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Effect of temperature and relative humidity on ultraviolet (UV_{254}) inactivation of airborne porcine respiratory and reproductive syndrome virus

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ABSTRACT

The objective of this research was to estimate the effects of temperature and relative humidity on the inactivation of airborne porcine reproductive and respiratory syndrome (PRRS) virus by ultraviolet light (UV_{254}). Aerosols of PRRS virus were exposed to one of four doses of UV_{254} under nine combinations of temperature ($n = 3$) and relative humidity ($n = 3$). Inactivation constants (k), defined as the absolute value of the slope of the linear relationship between the survival fraction of the microbial population and the UV_{254} exposure dose, were estimated using the random coefficient model. The associated UV_{254} half-life dose for each combination of environmental factors was determined as ($\log_{10} 2/k$) and expressed as UV_{254} mJ per unit volume. The effects of UV_{254} dose, temperature, and relative humidity were all statistically significant, as were the interactions between UV_{254} dose × temperature and UV_{254} dose × relative humidity. PRRS virus 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 ≤24%, and least susceptible at ≥80% relative humidity. The current study allows for calculating the dose of UV_{254} required to inactivate airborne PRRS virus under various laboratory and field conditions using the inactivation constants and UV_{254} half-life doses reported therein.

1. Introduction

Initially described in the late 1980s, porcine reproductive and respiratory syndrome (PRRS) is characterized by reproductive failure in sows, poor growth performance in growing pigs, and respiratory disease in pigs of all ages (Zimmerman et al., 2012). Since its emergence as a clinical entity in the late 1980s, PRRS virus has proven to be a persistent threat to the health and productivity of pig herds and the economic well-being of pig producers. Neumann et al. (2005) estimated the annual cost of PRRS to U.S. pig producers at $560.32 million per year. By comparison, prior to eradication, annual losses in the U.S. to classical swine fever (hog cholera) and pseudorabies virus were estimated at $364.09 million (Wise, 1981) and $36.27 million (Hallam et al., 1987), respectively (adjusted to year 2004 dollars).

Since the beginning of the PRRS virus pandemic in the 1980s, movement of the virus between neighboring herds in the apparent absence of direct contact (“area spread”) has been often reported (Robertson, 1991). Several epidemiological investigations showed that proximity to infected herds increased the risk of a herd acquiring PRRS virus. In France, Le Potier et al. (1997) found that 45% of herds suspected to have become infected through area

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spread were located within 500 m (0.3 miles) of the postulated source herd and only 2% were 1 km from the initial outbreak. In Denmark it was observed that the likelihood of herd positivity increased as the density and proximity of PRRS virus-positive neighboring herds increased (Mortensen et al., 2002).

In the last decade, researchers have confirmed the occurrence of airborne transmission of PRRS virus over significant distances. Initially, Dee et al. (2005b) demonstrated that infectious airborne PRRS virus could travel over distances ≥150 m. Thereafter, using a source population of 300 PRRS virus-infected pigs, Dee et al. (2009) demonstrated long-distance airborne movement by the successful recovery of infectious virus up to 4.7 km from the source. Subsequently, Otake et al. (2010) recovered infectious airborne PRRS virus at distance of 9.1 km from the source. Cumulatively, the epidemiological and experimental data suggest that airborne PRRS virus is a significant, and perhaps the primary, means of area spread.

Protection of pig barns from airborne spread of infectious agents is a recent concept. To date, researchers have primarily focused on preventing the introduction of airborne PRRS virus into barns using commercially available air filters, e.g., high-efficiency particulate air (HEPA) filters, minimum efficiency rating value (MERV) filters of various efficiencies, and fiberglass pre-filters. Overall, the results demonstrated that filtering incoming air with HEPA filters and MERV filters ≥14 prevented the transmission of PRRS virus (Dee et al., 2005, 2006a,b, 2010).

