RESEARCH REPORT
SWINE HEALTH

Title: Demonstration of Airborne PRRSv Inactivation by a Non-Thermal Plasma
NPB#16-198

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Industry Summary:

Two of the primary goals in the National Pork Board (NPB) strategic plan call for the development and deployment of mitigation strategies capable of eliminating the top domestic swine diseases, including decreasing the annual economic impact of porcine reproductive and respiratory syndrome (PRRS) by 20%. Particulate filters installed on hog barn ventilation air intakes increase operating costs through the need for periodic filter replacement, and can be rendered ineffective by any leaks that appear in what must otherwise be an airtight building structure. Other reported challenges of air filters include reduced cooling capacity and shorter than expected filter life/more frequently than expected filter replacement. In its 2016 request for proposals, NPB sought practical systems for inactivating PRRSV in commercial farm settings.

Plasmas represent an ionized state of matter in which atoms and molecules lose or acquire excess charge as they collide with free electrons, all of which results in a highly chemically reactive environment. While conventional combustion flames represent thermal plasmas, non-thermal plasmas are induced by electromagnetic and other means and lack the high temperatures of thermal plasmas. This project tests the effectiveness of a prototype non-thermal plasma (NTP) reactor to inactivate airborne PRRSv. PRRSv was suspended in the air at the entrance to a small wind tunnel using a mister supplied with a liquid solution containing active PRRSv. The PRRSv aerosols were then exposed to a region of non-thermal plasma within a prototype NTP reactor installed in the wind tunnel test section. Lab analyses of the PRRSv collected from the airstream upstream and downstream of the NTP reactor by identical liquid impingers provided pre- and post-treatment $TCID_{50}$, a measure of the abundance of an infectious viable agent. The reduction in $TCID_{50}$ following NTP treatment, after adjusting for the loss of PRRSv between the two collection points, represents the inactivation efficiency achieved by the NTP reactor for a given set of experimental conditions. The results showed that PRRSv was inactivated to a similar degree as a different virus, MS2 phage, at the same conditions, e.g., 1.3-log (> 90%) inactivation of PRRSv achieved at an applied voltage of 20 kV and an air flow rate of 12 cfm. Differential pressure across the reactor was minimal compared to that imposed by HEPA filters. A high porosity consumer-grade ozone filter positioned downstream of the reactor effectively reduced residual ozone concentrations down to levels commensurate with the ambient laboratory environment. The results demonstrate the potential of NTPs, once properly optimized, for the prevention of airborne PRRSv transmission into hog barns with ventilation air. An optimized NTP airstream sterilization system would: 1) reduce or eliminate the need

to retrofit facilities to establish an air-tight building envelope, 2) eliminate the expense and waste of replacing and disposing of used air filters, 3) offer protection from airborne viruses irrespective of viral mutations, 4) offer protection that is on-demand or tunable as meteorological or climatic conditions warrant.

Keywords:
PRRS, aerosol, plasma, virus, ventilation

Scientific Abstract:
Porcine reproductive and respiratory syndrome virus (PRRSv) has been detected in air more than 9 km downwind of infected swine. Applying air filtration to ventilation air supplied to hog barns involves structural retrofits to buildings that can be costly, in addition to the periodic replacement of used filters. Non-thermal plasmas (NTPs) are electrical discharges comprised of reactive radicals and excited species that inactivate viruses and bacteria. Our previous experiments using a packed bed non-thermal plasma reactor demonstrated effective inactivation of bacteriophage MS2 as a function of applied voltage and power, ranging from less than one-log inactivation at < 20 kV and a few watts to greater than two-log inactivation at 30 kV. The present study examined the effectiveness of the same reactor in inactivating aerosolized PRRSv. A PRRSv solution containing ~10^5 TCID50/ml was aerosolized at a rate of 3 ml/min by an air-jet nebulizer and introduced into air flows of 5 or 12 cfm followed by NTP exposure in the reactor. Twin impingers upstream and downstream of the reactor collected samples of the virus-laden air flow. Subsequent TCID50 assay and quantitative polymerase chain reaction (qPCR) analyses of the collected samples determined the pre- and post-treatment abundance of infective PRRSv (in TCID50/ml) as compared with the abundance of the viral genome (qPCR), whether infective or rendered inactive by NTP exposure. An optical particle sizer measured upstream and downstream aerosol size distributions, giving estimates of aerosol filtration by the reactor. The results showed that PRRSv was inactivated to a similar degree as MS2 at the same conditions, with the 1.3-log inactivation of PRRSv achieved at 20 kV and 12 cfm air flow rate. Differential pressure across the reactor was minimal compared to HEPA filters and a consumer-grade ozone filter reduced residual ozone concentrations down to levels commensurate with the ambient laboratory environment. The results demonstrate the potential of properly optimized NTPs for preventing infiltration of PRRSv into hog barns with ventilation air.

