EVALUATION OF A VEHICLE UNDERCARRIAGE DECONTAMINATION SYSTEM FOR INACTIVATING NEWCASTLE DISEASE VIRUS

by

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ABSTRACT

The major focus of the present study was to evaluate an affordable and portable undercarriage decontamination system for poultry farmers to disinfect their vehicles. These washing stations are necessary to disinfect vehicles moving between farms, avoiding spread of infectious material. Commercialized undercarriage decontamination systems are typically expensive and permanent due to their size, thus limiting use among small-scale farmers. Constructed of materials available to most farmers including PVC pipes and simple tools, the undercarriage decontamination system built for this study is cost friendly and portable. To evaluate the area coverage of the spray rig, litmus paper strips were affixed to various locations on the truck exterior and undercarriage and sprayed with a diluted citric acid solution. The spray rig effectively covered all areas of the truck with the citric acid solution. Next, galvanized steel coupons inoculated with Newcastle disease virus (NDV) were adhered onto the truck using magnets and passed through the decontamination system. Three coupons orientations (horizontal, vertical, and complex) were chosen to test the decontamination system's ability to target various locations on the truck. Two solutions, one peroxide agent and one detergent, were used to evaluate differences in effectiveness of a disinfectant versus a cleaning agent. The viral material from the steel coupons was pooled by orientation type and inoculated into 10-day old specific pathogen free eggs. Eggs were incubated and candled for five days to monitor mortality. Chorionic allotonic fluid was collected from each egg and used in hemagglutination assays. The median embryo infective dose for each treatment group and positive control were calculated based on hemagglutination assay results. The decontamination system successfully inactivated virus in the horizontal orientation but

failed in the vertical and complex locations. However, the system lowered the titer of NDV in these cases, achieving up to a two-log drop. Adjustments to the decontamination system are needed to improve its efficacy.

Chapter 1

LITERATURE REVIEW

New Castle Disease Virus

Newcastle disease virus (NDV) causes mortality and morbidity, affecting poultry worldwide. Virulent Newcastle disease (vND) is a form of the disease that causes very high mortality. In a 2003 outbreak of vND lasting 11 months in California, 3.16 million birds were depopulated, costing the industry $161 million dollars. As of April 12, 2019, a current outbreak of virulent NDV in California has resulted in 422 cases (USDA APHIS, 2019). The outbreak spread to commercial flocks and to neighboring states (California, 2019). In poultry, vND manifests in systemic illness. Infected birds suffer from symptoms such as sneezing, coughing, diarrhea, lethargy, tremors, and sudden death. Virus particles can be spread through the bodily fluids of sick birds. Common means of transmission and fomites are manure, crates, egg flats, equipment, and clothing, making proper biosecurity protocols imperative (USDA APHIS, 2018). Part of the paramyxovirus family, NDV is a non-segmented, negative sense RNA virus surrounded by an envelope. Virulence of NDV can be classified into three categories. The least virulent strains described as lentogenic cause mild symptoms and are commonly used in vaccines. Mesogenic NDV strains cause more serious respiratory infections and mortality. The most virulent are velogenic strains, also known as vND, which are responsible for high mortality rates in both vaccinated and unvaccinated flocks (Dortmans, 2011). The

serious consequences of a deadly NDV outbreak call for effective biosecurity measures to prevent its spread.

Biosecurity

The goal of biosecurity is to prevent the spread of infectious agents between animals. The various factors and strategies considered when creating a biosecurity plan can be divided into three major categories: isolation, resistance, and sanitation. When new animals enter an established flock, the flock is at risk of being exposed to any disease agent the new animal carries. Healthy animals can harbor infectious agents and act as carriers of disease. Screening and isolation of new animals is essential to keeping the flock safe. The second major factor is disease resistance in the flock or herd. Through selective breeding, genetic resistance to certain diseases can be favored in an animal population. A good immunization program can increase the disease resistance as well. The last component of biosecurity is sanitation. Sanitation procedures involve the cleaning and disinfection of potentially infected items and facilities. This includes clothing, shoes, equipment, vehicles, and the interiors of farm facilities (Hovingh, 2016). Cleaning and disinfection are two separate phases of sanitation.

Cleaning and Organic Load

Cleaning refers to the removal of organic material or debris from the surface of an item or area through dry or wet cleaning. Dry cleaning involves using a mechanical motion such as sweeping or brushing to remove dirt or contaminated material. Wet cleaning uses water and detergent or soap to eliminate organic material, which can decrease the effectiveness of disinfectants (USDA APHIS, 2018). The organic

material or debris on a surface is referred to as the organic load. The organic load can be comprised of fecal material, blood, serum or any bodily fluids, which prevent disinfectants from properly working in two ways. Firstly, the disinfectant will bind with the organic material, making it less effective. This also decreases the amount of active disinfectant available. Secondly, microorganisms are shielded by the organic material, preventing the disinfectant from inactivating the pathogens (Centers of Disease, 2016). Proper cleaning of a surface can reduce the pathogen load by 75%, making it a key step before the application of a disinfectant (Canadian Food, 2018).

Disinfection

Disinfection should follow cleaning to ensure the disinfectant has proper contact with the surface being sanitized. The goal of disinfection is to kill or inactivate infectious organisms present by physical or chemical means. Physical disinfection methods include heat or UV light, whereas chemical disinfection uses specific compounds that can be applied as a solution to kill harmful organisms (USDA APHIS, 2018). It is important to choose a disinfectant that targets the disease agents prevalent or commonly found in a specific flock or herd. In the present study, the peroxygen disinfectant used has been proven to be effective against avian influenza and NDV in addition to several other viral families (Independently Proven, n.d).