Ultraviolet inactivation of PRRS virus may also offer promise. Wheeler et al. (1945) reported the use of ultraviolet to inactivate airborne rubella virus and *Streptococcus pyogenes* in Army and Navy barracks. Likewise, Perkins et al. (1947) reduced the spread of airborne viral pathogens (“measles”) in school classrooms using ultraviolet. Riley (1961) demonstrated that ventilated air from hospital tuberculosis wards produced tuberculosis in guinea pigs, but not when the air was irradiated with ultraviolet light. In recent years, UV_{254} emitters have been engineered into areas where people congregate either by placing UV_{254} light tube grids into existing ventilation ductwork or by installing free standing UVC emitters (Brickner et al., 2003; Dumyuahn and First, 1999; McDevitt et al., 2008; Menzies et al., 1999; Noakes et al., 2006). Ultraviolet technology is appealing due to its low cost as compared to HEPA filtration (Brickner et al., 2003). However, effective implementation must be based on achieving a dose of UV_{254} sufficient to inactivate the target. No estimates of the effect of UV_{254} on airborne PRRS virus have been published. Therefore, the objective of this experiment was to evaluate the capability of ultraviolet (UV_{254}) to inactivate airborne PRRS virus under varying conditions of temperature and relative humidity.

2. Materials and methods

2.1. Experimental design

The objective of this study was to evaluate the effect of temperature and relative humidity on the inactivation of airborne PRRS virus by ultraviolet irradiation (UV_{254}). Aerosols of PRRS virus were exposed to four levels of UV_{254} under nine defined conditions of temperature and relative humidity (Table 1). Each combination of temperature and relative humidity was replicated 3 times. Samples of air collected after UV_{254} treatment were titrated for infectious PRRS virus and the data used to calculate the UV_{254} inactivation constants (k) and UV_{254} half-life (T 1/2) exposure doses for each combination of temperature and relative humidity.

Table 1
Temperature and relative humidity combinations of PRRS virus aerosols.

Temperature	Relative humidity		
	≤24%	25–79%	≥80%
≤15 °C 16–29 °C ≥30 °C	Airborne PRRS virus received 4 levels of UV_{254} treatment at each combination of temperature and relative humidity. Each combination was replicated 3 times.		

2.2. Porcine reproductive and respiratory syndrome virus

A type 2 PRRS virus isolate, MN-184 (kindly provided by Dr. Scott Dee, University of Minnesota, MN, USA) was propagated on MARC-145 cells, a clone of the African monkey kidney cell line MA-104 (Kim et al., 1993). Cells were grown in 162 cm^2 flasks (Corning Incorporated, Corning, NY, USA) using growth media; Dulbecco’s Modified Eagles Medium (DMEM), (Mediatech Inc., Manassas, VA, USA) supplemented with 0.25 μg/ml Amphotericin B (Sigma Chemical Co., St. Louis, MO, USA), 50 μg/ml gentamicin (Sigma), 0.5 M L-glutamine (Fisher Scientific, Hampton, NH, USA), 300 international units (IU) per ml penicillin (Sigma), 300 μg/ml streptomycin (Sigma), 1.0% nonessential amino acids (HyClone, Logan, UT, USA), 25 mM HEPES buffer (Sigma Chemical Co.) and 10.0% heat-inactivated fetal bovine serum (FBS) (Sigma Chemical Co.). When cells were confluent (72–84 h), the medium was discarded and the flasks inoculated with 5 ml DMEM (without L-glutamine) containing PRRS virus isolate MN-184 at a virus titer of $1 \times 10^{3.5}$ median tissue culture infective dose ($TCID_{50}$) per ml. Flasks were placed on a rocking platform in a 37 °C humidified 5% CO_2 incubator for 90 min and then 40 ml of supplemented DMEM (Mediatech Inc.) growth medium (now with 4% FBS) was added and the flasks returned to the incubator for 36 h. Cell culture supernatant was harvested by flask freeze-thaw and centrifugation (3000 × g for 20 min at 4 °C). Virus stock was stored in 25 ml aliquots at −80 °C.

2.3. Experimental procedures

2.3.1. Overview

The system was constructed such that, throughout the 45 min experiment, aerosolized PRRS virus continuously flowed from Reservoir One to Reservoir Two and then across a UV_{254} exposure field. A manifold in Reservoir Two functioned to equally distribute aerosolized PRRS virus into four quartz tubes placed parallel to each other in the