Introduction:
Traditional biosecurity measures almost exclusively address the transmission of infectious agents on surfaces. However, it has been established that PRRSv can be transmitted in ambient air kilometers from the source [1, 2]. NPB study 09-209 found new PRRSv outbreaks were recorded at 20% of farms with ventilation air filters installed as compared to 92% of farms without such filters in the control group. Nevertheless, adoption of filtered ventilation air in swine operations has not yet gained widespread acceptance for several practical reasons. High efficiency particulate arresting (HEPA) filter systems have 0.2 to 0.4 inches of water column (in. w.c.) pressure drop at under nominal operating conditions [3]. Such pressure drop across the filters produces a partial vacuum inside the facility that, if unaddressed, would induce infiltration of outside air into the building through the building envelope. To eliminate such air infiltration, capital costs for installing air filtrations systems include structural retrofits necessary to provide an airtight building envelope. Exhaust fans may need to be upgraded to increase their capacity, power, and efficiency, particularly when considering cooling capacity limits during summer's heat and humidity; since the viscosity of air increases with temperature, filtration systems have the most pronounced effects on ventilation rates during summertime when ventilation is most critical. And finally, air filtration systems increase operating costs associated with periodic, typically biannual replacement of the MERV-10 pre-

filters, used to capture the majority of dust and suspended particles in order to extend the lives of the higher efficiency, more expensive MERV 14 or MERV-16 filters [4].

Of the two defining characteristics of infectious aerosols - *transport* and *infectivity* [5] - particulate filters only address transport. However, non-thermal plasmas (NTPs) address both characteristics by 1) electrostatic removal of larger particles (>1 mm approx.) and 2) sterilization of the remaining smaller particles by direct plasma exposure. NTPs are stable electrical discharges containing excited and ionized species and radicals that are orders of magnitude more reactive than ozone (O_3) [6], the more familiar, less effective, and more persistent oxidant used by indoor air cleaners. Radicals and excited species are thought to vigorously attack the bacterial cell membrane or virus capsid, leading to damage that causes a loss in structural integrity and eventual pathogen inactivation. NTPs have already been proven for surface disinfection, inactivating biological pathogens on the surfaces of food products (Fig. 1) [7, 8] and treatment of skin diseases [9]. NTPs have also been thoroughly studied for destruction of gaseous pollutants, the radicals and excited species having been shown to destroy a wide variety of gaseous volatile hydrocarbons such as those emitted from industrial processes [6] as well as gaseous pollutants such as nitrous and sulfurous oxides (NOx and SOx) emitted from combustion [10]. However, the intersection of these two established applications of NTPs - disinfection of an air stream - is substantially complicated by the fact that viruses and bacteria act as charged aerosols suspended the gas stream, both the aerosols and the gas responding separately to the electric fields and flow of charged ions that form the foundation of NTPs.

Objectives:

The objective of this project was to measure the inactivation of aerosolized PRRSv by a prototype packed bed NTP reactor. The research questions to be answered are:

1. To what degree does the prototype packed bed NTP reactor inactivate airborne PRRSv?
2. Under the same operating conditions, does the prototype reactor inactivate PRRSv at a higher or lower efficiency than the viral surrogate MS2, and thus should PRRSv inactivation be considered a conservative benchmark as compared to MS2, or vice versa?

Materials & Methods:

Experimental apparatus

The NTP reactor testing was conducted using a small-scale non-recirculating wind tunnel located at the University of Minnesota. A photograph of the test setup is shown in Figure 1. The wind tunnel has an inside duct diameter of 3.5”, an overall length of 96” and can operate in a volumetric flow rate range from approximately 3 to 30 cfm. A blower is connected at the outlet of the wind tunnel and the volumetric flow rate is maintained by measuring the pressure drop across a calibrated orifice meter mounted near the tunnel exit and applying a necessary voltage to a blower motor downstream of the tunnel to control the flow. Temperature and relative humidity in the tunnel and absolute pressure are measured and taken into account in the volumetric flow rate measurements. The flow rate is calculated and maintained using PID feedback control in LabView. Data acquisition by the LabView software is facilitated by a National Instruments USB-6001 DAQ. A HEPA filter is located at the exit of the tunnel to prevent contamination or release of the PRRSV into the laboratory.

The NTP reactor was mounted in the test section of the wind tunnel (shown in Figure 1) and was tested at volumetric flow rates of 5 and 12 cfm. A large particle generator (LPG) atomizer was installed at the entrance of the tunnel and used to generate PRRS virus challenge aerosols. A PRRSV solution from a 65 ml syringe was feed into the LPG atomizer using a syringe pump set at a constant liquid feed rate of 3 ml/min.