Classes of Disinfectants

Soaps and detergents: Soaps and detergents are necessary for the cleaning phase prior to use of a disinfectant. They can be used to decontaminate surfaces by removing organic material and dirt from items or surfaces. As previously discussed, this step is essential to limit the inhibitory effects of the organic load. In the case of

virus decontamination, soaps and detergents can play a large role in the disinfection process. Detergents can degrade the lipid components found in enveloped viruses such as Newcastle disease or avian influenza (De Benedictis et al, 2007). This characteristic and their non-corrosive nature make detergents an attractive chemical disinfectant to use on metal surfaces.

Oxidizing Agents: Oxidizing agents are a class of disinfectant mainly containing peroxides which produce free hydroxyl radials that oxidize lipids and nucleic acids. Organic material is shown to inhibit or decrease the efficacy of oxidizing disinfectants. Additionally, these compounds are effective on hard surfaces, but can be corrosive to metals (De Benedictis et al, 2007). Disinfectants under this category are broad-spectrum and target a variety of pathogens. Examples of peroxide-based disinfectants include hydrogen peroxide and peracetic acid. Hydrogen peroxide and peracetic acid are both proven to be bactericidal, viricidal, fungicidal and even sporicidal at high enough concentrations (Dvorak, 2008). Specifically, a popular disinfectant called Virkon S combines a peroxide, organic acid, and surfactant for disinfection. The surfactant acts to decrease the surface tension on the material, allowing for better contact with water-based disinfectant compounds. Unlike other oxidizing agents, Virkon S has shown to be relatively stable in the presence of organic material (Schuenemann et al, 2017). Additionally, Virkon S is a broad-spectrum disinfectant with efficacy against 22 viral families, 400 bacterial strains, 60 strains of fungi, including avian influenza and vND (Independently Proven, n.d).

Phenols: Phenols are carbolic acid derivatives, usually made from coal tar or synthetic formulations. They kill microorganisms by denaturing proteins and changing the cell wall permeability by damaging membrane-bound enzymes (Dvorak, 2008.

Phenols have been seen to maintain effectiveness in the presence of an organic load. Common examples of phenols include TekTrol, Pine-Sol, and Lysol (University of Colorado, 2008).

Acids and Alkalis: Acids change the pH of the surface being disinfected, making it an inhospitable environment for microorganisms. Acids can also precipitate proteins and damage nucleic acids found in microorganisms. Common acid disinfectants include acetic acid and citric acid. Alkalis include sodium hydroxide, sodium carbonate, and calcium oxide. These disinfectants harm microorganisms by disrupting the lipids found in the envelopes or cell membranes (Dvorak, 2008).

Halogens: Halogenic disinfectants can be divided into two major categories, chlorine compounds and iodophors. Chlorine compounds include sodium hypochlorite, the active ingredient in household bleach (University of Colorado, 2008). These compounds are effective against enveloped and non-enveloped viruses, bacteria, and fungi. Chlorine compounds denature proteins through oxidation. Iodophors can be used both as a disinfectant and antiseptic on the skin and possess broad spectrum disinfection capabilities (Dvorak, 2008).

Aldehydes: Common examples of aldehydes include formaldehyde and glutaraldehyde. This class of disinfectant has broad spectrum capacity and is effective against bacteria, viruses, fungi and spores. Similarly, to the other disinfectants, aldehydes damage proteins and nucleic acids in the microorganism. Aldehydes are not corrosive to metals, but are potentially carcinogenic to humans (Dvorak, 2008).

Vehicles as Carriers of Disease

The routes of disease transmission can be divided into two major categories: direct and indirect. Direct transmission involves the spread of disease from an infected

animal's bodily fluids or from physical contact with the animal. This can also include apparently healthy animals which have a latent infection and shed the pathogen, spreading it to others in the flock or herd. Indirect transmission requires an intermediate that carries the infectious material. If the carrier is another living being, it is considered a vector. Common examples of vectors include birds, rats, mosquitos, and insects. However, infectious material can also be spread on non-living objects known as fomites. Major fomites in farm settings are equipment, footwear, clothing, and vehicles (Canadian Food, 2018). More specifically, vehicles pose a major risk for the spread of infectious diseases.

According to the Canadian Food Inspection Agency, the modes in which pathogens are spread during animal transport can be divided into five scenarios (2018). First, animals can enter a contaminated, poorly washed transport vehicle and contract the infectious agent. Disease transmission can also occur when a properly disinfected vehicle becomes contaminated at an infected farm during loading or unloading of animals. Infectious material can be introduced to the farm or premise itself from a contaminated transport vehicle and remain in the environment. Commonly, animal transport vehicles travel to similar sites, which may carry disease from other farms. For instance, these may include slaughterhouse facilities, auctions, or processing plants. Lastly, animals can be exposed to pathogens from contaminated equipment or personnel accompanying the transport vehicle. In any of these five scenarios, a transport vehicle poses the risk of spreading disease to and from specific facilities.

Few studies evaluate the role of vehicle interiors on viral particle spread and consequential infection in live animals. One study analyzed how animal transport

vehicle interiors may spread Porcine Reproductive and Respiratory Syndrome (PRRS) to susceptible pigs. The researchers found that healthy pigs could contract PRRS from a contaminated trailer and pigs infected with PRRS could also contaminate a trailer to detectable levels (Dee et al, 2004). Another article studied the presence of Porcine Epidemic Diarrheal Virus (PEDV) on transport vehicles during the unloading process at slaughter facilities. The floors near the rear door of 575 livestock trailers were sampled and tested for virus. The authors report that 38 trailers were contaminated with PEDV before unloading, while 28 trailers were contaminated during the unloading process. An interesting finding the authors note is that contamination during unloading was more frequent if personnel from the harvest facility entered the trailer. Additionally, if a clean trailer unloaded after a contaminated trailer unloaded, it was highly likely the clean trailer would become contaminated post unloading. The authors conclude by advocating for strict biosecurity protocols at common sites such as harvest facilities or auctions (Lowe et al, 2014). Due to these various routes in which disease can spread between farms through transport vehicles, it is imperative to properly clean and disinfect these vehicles used in animal agriculture.

