RESEARCH ABSTRACT

SWINE HEALTH

Title: Inactivation of PRRSV using ultraviolet light - **NPB #07-119**

Investigator: Jeffrey Zimmerman (jjzimm@iastate.edu)

Co-investigators: Steven J. Hoff, PhD PE; William Jenks, PhD, Jacek Koziel, PhD, PdF; J van Leeuwen, Deng

Institution: Iowa State University

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SCIENTIFIC ABSTRACT

The objective of this study was to calculate the inactivation of PRRSV by dose of UV_{254} in a "static" (i.e., virus-in-liquid solution) system. This study is the first step in evaluating the use of UV_{254} for the inactivation of airborne pathogens in commercial swine facilities.

Viruses The study was conducted using PRRSV isolate MN-184 (kindly provided by Dr. Scott Dee, University of Minnesota) propagated on MARC-145 cells. Reovirus strain $T3D^C$ (kindly provided by Dr. Cathy Miller, Iowa State University) grown on L929 cells was included in the experiment. A double-stranded RNA virus, Reovirus type 3 extremely hardy and highly resistant to inactivation by UV_{254}. Inclusion of a UV_{254}-resistant pathogen in the experiment was intended to increase the external validity of the study by providing data for contrast and comparison.

Equipment Commercially-available ultraviolet (UV_{254}) lamps (American Ultraviolet Co., Lebanon IN) were mounted in an environmental chamber (Percival Scientific, Perry IA) capable of maintaining any pre-selected temperature between 0 and 60°C. The dose of UV_{254} to which the samples were exposed was measured using UV_{254} radiometer sensors (Technika,Co., Scottsdale AZ). *No equipment was purchased through NPB #07-119*.

Treatments The experiment was conducted in the environmental chamber with the temperature held at 4°C. Five samples of each virus were exposed to each of 10 UV_{254} doses [0.000 (negative controls), 0.025, 0.050, 0.075, 0.100, 0.125, 0.150, 0.200, 0.250, and 0.300 Joules/cm^2]. Immediately following exposure, samples were stored at -80°C until assayed. Microtitration infectivity assays were conducted to quantify the amount of infectious virus in remaining in each sample post-treatment.

Microtitration infectivity assays To quantify infectious PRRSV, confluent MARC-145 cells were inoculated with 10-fold serial dilutions of the sample and incubated for 1 hour at 37°C under 5% CO_2. The inoculum was then removed and replaced with DMEM supplemented with 4% FBS and antibiotics. Cells were incubated for an additional 24 hours, then fixed with 80% acetone in water, dried, and stained with anti-PRRSV monoclonal

antibody SDOW-17F. Reactions were visualized under a fluorescent microscope and the titer ($TCID_{50}$) of infectious PRRSV calculated using the Spearman-Kärber method.
Infectious reovirus was quantified using a plaque assay. Confluent L929 cells were exposed to 10-fold serial dilutions of the sample and incubated for 1 hour at 37˚C under 5% CO_2. Thereafter, cells were overlayed with 2% agar diluted in complete medium and incubated for an additional 48 hours. After the 48 hour incubation, the plaques were counted and the concentration ($TCID_{50}$) of infective virus was determined using the Spearman-Kärber method.
Data Analysis The *k-value* (inactivation constant) is used to describe the susceptibility of virus to UV_{254}. Higher *k-values* indicate greater susceptible to inactivation by UV_{254}.
The k-value is calculated as the slope of the line describing the inactivation of the virus $[\log\left(\frac{Nt}{N}\right)]$ where N = initial viral concentration and Nt = concentration following treatment with a specific dose of UV_{254}. The *k-values* for PRRSV and reovirus were estimated to be 0.0893 and 0.0103, respectively.
Conclusions PRRSV in solution is highly susceptible to UV_{254} irradiation. These data justify the next phase of this research: evaluation of the inactivation of airborne PRRSV using UV_{254}.