Dry and filtered dispersion air at a volumetric flow rate of 1.5 lpm was passed through the atomizer rotameter and connected to the dispersion air of the atomizer to aerosolize the liquid leaving the atomizer nozzle. After the PRRSV aerosol was drawn into the chamber it was diluted and dried with air entering the chamber and mixed thoroughly using an orifice mixing plate causing turbulence and creating a uniform aerosol concentration in the tunnel. Isokinetic probes were used upstream and downstream of the NTP reactor to collect particle number concentrations with a portable optical particle counter (OPC, TSI AeroTrak model 9306-V2). An aerosol diluter was used to prevent coincidence errors in the OPC. Liquid samples collected over 20 minute sample periods were subsequently collected by drawing air through isokinetic probes connected to liquid impingers located upstream and downstream of the reactor with each impinger operating at a nominal volumetric flow rate of 9 lpm. The pressure drop across the NPT reactor was measured at the wind tunnel test flow rates of 5 cfm and 12 cfm.

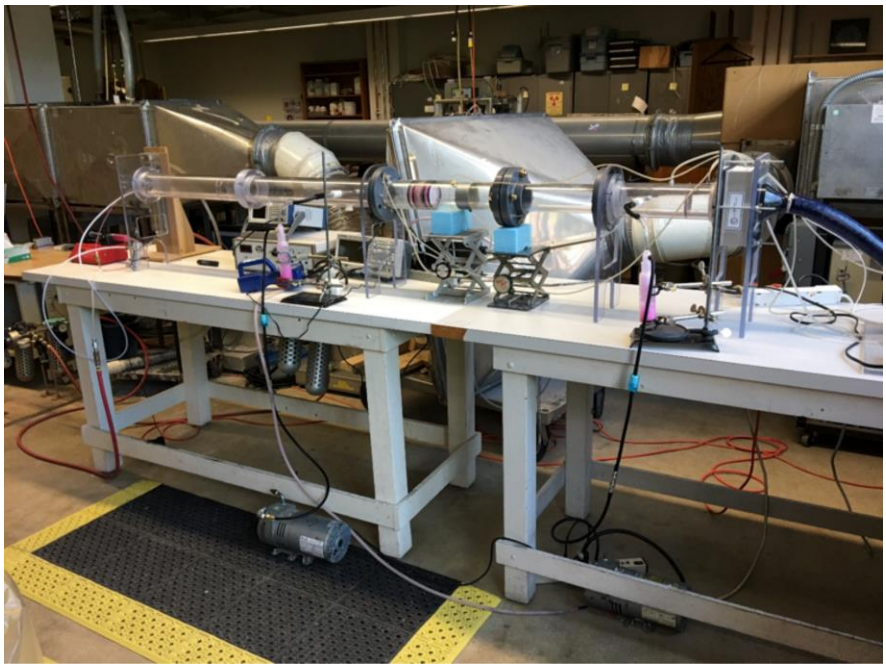

Figure 1. Photograph of the small-scale wind tunnel setup at Univ. of Minnesota with the NTP reactor mounted in the middle test section. The inlet is on the left side and the flow goes from left to right through the tunnel and HEPA filter where it is drawn out through a vacuum blower (not shown).

Figure 2 is a close-up schematic of the DBD packed-bed NTP reactor. The reactor is composed of three Plexiglas tubes with two smaller tubes (3.5-inch OD, 3-inch ID, 6-inch length). The smaller tubes slide freely relative to the larger tube (4-inch OD, 3.75-inch ID, 8-inch length) due to a clearance of 0.125-inch. Two rubber O-rings, which seat in the grooves on the OD of the smaller tube permit an air tight sliding mechanism. As indicated in Figure 2, a circular perforated brass plate is installed at the end of each sliding tube to serve as the ground electrode and to evenly distribute the inlet and outlet flow of the reactor. The design of the sliding electrode allows for packed bed depth adjustment ranging between 0.25 to 6 inches. A brass ring (0.035-inch thickness, 1-inch width) adhered to the OD of the larger Plexiglas tube serves as the positive electrode for the AC high voltage supply. In Figure 2, two flow plugs made of Styrofoam (2-inch OD) is positioned at the center of the reactor to direct the airflow with viral aerosols through an annular region in which the plasma is concentrated. The DBD reactor utilizes the

microdischarge generated through a dielectric barrier made of Plexiglas. The packed-bed, consisting of 500 inert borosilicate glass beads (0.25-inch diameter), further enhances the microdischarge by partial discharges at the contact points between the glass beads for effective electron-viral aerosol collisions and inactivation process.