Current Solutions Available

Current guidelines for transport vehicle disinfection include various steps such as cleaning, washing, disinfection, and drying. Proper cleaning requires the removal of organic material by brushing or scraping, followed by flushing with water. This step alone can reduce pathogen load by 75% (Canadian Food, 2018). Cleaning can be accompanied by washing and use of a detergent to further dislodge organic material. After the vehicle appears to be free of debris or manure, a disinfectant should be applied and allowed to set for the recommended contact time. Lastly, vehicles should

be set to dry, permitting further pathogen inactivation through heat or lack of moisture. Decontamination protocols may be any combination of these various steps depending on the individualized risks (Canadian Food, 2018). Proper disinfection of transport vehicles can be time consuming, labor intensive, and expensive. The USDA Foreign Animal Disease Preparedness and Response plan recommends using a designated wash station to clean and disinfect vehicles (2018). Vehicle wash stations vary in size, price, and accessibility.

Various models of vehicle wash stations are available on the market. Hydro-chem systems offers a biosecurity wash system design that targets the undercarriage, wheels, and chassis of trucks or trailers. In this design, a trailer slowly moves through a large house-like structure that contains the sprayers, sanitizing the vehicle in one step. A typical wash costs between five to seven dollars. The same company offers wash systems which target the undercarriage of vehicles (Hydro Chem, n.d). Another company, Stanton Systems, offers a tire wash model called the Portable STB 30, which targets the wheels of a vehicle. This model is capable of tire rotation when cleaning and removing dust and mud, but lacks a disinfection step (Stanton Systems, n.d). Meier- Brakenberg offers 4.8 m (16 ft.) disinfection arch which vehicles can pass through marketed as the DESTORmobil500. The entire system can be constructed by two people in about 20 minutes and is one of the few portable models on the market (Meier- Brakenberg, 2019). The efficacy of these large-scale wash systems has not been tested. Permanent wash systems may save time, but their size and cost may discourage producers from investing in them. Other solutions to this problem are necessary to provide affordable and portable options for producers.

Objective

The objective of the present study was to evaluate an affordable and portable undercarriage decontamination system for poultry farmers to disinfect their vehicles between farms. The design of the decontamination system was created by University of Delaware engineering students in 2016 and improved over the years. The range and coverage of the sprayers was evaluated using a diluted citric acid solution and litmus paper strips adhered to a truck. After preliminary data was collected, the efficacy of the decontamination system in inactivating Newcastle disease virus using a detergent and disinfectant was evaluated.

Chapter 2

MATERIALS AND METHODS

Overview

In the present study, the efficacy of an open source, low cost and portable vehicle undercarriage decontamination system was tested under field conditions. A detergent and disinfectant were both used with the system to inactivate or decrease the titer of Newcastle disease virus (NDV). Initially, to evaluate the area coverage of the spray rig, litmus paper strips were adhered to various locations on the truck exterior and undercarriage and sprayed with a diluted citric acid solution. Next, galvanized steel coupons inoculated with NDV were adhered onto the truck using magnets and the truck driven through the decontamination system. Three coupons orientations (horizontal, vertical, and complex) were chosen to test the decontamination system's ability to target various locations on the truck. One disinfectant, a peroxide agent and one detergent, were used to determine the differences in decontamination effectiveness between a disinfectant and detergent. The viral material from the steel coupons was pooled by orientation type and inoculated into 9-11-day old specific pathogen free eggs. Eggs were incubated and candled for five days to monitor mortality. Chorionic allantoic fluid was collected from each egg and used in hemagglutination (HA) and hemagglutination-inhibition assays (HI). The median embryo infective dose 50 (EID_{50}) for each treatment group and positive control were calculated based on hemagglutination assay results.

Chemical Test Strip Trials

Litmus paper strips were adhered to the truck via magnets in three orientations (horizontal, vertical, and complex) with six locations per orientation and two strips at each location. Horizontal strips were parallel to the ground and vertical strips were at 90 degrees to the ground. Complex strips were either blocked by truck parts or inverted horizontally. A 0.5% citric acid dilution was used as the treatment. The truck,

with litmus paper strips attached, was driven through the sprayer. A color change on the litmus paper was recorded as a positive result, while no color change was a negative result.

Virus Titration

A LaSota strain of Newcastle disease was used as a surrogate for avian influenza virus and was titrated using standard techniques and determined to have a titer of $10^{8.1}$ EID_{50}. The same seed stock virus was used for all phases of the project.

Virus Preparation and Coupon Inoculation

On the day of use, virus was thawed at room temperature and diluted 1:3 with phosphate buffered saline (PBS) to make the virus working stock. All galvanized steel coupons (2.2 cm x 2.2 cm x 0.2 cm) were washed three times with detergent and rinsed before use. All coupons were sterilized at 121° C (249° F) for 30 minutes before use. On the day of the trial, autoclaved metal coupons were placed in a biosafety hood on sterilized aluminum foil. Virus working stock was further diluted with fetal bovine serum (FBS) to achieve a 5% FBS and virus mixture. Each coupon was inoculated with 0.1 ml of the 5% FBS-viral mixture and allowed to dry in the biosafety hood at room temperature for 60-90 minutes. The dried coupons were transferred to cardboard boxes using sterile forceps. Six inoculated coupons were immediately placed into a six-well plate to later serve as the positive control.