INTRODUCTION
The long-term objective of this work is to develop a cost-effective method for the inactivation of airborne PRRSV. Completion of this objective is considered a cornerstone in the prevention of PRRSV "area spread". HEPA-filtration has been implemented on a limited basis in boar stud facilities, but it will never be a practical solution for commercial producers because HEPA-filters are extremely expensive and have a relatively short "service life."
Potentially, cost-effective inactivation of airborne PRRSV could be achieved by the use of UV_{254} light. Compared to HEPA-filtration, ultraviolet light emitters and other hardware are inexpensive and easily adapted to existing ventilation systems.
UV_{254} inactivation has been a proven method of disinfection since the 1930s (Wells and Brown, 1936). Since then, UV_{254} inactivation has been engineered into areas where people congregate (Beggs and Sleigh, 2002), generally by placing UV_{254} light tube grids into existing ventilation ductwork. Although data is available on the UV_{254} inactivation of several human viral pathogens (Nwachuku et al., 2005, Thurston-Enriquez et al., 2002), no data is available on the UV_{254} inactivation of PRRSV or other viral pathogens of animals.
Therefore, the core question of this research is: “Can UV_{254} inactivate airborne PRRSV in a treatment time that is consistent with air turnover rates and environmental conditions in commercial swine barns?” If the answer is “yes,” UV_{254} treatment would be applicable to both the inflow and outflow air at typical existing and new mechanically-ventilated barns.

OBJECTIVES
Objective 1: Year One. Calculate the relationship between dose of UV_{254} and inactivation of PRRSV in solution. As described elsewhere in this report, this work has been done using “off-the-shelf” ultraviolet hardware available from commercial manufacturers.
Objective 2: Year Two. Determine the effect of relevant parameters (air flow rates, temperature, relative humidity, etc.) on the UV_{254} inactivation of airborne PRRSV.

MATERIALS & METHODS

1. The equipment to conduct this research, e.g., UV_{254} fixtures (American UV Co. - $1,200), rheostat ($800), environmental chamber (Percival Scientific - $4,500), and UV sensors and associated software (Technika - $5,378) (Figure 1), were acquired at no cost to NPB.

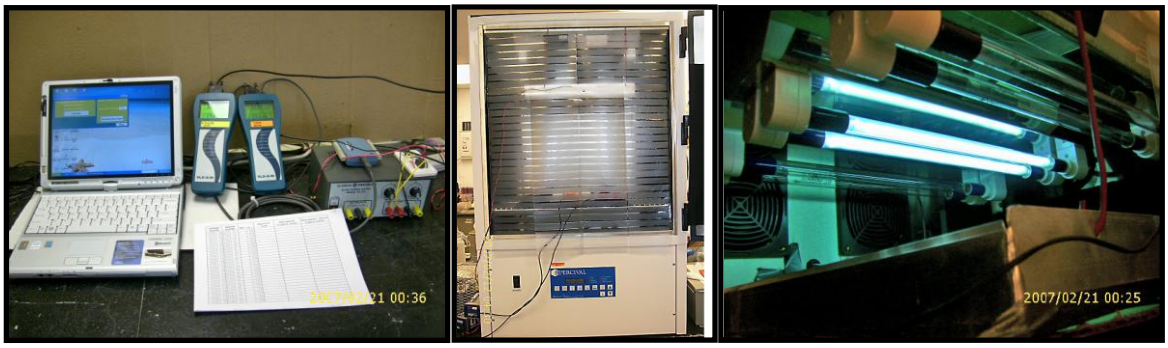

Figure 1. UV exposure data monitoring/acquisition equipment (left). Percival environmental chamber with door open and protective shielding in place (middle). UV_{254} emitter (right).

2. PRRSV (isolate MN-184 kindly provided by Dr. Scott Dee) and reovirus type 3 (kindly provided by Dr. Cathy Miller, Iowa State University) were used in the experiment.
3. UV_{254} exposures were performed by placing 2 mls of PRRSV or reovirus in wells on 8-well plates, then exposing plates to one of 10 UV_{254} exposure levels [0.000 (negative controls), 0.025, 0.050, 0.075, 0.100, 0.125, 0.150, 0.200, 0.250, and 0.300 Joules/cm^2]. Figure 2 shows the process of loading treatment samples and the negative control (covered in aluminum foil) on 8-well plates.
   - One well on each plate served as a UV-unexposed control; this well was covered with aluminum foil. Each exposure level was replicated 5 times.
   - The UV_{254} exposure dose was monitored in real-time using radiometers and associated software (Technika). When the desired dose was reached, lamps were powered down.
   - To reduce bias, the order in which UV_{254} treatments were performed was randomized by listing exposure doses sequentially from low (0.025 J/cm) to high (3.0 J/cm) then randomizing the order in which they were performed (ramdon.org).