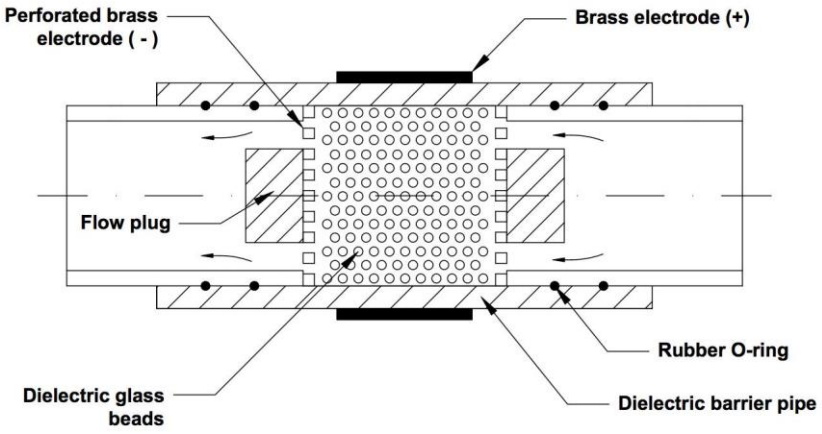

Figure 2. Schematic of the DBD packed-bed NTP reactor.

Virus propagation and titration

PRRS VR2332 strain was propagated in MARC-145 cells and titrated to 10^5 $TCID_{50}$/mL. The virus was aliquoted and frozen at -80°C until used.

Aerosolization test

For each test, a syringe was filled with 65 mL of PRRSV at 10^5 $TCID_{50}$/mL, placed into a New Era pump system, model NE-1000 multi-phaser, and aerosolized into the testing chamber at 3 mL/min via an air-jet nebulizer for 20 minutes. Room temperature and relative humidity level were measured during each test. Table 1 presents the test conditions, for which 2 replicates were taken for control conditions without the reactor and with the reactor turned off, and 3 replicates were taken with the reactor turned on

Air sample collection

Air samples were collected with impingers, placed upstream and downstream of NTP reactor. Each impinger was filled with 20 mL of DMEM supplemented with 1.5 mg/mL of bovine serum albumin fraction V 7.5%, 1X antibiotic-antimycotic, 0.0015 mg/mL of Trypsin-TPCK, and 0.05 mg/mL of gentamicin. After 20 minutes sampling time, the volume of supplemented DMEM in each impinger was measured, recorded, aliquoted into 3 tubes and frozen at -80°C until tested.

Quantitative RT-PCR

Samples were quantified for PRRSV using quantitative RT-PCR as previously described [11]. A standard curve based on transcript RNA with a quantitative linear range from 1×10^3 copies/µL to 1×10^6 copies/µL was used to determine the RNA copy number/mL. The copy number/mL results were consecutively converted to copies/m^3 of air. RT-PCR analyses were conducted by the Veterinary Diagnostic Lab at the Univ. of Minnesota.

Sample titration

Samples were serially diluted 5-fold. 100 uL of the serial dilution was plated onto 96-wells plates containing MARC-145 cells in 4 replicates. Plated samples were incubated for 7 days at 37°C with 5% CO_2. At day 7, plates were removed and CPE was read and recorded. TCID50/mL was calculated using the Spearman-Karber method and consecutively converted to TCID50/m^3 of air. PRRSv titration was conducted by the Veterinary Diagnostic Lab at the Univ. of Minnesota.

Table 1. Summary of testing conditions.

NTP Reactor Position	NTP Reactor Voltage (kV)	Chamber Air Flow (cfm)
No reactor	N/A	12
No reactor	N/A	5
OFF	N/A	12
OFF	N/A	5
ON	12	12
ON	12	5
ON	16	12
ON	16	5
ON	20	12
ON	20	5
ON	30	12
ON	30	5

Results

Testing took place in two phases, in May and August, over the course of four months to allow time for samples collected in the first phase to be analyzed by the Veterinary Diagnostic Lab at Univ. of Minnesota and "lessons learned" applied in the second phase. For example, there was initial concern that the sensitivity of PRRSv titration, which is limited by the initial titer of infective PRRSv that is aerosolized, might lead to a situation where no infective virus was detected, resulting in uncertainty as to whether such a result represented a "perfect test" or a failed one. As a result, phase 1 employed the lowest voltages to make sure to the extent possible that as little of the virus was inactivated. Test conditions in the second phase employed higher voltages after viral analyses showed the results of phase 1 yield partial inactivation.

In the first phase (May data), the NTP reactor was operated at voltages of 12, 16, and 20 kV, and the highest NTP deposited power was 1.7W achieved at 20kV. In the second phase (August data), voltages of 30 kV were applied in the reactor using a second power supply, resulting in about 23 W power consumption by the NTP reactor. Table 2 (below) summarizes the average PRRSv inactivation efficiency achieved by the

prototype reactor for data that passed our data quality controls in the phase 1 (May) and phase 2 (August) test results.