Disinfectant and Detergent Preparation

Concentrations of the disinfectant and detergent were determined based on manufacturer's recommendations for vehicle disinfection. The peroxygen agent was diluted with city water to achieve a 1% concentrated solution (LanXess, n.d). The detergent was diluted to a 5% concentrated solution (Simple Green, n.d.). Water used was exposed to air for three days prior to the trial to ensure no chlorine remained in the water.

Undercarriage Disinfection Trial Procedure

Galvanized steel coupons inoculated with NDV were adhered to the truck using magnets, which were placed on the truck before the trial began. The coupons were placed in three orientations (horizontal, vertical, and complex) with six locations per orientation and two coupons at each location (A and B repetitions). The truck was driven through the undercarriage decontamination system at a speed that allowed exposure to the system for one minute and then left untouched for ten minutes to allow for proper disinfectant contact time. After ten minutes, the coupons were collected into six-well plates and 2.0 ml of Difco D/E Neutralizing broth was added to each well to stop further reactions. The coupons were transferred to the laboratory for further processing. Three trials each of the peroxide disinfectant and detergent were completed. The order of the trials was randomized, and the truck was rinsed with clean water after each trial to avoid mixing of the disinfectant agents.

Horizontal		
Coupon ID	Horizontal Location on Truck	Number of Coupons
HA1/ HB1	Top of the front suspension A arm	2
HA2/ HB2	Transmission support	2
HA3/ HB3	Back wheel spring	2
HA4/ HB4	Differential	2
HA5/ HB5	Back bumper	2
HA6/ HB6	Draw bar	2

Vertical		
Coupon ID	Vertical Location on Truck	Number of Coupons
VA1/ VB1	Top front of wheel	2
VA2/ VB2	Drivers door body mount	2

VA3/ VB3	Front of back wheel reverse (body)	2
VA4/ VB4	Behind back wheel (body)	2
VA5/ VB5	Behind back wheel (frame)	2
VA6/ VB6	Outside back wheel	2

Complex		
Coupon ID	Complex Location on Truck	Number of Coupons
CA1/ CB1	Back front bumper (vertical)	2
CA2/ CB2	Back of brakes (vertical)	2
CA3/ CB3	Above transmission (inverted)	2
CA4/ CB4	Inside frame driver's door	2
CA5/ CB5	Between body + frame	2
CA6/ CB6	Above rear wheel (inverted)	2

Table 1. List of coupon identifiers, location on truck and number of coupons per location.

Coupon Processing and Virus Inactivation

In a biosafety hood, the 6-well, plated coupons were scraped with a pipette and the fluid aspirated from the well was jetted back onto each coupon three times to dislodge virus from the coupon. All fluid from the six wells of one plate were pooled into one tube and processed separately for each plate. A 10-fold serial dilution from 10^{-1} to 10^{-3} from each tube was performed with PBS with antibiotics [penicillin (5,000 IU/mL) / streptomycin (5,000 µg/mL)]. Once the dilutions were complete, the dilution tubes stood for 60 minutes at room temperature to allow antibiotics sufficient time to kill possible bacterial contamination. To test for virus inactivation, 0.2 ml of each dilution was inoculated into the chorioallantoic sac (CAS) of five or six, 9-11 day old,

embryonated specific pathogen free (SPF) chicken eggs. The eggs were incubated at 37.5° C and candled daily for 5 days, with eggs with embryo mortality removed for refrigeration at 4° C. Mortality was recorded each day and on the fifth day, eggs were placed at 4° C overnight. Egg mortality during the first 24-hour post inoculation were considered non-specific death and not tested.

Positive Virus Control

One plate, containing six inoculated coupons (virus working stock and FBS) served as the positive virus control to test for virus recovery after drying and environmental impacts from field conditions. Two ml of Difco D/E Neutralizing Broth was added to each well of the positive control plate simultaneously with the experimental plates. The coupons were scraped with the pipette and the fluid aspirated from the well was jetted back onto the coupon three times to dislodge virus from the coupon. All fluid from the six wells of the positive control was pooled into one tube for the positive control group. Then a 10-fold serial dilution from 10^{-1} to 10^{-8}, was performed using PBS with antibiotics. Once the dilutions were complete, the dilution tubes sat for 60 minutes at room temperature to allow antibiotics enough time to kill possible bacterial contamination. Then 0.2 ml of the positive control virus dilutions were inoculated into 5 eggs per dilution. Eggs were incubated at 37.5° C and candled for five days. Mortality was recorded each day and on the fifth day, eggs were placed at 4° C overnight. Eggs not inoculated with virus were saved to use as negative controls.

Hemagglutination and Hemagglutination Inhibition Assays

To test for the presence of virus, chorioallantoic fluid (CAF) was collected from each egg and tested for hemagglutination activity (HA). CAF was collected using sterile needles and syringes, avoiding cross contamination between eggs. Then 50 µL of PBS was added to each well in 96-well plates followed by 50 µL of CAF added to the first well and diluted 1:2 across eight wells of the microtiter plate using a

multichannel pipet. Experimental groups were separated by a row of negative control CAF to use for comparison. After dilutions, 50 µL of 0.5% washed chicken red blood cells (RBC) was added to each well. Washed 0.5% RBC solution was achieved by mixing whole blood with 50% Alsever's solution (Sigma- Aldrich, St. Louis, MO) and centrifuging at 1,000 g for 10 minutes and re-suspending cells in PBS three times. The concentrated RBC were diluted with PBS to produce a 0.5% final dilution. The plate was covered and sat for 30-45 minutes for RBCs to agglutinate or settle. Once the negative control rows showed distinct buttons, the remainder of the plate was read and recorded. Cloudy wells with no distinct buttons were recorded as positive for viral agglutination. HA's were repeated for positive results to confirm virus presence. The data from HA results for each egg were used to find the mean EID_{50} for each test group and positive control using the Reed and Muench method (1938). Neutralizing indices (NI) for each test group were calculated by subtracting the mean EID_{50} of a test group (t_a) from the EID_{50} of the positive control (t_{pc}) for that trial. A disinfectant and coupon orientation test group passed the trial if the positive control titer was ≥ 0.4, the NI was ≥ 2.8, and no positive HAs were found.

