005, 2009, 2010). For PRRSV, the effects of UV₂₅₄ dose, temperature, and relative humidity on the recovery of infectious PRRSV were all statistically significant, as were the interactions between (UV₂₅₄ dose x temperature) and (UV₂₅₄ dose x relative humidity). PRRSV was more susceptible to ultraviolet as temperature decreased; most susceptible to ultraviolet inactivation at relative humidity between 25 and 79%, less susceptible at relative humidity \leq 24%, and least susceptible at \geq 80% relative humidity.

Although the data showed that higher relative humidity increased the dose of UV₂₅₄ required to inactivate the virus, the inactivation constants are all relatively small. That is, the data indicated that airborne PRRSV was highly susceptible to UV₂₅₄ inactivation at all temperatures and relative humidities examined in this project. Using the smallest inactivation constant ($k = 2.49$, Table 1) the dose of UV₂₅₄ required to inactivate 99.9% of airborne PRRSV may be calculated as = 1.21 mJ per unit area.

In contrast, no BVDV and very limited influenza A virus was recovered under the identical conditions; suggesting that airborne transmission of these isolates is highly unlikely (BVDV) or limited to specific ambient conditions (influenza A virus).

The influenza virus results are of interest because of renewed attention regarding possible aerosol spread of influenza. Three modes of transmission have been postulated, 1) aerosol transmission, 2) large droplet transmission, and 3) fomites (Tellier, 2009). Airborne transmission is the most controversial because there are limited studies on the aerosol transmission of influenza and there are no studies in which influenza virus has been generated, transferred a distance, re-captured and infectious virus quantified. Researchers have used quantitative RT-PCR to detect airborne influenza virus, but PCR does not differentiate between infective and non-infective virus (Fabian, 2008). Our results suggest that airborne influenza virus detected by PCR represents non-infectious virus.

SUMMARY

Airborne BVD and influenza A viruses were quickly inactivated under the conditions of temperature and relative humidity achieved in this experiment. Therefore, airborne transmission of these isolates is either highly unlikely (BVDV) or limited to specific ambient conditions (influenza A virus). In contrast, PRRSV was recovered at all combinations of temperature and relative humidity. Even so, UV₂₅₄ inactivation constants estimated for PRRSV showed the virus to be highly susceptible to ultraviolet. These data suggested that inactivation of airborne PRRSV could be achieved with ultraviolet or similar technology (photocatalysis) if sufficiently robust systems could be developed for the field.

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