Objective 1: To what degree does the prototype packed bed NTP reactor inactivate airborne PRRSv?
Objective 2: Under the same operating conditions, does the prototype reactor inactivate PRRSv at a higher or lower efficiency than the viral surrogate MS2, and thus should PRRSv inactivation be considered a conservative benchmark as compared to MS2, or vice versa?

Figure 3 compares PRRSv inactivation data obtained during the present project to MS2 phage inactivation data obtained at Univ. of Michigan in a USDA-funded project, all for equivalent power settings for the NTP reactor and similar air flow rates.

Figure 4 shows the much improved precision of the August PRRSv inactivation data, showing how two different methods of calculating inactivation efficiency results in closely clustered results and comparable trends with respect to applied NTP voltage.

Figure 5 is a photograph of the accumulation of MEM within the packed bed NTP reactor sometimes found at the end of select 20-minute tests.

Figure 6 presents results from testing conducted at the University of Michigan after phase 2 of testing at University of Minnesota in which it was sought to assess the effect of an accumulation of MEM within the packed bed NTP reactor on MS2 inactivation.

Table 2. Summary of PRRSv inactivation efficiency results determined based on the ratio of post-treatment (downstream) to pre-treatment (upstream) abundance of infective PRRSv normalized by either a) ratio of downstream to upstream abundance of infective PRRSv without NTP treatment (NTP-Off), or b) ratio of downstream to upstream abundance of genome copies determined by qPCR. Air flow rates of 5 and 12 cfm, applied NTP reactor voltages of 12, 16, 20, and 30 kV. Highlighted values represent rejected, unphysical results implying higher infectivity for post-treatment samples.

Inactivation by NTP Reactor	August Tests		May Tests		
	30kV	20kV	12kV	20kV	16kV
NTP-Off 5cfm	66.20%	29.71%	71.27%	-27.10%	68.16%
qPCR 5cfm	61.01%	53.47%	83.88%	-51.02%	80.03%
NTP-Off 12cfm	73.98%	26.55%	-71.79%	94.63%	35.24%
qPCR 12cfm	71.31%	47.23%	71.59%	98.63%	71.15%

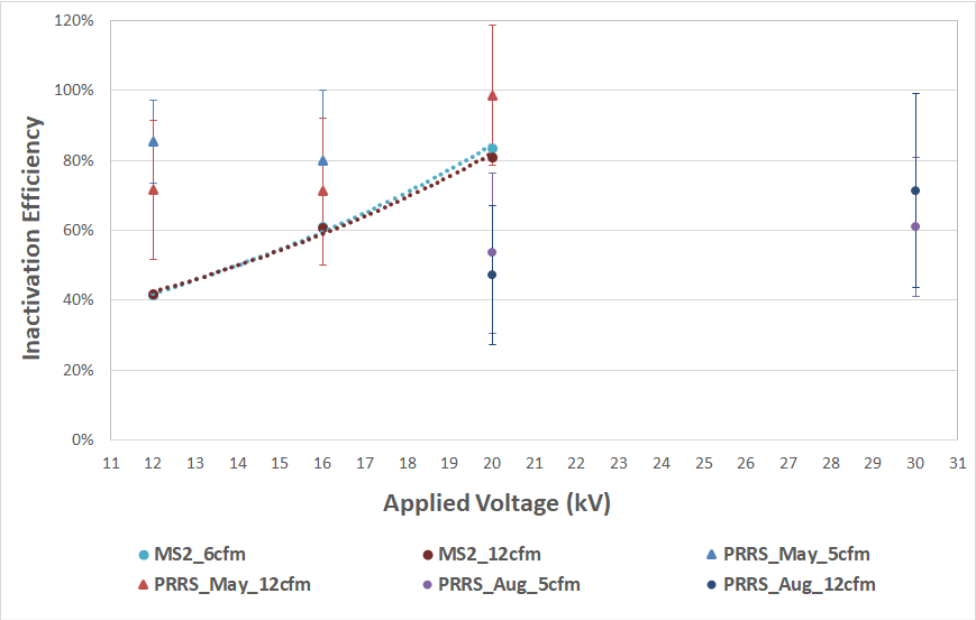

Figure 3. Comparison of phase 1 (May) and phase 2 (August) PRRSv inactivation by NTP treatment at two air flow rates (5 or 6 cfm and 12 cfm) and four voltages (12, 16, 20, and 30 kV), as compared to earlier results for MS2 phage inactivation at the same conditions (30 kV not shown).