$$NI = t_{pc} - t_a$$

Positive results from the HAs were tested for hemagglutination inhibition with NDV antisera to ensure that NDV virus was responsible for the positive HA results. On the day of use, the antisera was thawed and diluted with PBS to achieve a 1:5 dilution. All wells of the 96-well plates were filled with 25 µL of PBS. Then 25 µL of CAF was added to the first well and diluted 1:2 across eight wells of the microtiter plate using a multichannel pipet. Experimental groups were separated by a row of negative control CAF and positive control CAF to use for comparison. After dilutions, 25 µL of antisera was added to each well and sat for 30 minutes at room temperature to allow time for the antisera to interact with the virus. Then 0.5% washed chicken red blood cells (RBCs) were added to each well and allowed to agglutinate or settled for 30-45 minutes. A button forming at the bottom of the well was indicative of the NDV antisera interacting with the NDV preventing agglutination. This demonstrates

hemagglutination inhibition and was recorded as a positive result verifying the hemagglutination shown in the HA testing was a result of NDV virus growth. Negative controls also formed buttons due to lack of virus present.

RESULTS

To evaluate the efficacy and range of the spray rig, litmus paper strips were mounted onto a truck in various locations and a 0.5% diluted citric acid solution was used in the spray rig to interact with the litmus paper. Two experiments were conducted, focusing on different regions of the truck or coupon orientation with three trials per experiment. Experiment one included six locations per litmus paper orientation (horizontal, vertical, or complex). All locations passed the trial with obvious color change except location C6, which was located inverted above the rear wheel well (Table 2). To better understand the limitations of the spray rig, experiment two was conducted, which included ten litmus strip locations in three main regions of the truck: the body, undercarriage, and wheel well. In experiment two, all locations passed with obvious color changes in all three trials (Table 3). Based on these results, six coupon locations were chosen per coupon orientation (horizontal, vertical, or complex) and tested using a detergent and disinfectant.

Before the second phase of the experiment, the mean embryo infective dose (EID_{50}) per ml virus titer of the NDV seed stock was calculated using the Reed and Muench Method (1938). The resulting titer for the NDV seed stock was $10^{8.1}$ EID_{50} /ml. The decontamination system was tested with NDV inoculated coupons in three orientations on the truck undercarriage and body. Three trials were completed for both detergent and disinfectant. Fluid from the coupons was used to determine the titers for the disinfectant and detergent groups, positive controls, and detergent cytotoxic control. Only one egg died in the detergent cytotoxic control. This low mortality

indicates the detergent is safe for the embryos and did not influence the other test groups. Each trial had a valid positive control virus titer ($10^{4.2}$, $10^{4.7}$, 10^5 EID_{50} /ml) above the minimum limit of 10^4 EID_{50} /ml.

To compare among trials, the neutralizing index (NI) for each treatment group was calculated and compared (Appendix A). A trial was considered successful if no positive results in hemagglutination activity was observed, the neutralizing index was ≥ 2.8 and the positive control titer was $\geq 10^4$ EID_{50} /ml. These guidelines are based on the EPA's Product Performance Test Guidelines Disinfectants for Use on Hard Surfaces (2012). The neutralizing index accounts for the positive control in each trial, making it a useful tool to compare across various trials.

In trials using the detergent, coupons in the horizontal orientation passed the guidelines in trials A1, B1, and B2 (Figure 1). Trial 2, replication A in the horizontal orientation had a sufficient NI (> 4.0), but had positive HA results, therefore did not meet the guidelines. Trial 3, replication A failed due to a low NI value and positive HA results. Although trials HA2 and HA3 did not meet the guidelines, a drop in virus titer was observed in both cases. The disinfectant in the horizontal coupon orientation passed in trials and replications A1, B1, A2 and B3. Trials A2 and A3 had a NI above 2.8 (3.1, > 3.5 respectively), but had positive HA results, not meeting the EPA guidelines. In the vertical coupon orientation, no trials met the EPA guidelines for either the detergent or disinfectant. However, trials B1, A2, and B2 for the disinfectant had NIs ≥2.8 (2.8, > 3.8, 3.1 respectively), indicating a decrease in virus presence. Similarly, all trials for both the disinfectant and detergent in the complex coupon

orientation failed to meet the guidelines. Trials and replications A1 and B3 for the disinfectant had sufficient NIs (3.8, > 3.5 respectively), but had positive HA results.

The mean neutralizing indices for each experimental group were calculated. Paired student t tests were performed to compare the NIs between disinfectant and detergent, coupon orientation, and between trials. No statistical difference was found between trials. The horizontal coupon orientation was found to be significantly different from both the vertical and complex coupon orientations (Table 4). There was no difference between the vertical and complex coupon orientations. Additionally, the average NI of the chemical agents was significantly different (Table 5). The peroxygen agent had a higher NI than the detergent.