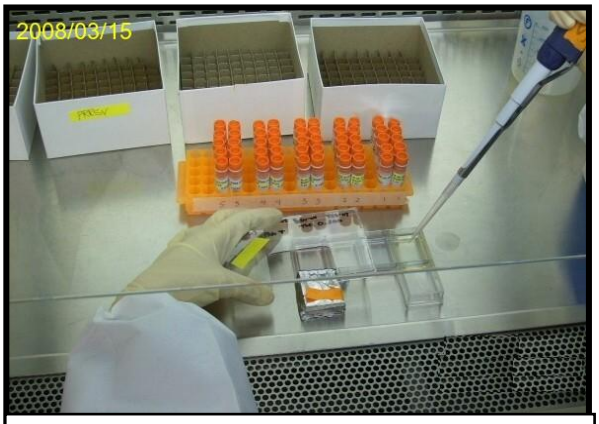

Figure 2: Loading virus into the 8 well plate. Note the aluminum-covered negative control well.

4. Microtitration infectivity assays were conducted to determine the concentration of infectious PRRSV and infectious reovirus after exposure to UV_{254}.

RESULTS

For each UV_{254} exposure dose, percent virus inactivation was expressed as:

$$\frac{(\text{quantity of infectious virus in exposed sample})}{(\text{quantity of infectious virus in unexposed (negative control) samples})} \times 100$$

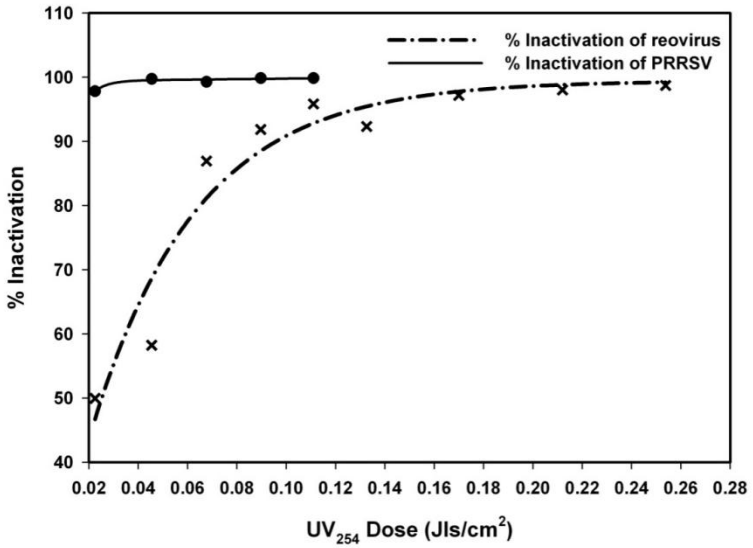

Figure 3. Percent virus inactivation by dose of UV_{254} (Jls/cm^2)

Virus-specific k-values were calculated as described above. The *k-value* for PRRSV was calculated to be 0.0893 and the k-value for reovirus was 0.0103, i.e., PRRSV was much more susceptible to UV_{254} inactivation than reovirus (see Figure 4).

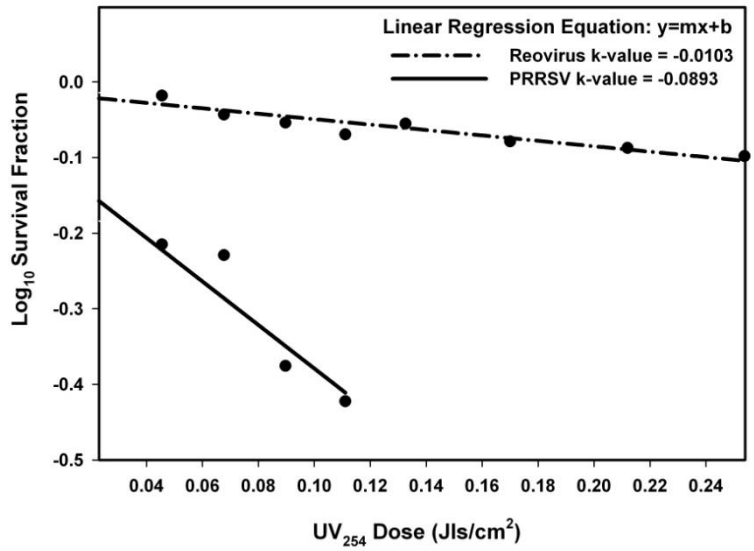

Figure 4. K-values estimated for PRRSV and reovirus

DISCUSSION

This is the first step in evaluating the use of UV technology for the protection of commercial swine herds against airborne pathogens. As shown in Figures 3 and 4, PRRSV is readily inactivated by exposure to UV_{254}. The fact that PRRSV is highly susceptible to inactivation by UV_{254} suggests that this line of investigation should be pursued.

REFERENCES

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