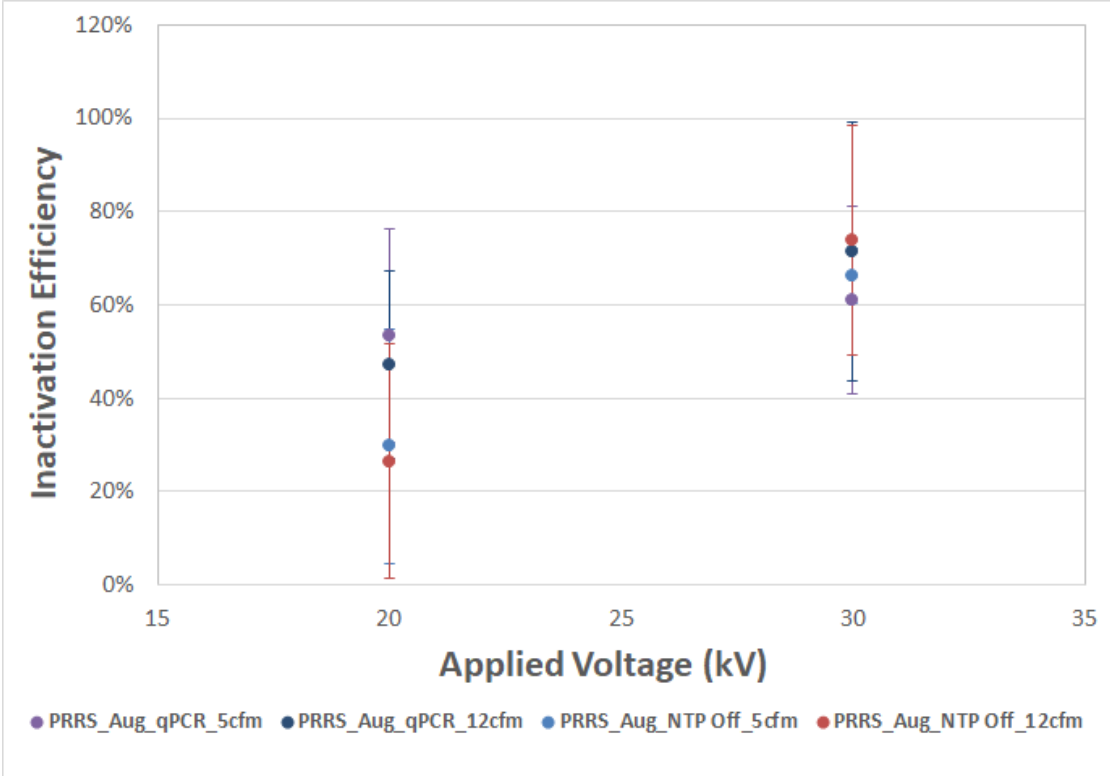

Figure 4. Results of phase 2 (August) PRRSv inactivation by NTP treatment at two air flow rates (5 and 12 cfm) and two voltages (20 and 30 kV, corresponding to 1.7 and 23 W, respectively). Compared are the results of two different methods for calculating inactivation efficiency: reduction in $TCID_{50}$ based on pre-

treatment and post-treatment samples, normalized by either a) $TCID_{50}$ reductions taken at the same locations with the reactor off, or b) RT-PCR reductions taken at the same locations with the reactor on.

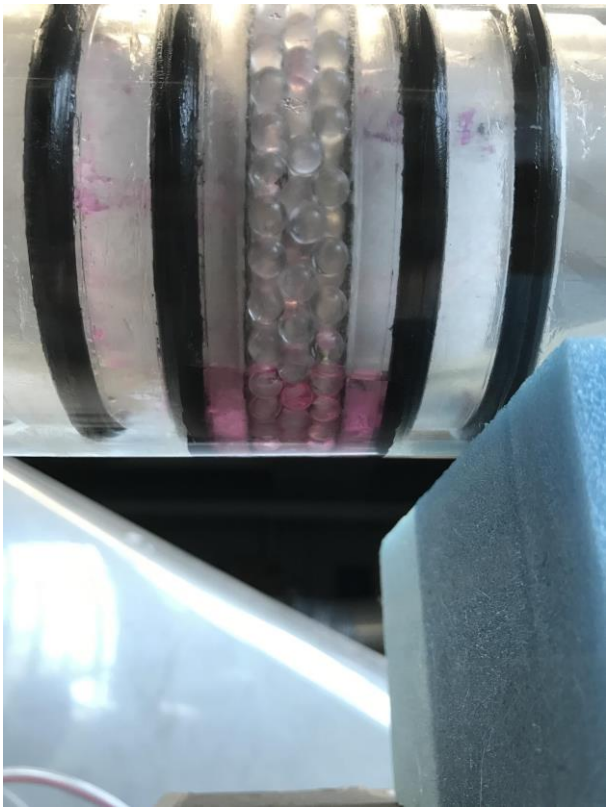

Figure 5. Photo showing the accumulation of MEM (pink liquid) after a 20 minute test, the result of incomplete evaporation of PRRSv-bearing MEM droplets before reaching the packed bed NTP reactor.