Test Strip ID	Trial 1	Trial 2	Trial 3
H1	✔	✔	✔
H2	✔	✔	✔
H3	✔	✔	✔
H4	✔	✔	✔
H5	✔	✔	✔
H6	✔	✔	✔
V1	✔	✔	✔
V2	✔	✔	✔
V3	✔	✔	✔
V4	✔	✔	✔

V5	✔	✔	✔
V6	✔	✔	✔
C1	✔	✔	✔
C2	✔	✔	✔
C3	✔	✔	✔
C4	✔	✔	✔
C5	✔	✔	✔
C6	~	~	~

Table 2. Litmus paper was used to evaluate the liquid distribution pattern from the vehicle disinfection system for Experiment 1. A checkmark (✔) indicates a color change was observed in the litmus paper at that location on the truck. The tilde (~) represents no color change in the litmus paper.

Body				
Location ID	Location Description	Trial 1	Trial 2	Trial 3
B1	In front of front wheel	✔	✔	✔
B2	Above front wheel	✔	✔	✔
B3	Behind front wheel	✔	✔	✔
B4	Above handle	✔	✔	✔
B5	Right of back door	✔	✔	✔
B6	Left of gas cap	✔	✔	✔
B7	Left of back wheel	✔	✔	✔

B8	Top of back wheel	✔	✔	✔
B9	Right of back wheel	✔	✔	✔
B10	Left of tail light	✔	✔	✔

Wheel Well				
Location ID	Location Description	Trial 1	Trial 2	Trial 3
W1	Left vertical	✔	✔	✔
W2	Left mid panel	✔	✔	✔
W3	Right mid panel	✔	✔	✔
W4	Right vertical	✔	✔	✔
W5	Left inverted	✔	✔	✔
W6	Right inverted	✔	✔	✔
W7	Midpoint	✔	✔	✔
W8 (V4)	Behind back wheel	✔	✔	✔
W9 (C6)	Above rear wheel inverted	✔	✔	✔
W10	Between W6 and W4	✔	✔	✔

Undercarriage				
Location ID	Location Description	Trial 1	Trial 2	Trial 3
U1	Vertical above bumper	✔	✔	✔
U2	Horizontal left of H5	✔	✔	✔
U3 (H5)	Back bumper	✔	✔	✔

U4 (H6)	Draw bar	✔	✔	✔
U5 (H1)	Top of A arm	✔	✔	✔
U6 (H2)	Transmission support	✔	✔	✔
U7 (H3)	Back wheel spring	✔	✔	✔
U8 (V3)	Front of back wheel reverse	✔	✔	✔
U9 (V5)	Behind back wheel (frame)	✔	✔	✔
U10 (C1)	Back of front bumper	✔	✔	✔
U11 (C2)	Back of brakes	✔	✔	✔
U12 (C3)	Above transmission inverted	✔	✔	✔
U13 (C4)	Inside frame driver door	✔	✔	✔
U14 (C5)	Between body + frame	✔	✔	✔

Table 3. Litmus paper was used to evaluate the liquid distribution pattern from the vehicle disinfection system for Experiment 2. A checkmark (✔) indicates a color change was observed in the litmus paper at that location on the truck. The tilde (~) represents no color change in the litmus paper.

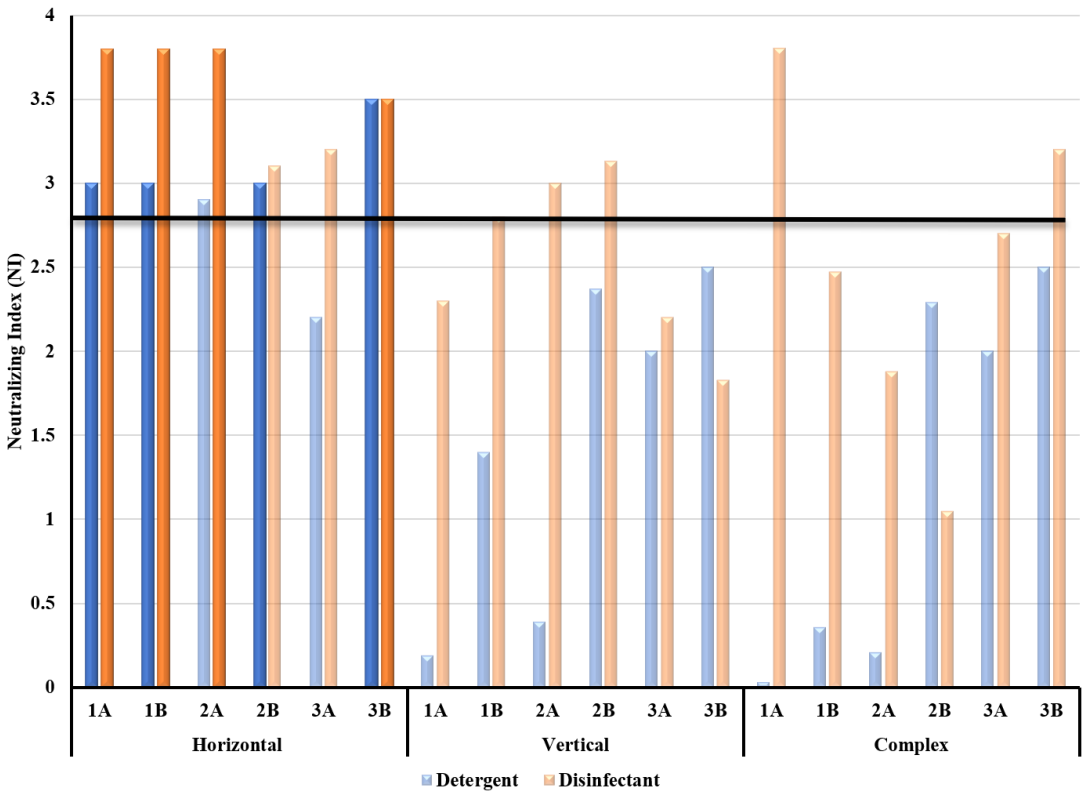

Figure 1. Graphical representation of the calculated neutralizing index ($NI = t_{pc} - t_a$) of the three coupon orientations. Each orientation included two replications (A and B) and two trials were performed (1 and 2). The line at 2.8 represents the minimum value for the neutralizing index to consider the disinfectant effective. Ghosted bars indicate a failed test.