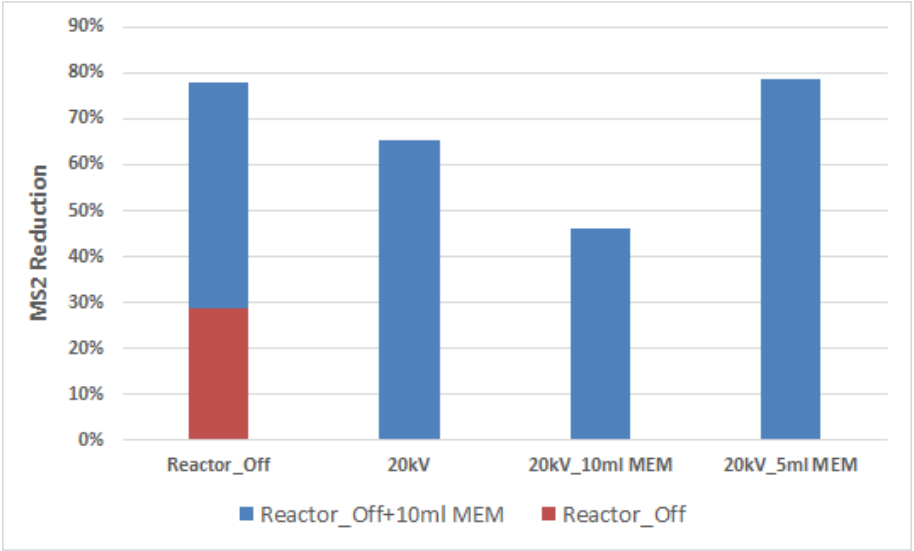

Figure 6. Results showing reduction (filtration + inactivation) of MS2 phage in response to 10 ml and 20 ml of MEM added to and held within the NTP packed bed. MEM was added to the packed bed first, before introduction of MS2 phage, aerosolized from VDB solution.

Discussion:

Figure 3 illustrates the increasing trend in inactivation efficiency with increasing applied voltage, as expected, and the generally lower inactivation efficiency at the higher air flow rate (12 cfm) as compared to the lower air flow rate (5 cfm). These trends are as expected and agree with those observed in previous results involving inactivation of MS2 phage conducted at University of Michigan. Inactivation of infectious agents by chemical oxidants generally is considered to follow a Chick-Watson or modified Chick-Watson model in which the ratio of the abundance of infective virus post-treatment compared to pre-treatment (C_{post}/C_{pre}) varies exponentially with the concentration of the inactivating agent and duration of exposure:

$$C_{post}/C_{pre} = \ln(-kC_{inact}t) \quad (1)$$

where C_{inact} is the concentration of the inactivating agent, t is duration of treatment, and k is a kinetic constant. Increasing applied voltage to the NTP reactor increases the concentration of reactive ions and electrons in the plasma and decreasing the airflow rate through the reactor increases the exposure time of the entrained PRRSv aerosols to the plasma, both of which would be expected to increase inactivation efficiency and decrease C_{post}/C_{pre}. Evident from Figure 3 is the fact that under similar experimental conditions, the effectiveness of the NTP in inactivating PRRSv aerosols was somewhat higher than the demonstrated effectiveness for inactivating MS2 with the same device at the same conditions; however, the large uncertainty in the results as indicated by the large confidence intervals allows only for a conclusion that the two viruses were likely inactivated comparably

For several tests, particularly those conducted in May, $TCID_{50}$ or genome copies analyses did not pass our data quality controls. Specifically, in some instances either the abundance of infective PRRSv or the number of genome copies was found to be higher at the downstream location than at the upstream location, including a few instances of this occurring when the reactor was not powered. Filtration of the aerosols within the packed bed alone has in the past been found to result in about a 30% reduction in number of genome copies. Degradation of the airborne PRRSv by attack from reactive species within the non-thermal plasma would not be expected to result in either an increase $TCID_{50}$ or an increase in the number of PRRSv genome copies at the downstream sampling location. More consistent values of inactivation efficiency were obtained in phase 2 of testing (August), as is evident from the absence of negative values of inactivation efficiency (Table 2) and the greater precision among multiple replicates of a single test condition seen in Figure 4.

Incomplete evaporation of the PRRSv-laden droplets of MEM was consistent throughout both phase 1 and phase 2 tests. Prior tests at University of Michigan involving MS2 phage achieved complete or near complete droplet evaporation after several modifications to the experimental apparatus: lengthening the distance between the introduction of the droplets into the airstream and the NTP reactor; supplementing ambient air with dry compressed air, thereby lowering relative humidity and speeding evaporation; producing smaller droplets via an ultrasonic atomizer. These remedies were not available or not possible for the current project. As a result, the significant unanswered question regarding the present results is what are the impacts, if any, of the persistent presence of liquid MEM in fine droplets exposed to the non-thermal plasma and accumulating in the NTP reactor itself.

MEM is a solution of glucose in water. One hypothesis is that residual MEM on aerosols or as aerosols passing through the NTP reactor may have the effect of protecting PRRSv from oxidation by the reactive species generated by the plasma. Whereas H_2O alone, when dissociated, produces additional reactive species in the form of H^+ and OH^-, hydrocarbons would act more as a sink for the plasma-generated species, consuming them in the course of their complete oxidation. There exists a separate branch of plasma environmental remediation that involves using plasmas to oxidize chemical contaminants in water supplies or wastewater. Support for this hypothesis came from preliminary tests prior to phase 1. Prior testing at Univ. of Michigan using MS2 phage determined that ozone (3) produced by the NTP reactor had the potential to accumulate in the downstream impinger fluid, a virus dilution buffer (VDB) consisting small amounts of sodium and phosphorous salts dissolved in water. The dissolved ozone was found to continuously inactivate MS2 phage in the impinger during and after testing, potentially leading to a high bias in inactivation efficiency results. Sodium sulfite was routinely added to the VDB during MS2 testing, serving to consume O3 and prevent inactivation of MS2 phage while in the impinger. However, when similar tests were conducted exposing PRRSv in MEM to dissolved O3, the results showed no inactivation of PRRSv in MEM by O3, suggesting that O3 was consumed by oxidation reactions with the hydrocarbons in MEM and therefore unavailable to chemically attack the PRRSv in solution.

A second potential effect of residual MEM could be in influencing the electrical properties of the packed bed and the strength or distribution of the plasma therein. In the packed bed, the plasma exists in the void spaces between the beads where the electric field increases above the threshold where electrical breakdown occurs in air. Accumulation of MEM in these spaces could provide a conductive electrical pathway that prevents such electric fields from forming. This, too, would be expected to reduce the apparent inactivation efficiency of tests involving MEM as compared to tests in which evaporation of the liquid phase was more complete.

In order to test these hypotheses and their potential effects, tests were conducted after phase 2 at Univ. of Michigan in which the packed-bed NTP reactor was pre-soaked with known amounts of MEM, after which tests were conducted to measure the inactivation of MS2 phage aerosols. Figure 6 shows the results of these tests in the form of MS2 reduction, comprising both physical filtration and NTP inactivation, comparing samples collected at the upstream pre-treatment location against those collected at the downstream post-treatment location. The addition of 10 ml of MEM to the packed bed increased the physical filtration of the MS2 phage aerosols substantially, the upstream-to-downstream reduction increasing from less than 30% (packed bed alone) to more than 70% (packed bed pre-soaked with 10 ml of MEM). The augmented physical filtration of the pre-soaked packed-bed NTP reactor yielded greater reduction in MS2 phage (78%) than the reduction resulting from non-thermal plasma treatment in the dry NTP reactor (65%). Further, the reduction in MS2 phage achieved within an NTP reactor pre-soaked with 10 ml of MEM (inactivation + augmented filtration) was *less than* that achieved in the dry packed bed reactor (inactivation + baseline filtration, 45%). That baseline filtration + inactivation reduced MS2 phage concentrations more than augmented filtration + inactivation suggests that the presence of residual MEM in the packed bed reactor, through some combination of filtration and chemical oxidation, causes a negative bias in measured inactivation efficiency. With regard to the electrical effects, it seemed that the residual MEM in the packed-bed NTP reactor can increase the amplitude of transferred current without changing the amplitude and waveform of the applied voltage. With 20kV applied to the dry packed-bed NTP reactor, the transferred current had an amplitude of 0.40mA. The addition of 5 ml of MEM to the packed bed increased the current amplitude to 0.48mA, and the addition of 10 ml of MEM to the packed bed further increased the current amplitude to 0.56mA. This was reasonable since the accumulation of liquid in the packed bed could reduce the resistance of the reactor and thus increasing the amplitude of transferred current. This discharge

environment, however, may not be favorable for plasma generation, since more current would pass through the conductive liquid without ionizing air in the packed-bed.

In summary, this study provides data on the airborne inactivation of PRRSV using a novel approach of treating contaminated air, which is the use of NTPs. Reduction rates by the technology were modest but provided proof of concept that airborne PRRSv can be inactivated by NTPs. Overall our results show promising results to inactive airborne PRRSv and offer potential alternatives to producers to enhance their biosecurity practices.

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