Chemical Agent	Mean Neutralizing Index (NI)
Peroxygen	2.9[a]
Detergent	2.1[b]

Table 4. Mean neutralizing indices for the chemical agents across three trials. Different [abc]Superscripts indicate a significant difference.

Coupon Orientation	Mean Neutralizing Index (NI)
Horizontal	3.6[a]
Vertical	2.0[b]
Complex	1.9[b]

Table 5. The mean NI was calculated across the three trials. A paired student's t test was used to compare the NI by coupon orientation. Coupon orientations with different [abc]Superscripts are significantly different. The p value for the horizontal and complex t test was 0.0001. The p value for horizontal and vertical t test was 0.0002.

Chapter 3

DISCUSSION

The level of virus inactivation and neutralizing indices varied among the three coupon orientations (Appendix A). The only replications which passed the EPA guidelines for disinfectants were in the horizontal coupon orientation for both the detergent and disinfectant (detergent: HA1, HB1; disinfectant: HA1, HB1) although they also successfully passed single replications (detergent: HB2, HB3; disinfectant: HA2, HB3). Similarly, the horizontal coupon orientation was statistically different from the vertical and complex orientations, while vertical and complex were not significantly different (Table 5). The success of the horizontal orientation was most likely due to the ability of the detergent or disinfectant to lie on the surface of the coupon, increasing the contact time and therefore its efficacy. For both the detergent and disinfectant, no trials passed the EPA guidelines in the vertical or complex coupon orientations. In the vertical orientation, lack of virus inactivation could be attributed to decreased contact time from the disinfectant dripping off the coupon. Complex coupon orientations included coupons which were inverted or out of direct line of the sprayers. Some of these coupons are not directly sprayed due to blockage from parts of the truck. All coupon locations were tested initially with litmus paper strips and all locations were adequately covered by the sprayers (Table 2 and 3). However, the complex locations may not be directly sprayed and could be covered with disinfectant indirectly through run off. Litmus paper is more sensitive to exposure than virus samples and treatment run off would be sufficient to generate a color change.

Additionally, inverted coupons under the complex category may suffer from decreased contact times as disinfectant drips off due to gravity.

Differences in the performance of the disinfectant versus detergent were observed as hypothesized. Overall, the peroxygen agent had consistently higher neutralizing indices in all coupon orientations when compared to the detergent and was significantly different. Disinfectants are normally intended to be used as part of a cleaning and disinfection process rather than being used independently. The use of a detergent along with removal of organic material through cleaning has shown to reduce the pathogen load by 80%, whereas using a disinfectant decreases it by 99% (Canadian, 2018). Despite these results, a producer could potentially favor using a detergent over a disinfectant because of the corrosive nature of some disinfectants over time to metal surfaces.

Although only eight repetitions in the horizontal coupon orientation passed the EPA guidelines, neutralizing indices greater than 2.8 in the other repetitions are of importance. Any decrease in the virus titer can reduce the risk of spreading NDV or other diseases of interest to the poultry industry, even if complete inactivation is not accomplished. In the vertical coupon orientation, three repetitions using the disinfectant agent had neutralizing indices above the EPA guidelines (B1, A2, B2). In the complex coupon orientation, two repetitions using the disinfectant had a NI above 2.8 (A1, B3). Although complete inactivation of virus was not achieved, the decontamination system is still a powerful tool that producers can use to limit the spread of virus on and off the farm. By lowering the titer of the virus and inactivating

it in some areas, the decontamination system decreases the chance of virus spreading between farms through vehicle contamination. This allows producers to increase the biosecurity on their farm, while keeping the larger community safe from contagious viruses.

Sources of error during the three trial days may have impacted the efficacy of the decontamination system. In order to access the metal coupons on the undercarriage of the truck, the truck was lifted on to ramps. This occurred immediately after moving through the sprayer system and was included in the ten-minute contact time. The jerking motion of driving the truck onto the ramps may have removed some of the sprayed disinfectant, therefore decreasing the amount of disinfectant and the contact time. This problem was alleviated in following trials by allowing the truck to sit for at least eight minutes before moving it onto the ramps and collecting the coupons at the ten-minute mark.

Another factor which varied among trials was the daily temperature outdoors. The experimental phase of this project occurred over the summer, with highs reaching 34° C (94° F) on one of the trial days. Heat can affect both the virus and disinfectants in opposing ways. It has been shown that NDV can be inactivated through heat. However, based on current literature, temperatures would have to reach 56° C (133° F) for three hours or 60° C (140° F) for 30 minutes to accomplish complete inactivation of the virus (Spickler, 2016). Additionally, high temperatures can affect the effectiveness of disinfectants. High temperatures lead to increased evaporation of

the disinfectant, resulting in a lowered contact time (Dvorak, 2008). The capacity in which heat affected the virus or disinfectants among the three trial days is unknown.

When disinfecting equipment and vehicles a major area of concern is the organic load. The organic load is any soil, manure or other organic material that may be present on the item being disinfected. The organic load can decrease the effectiveness of some disinfectants by acting as a barrier to pathogens contained in the organic material (Dvorack, 2008). In addition, the organic material can create a chemical reaction with the disinfectant, decreasing its sterilizing properties (Centers of Disease, 2016). Organic load is a variable difficult to measure, especially in the experimental design of the present study. The truck used was consistent among the three trial days, however, the exact amount of organic load on the truck each day is unknown, but visually did not appear to change. Additionally, the truck was not thoroughly cleaned before each trial in order to emulate field conditions. The coupons were free of organic material when adhered to the truck, but the liquid disinfectants caused organic material to run off and interact with some of the coupons. This interaction of organic material and the disinfectants may have decreased their efficacy.

Improvements to the undercarriage decontamination station can be made to increase its efficacy. The limitations of the decontamination system lie in its ability to target the vertical and complex coupon orientations. To remedy this problem, the detergent or disinfectant could be applied via foam instead of in the liquid state. Foams possess the ability to adhere to surfaces more effectively and for longer durations as compared to their liquid counter parts (Keijzer, n.d). This quality could

potentially increase the contact time of the disinfectant on the vertical and complex surfaces. Adaptions to the decontamination system may be needed to accommodate the foam in future studies. Another improvement to the system would be to paint the water tank black and allow the water to heat naturally in the sun. As previously discussed, NDV can be neutralized by heat. However, water temperatures would have to be monitored to prevent any negative interactions with the disinfectants. Lastly, other classes of disinfectants can be tested with the decontamination system. For instance, the efficacy of phenols or iodophors has not been tested with the decontamination system.

Chapter 4

CONCLUSION

Transportation vehicles pose a major risk to spreading contagious diseases between farms, putting producers and the larger community at jeopardy. The objective of the current study was to evaluate the efficacy of a portable, and low-cost decontamination system at inactivating Newcastle disease virus. Galvanized steel coupons inoculated with NDV were adhered onto a truck, passed through the sprayer, and treated with a detergent or peroxygen agent. Three coupon orientations were evaluated: horizontal, vertical, and complex. On average, the horizontal coupon orientation had significantly higher neutralizing indices as compared to the vertical and complex orientations. Additionally, the peroxygen agent had a significantly higher neutralizing index than the detergent. The detergent and disinfectant both passed the EPA's Product Performance Test Guidelines Disinfectants for Use on Hard Surfaces in the horizontal coupon orientation for one trial. The vertical and complex coupon orientations did not pass the guidelines but decreases in NDV titers were observed.

Although complete inactivation of NDV was not achieved, the decontamination system remains an important tool to decrease viral titer load on vehicles and can decrease the risk of spread between farms. Limitations of the experiment include the presence of an organic load, high outdoor temperatures, and movement of the truck during disinfectant contact time. In the future, the decontamination system will be adapted to use foam to apply the disinfectants in efforts to increase the contact time and effectiveness.

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Appendix A

VIRUS RECOVERY AND NEUTRALIZING INDICIES

Disinfectant Agent	Trial Number/ Replication	Coupon Orientation	Virus Recovered from Coupons	Positive Control Log_{10} Titer	Final Log_{10} Titer After Disinfection	Neutralizing Index (NI)
Detergent (Simple Green)						
	1A	Horizontal	No	4.2	< 1.2	> 3.0
	1B	Horizontal	No	4.2	< 1.2	> 3.0
	1A	Vertical	Yes	4.2	4.01	0.19
	1B	Vertical	Yes	4.2	2.8	1.4
	1A	Complex	Yes	4.2	4.17	0.03
	1B	Complex	Yes	4.2	3.84	0.36
Detergent (Simple Green)						
	2A	Horizontal	Yes	4.2	1.3	2.9
	2B	Horizontal	No	4.2	< 1.2	> 3.0
	2A	Vertical	Yes	4.2	3.81	0.39
	2B	Vertical	Yes	4.2	1.83	2.37
	2A	Complex	Yes	4.2	3.99	0.21
	2B	Complex	Yes	4.2	1.91	2.29
Detergent (Simple Green)						
	3A	Horizontal	Yes	4.7	2.5	2.2
	3B	Horizontal	No	4.7	< 1.2	> 3.5
	3A	Vertical	Yes	4.7	2.7	2
	3B	Vertical	Yes	4.7	2.2	2.5
	3A	Complex	Yes	4.7	2.7	2
	3B	Complex	Yes	4.7	2.2	2.5

Table 6. Viral recovery from hemagglutination assays, viral titers, and neutralizing indices based on coupon orientation for the detergent.

Disinfectant Agent	Trial Number/ Replication	Coupon Orientation	Virus Recovered from Coupons	Positive Control Log_{10} Titer	Final Log_{10} Titer After Disinfection	Neutralizing Index (NI)
Peroxygen Agent (VirkonS)						
	1A	Horizontal	No	5	< 1.2	> 3.8
	1B	Horizontal	No	5	< 1.2	> 3.8
	1A	Vertical	Yes	5	2.7	2.3
	1B	Vertical	Yes	5	2.2	2.8
	1A	Complex	Yes	5	1.2	3.8
	1B	Complex	Yes	5	2.53	2.47
Peroxygen Agent (VirkonS)						
	2A	Horizontal	No	5	< 1.2	> 3.8
	2B	Horizontal	Yes	5	1.9	3.1
	2A	Vertical	Yes	5	2	3
	2B	Vertical	Yes	5	1.87	3.13
	2A	Complex	Yes	5	3.12	1.88
	2B	Complex	Yes	5	3.95	1.05
Peroxygen Agent (VirkonS)						
	3A	Horizontal	Yes	4.7	1.5	3.2
	3B	Horizontal	No	4.7	< 1.2	> 3.5
	3A	Vertical	Yes	4.7	2.5	2.2
	3B	Vertical	Yes	4.7	2.87	1.83
	3A	Complex	Yes	4.7	2	2.7
	3B	Complex	Yes	4.7	1.5	3.2

Table 7. Viral recovery from hemagglutination assays, viral titers, and neutralizing indices based on coupon orientation for the disinfectant.