A STUDY ON THE CHEMICAL AND PHYSICAL INACTIVATION OF AVIAN BORNAVIRUS

An Undergraduate Research Scholars Thesis

by

JENNA BREEANNE KRANZ

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Research Advisor: Dr. Jeffrey MB Musser

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ABSTRACT

A study on the chemical and physical inactivation of Avian bornavirus. (May 2014)

Jenna Breeanne Kranz
College of Veterinary Medicine & Biomedical Sciences
Texas A&M University

Research Advisor: Dr. Jeffrey MB Musser
Department of Veterinary Pathobiology

Avian bornavirus (ABV) is the etiological agent of Proventricular Dilatation Disease, a fatal neurological disease of pet and aviary kept Psittacine birds (parrots). ABV is believed to be transmitted through the fecal-oral route. Viral susceptibility to environmental factors, such as pH and drying, and to disinfectants is unknown. Knowledge of this susceptibility is needed by bird owners and aviculturists to assist in reducing environmental contamination and transmission of the virus. In this study, the effectiveness of three commonly used disinfectants, BacDown® Detergent Disinfectant, F10SC Veterinary Disinfectant, and Virkon® S, and the survivability of ABV at differing pH environments and to drying was evaluated.

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CHAPTER I

INTRODUCTION

Avian bornavirus (ABV) is the etiological agent of Proventricular Dilatation Disease, a fatal neurological disease in pet and aviary Psittacine birds (parrots).[1] ABV is believed to be transmitted through the fecal oral route.[2] Research on the inactivation of the virus by disinfectants, pH, and drying is lacking, even though commercial disinfectant products are used in the disinfection and cleaning of cages, homes, and aviaries as a means to reduce viral load so as to prevent the spread of the virus. Improper use of disinfectants, lack of footbaths and hand washing, and inadequate disinfection procedures increases the spread of a similar avian virus, Newcastle disease virus, in poultry production facilities;[3] Newcastle disease virus and ABV are both enveloped, negative-sense, single-stranded RNA viruses in the Order *Mononegavirales*, which suggests that they would react similarly to disinfectants and disinfection procedures.[2] The effect of differing acidic and alkaline environments on the mammalian Borna Disease Virus shows that the virus was resistant to solutions with pH ranging from 5 to 12.[4]

For this study, three commercially available disinfectants recommended by the avian veterinarians at Texas A&M University were examined. BacDown® (Decon Labs, Inc., Pennsylvania) is a quaternary ammonium compound already in use at the Schubot Aviary, College of Veterinary Medicine, TAMU. F10SC Veterinary Disinfectant (PTY, LTD, South Africa) is a combination of a quaternary ammonium compound and a biguanide that is in wide spread use in commercial aviaries. Virkon® S (Antec International LTD, England) is an oxidizing agent recommended by USDA against viral foreign animal diseases. Identification of a

disinfectant and contact time that effectively inactivates ABV will provide the scientific data with which to establish disinfection protocol recommendations so as to decrease the spread of ABV and help reduce the incidence of Proventricular Dilatation Disease.

CHAPTER II

METHODS

Growing clean duck embryo fibroblasts (DEFs)

Aliquots of clean DEFs were quick thawed at 37°C for 3 minutes, added to a 50mL conical tube along with DMEM (Gibco; Grand Island, NY) media, and centrifuged 10 minutes 1500xG (3000 RPM) at 4°C. The supernatant was removed and DMEM FBS 10% was added to the pellet and mixed. The cell/media mixture was then aliquoted as follows: 15mL cell/media mixture in each of 2 T-75 flasks (~2 vials/flask) and 7.5mL cell/media in each of 2 T-75 flasks (~1 vial/flask). 7.5mL of DMEM 10% was also added to each of the 2 flasks containing 7.5mL cell/media mixture. All 4 flasks were incubated at 37°C in 5% CO_2.

Cytotoxicity of disinfectants

DEF cells were grown in a 75cc flask as previously described. When approximately 80 to 90% confluent, the media was removed and the cells were washed with 3.0mL of 0.25% trypsin-EDTA (Sigma-Aldrich) which is then discarded. Two mL of 0.25% trypsin-EDTA was again added and the cells are incubated for 2 minutes. The cells were examined every 30 seconds thereafter, and when the cells were detached, 13mL of the 10% bovine calf serum DMEM was added. From this, 6mL was added to 34mL of 10% bovine calf serum DMEM. From this solution, 0.2mL was added to each well of a 96 well microplate (BD Falcon™, BD) excluding one row of 8 wells to be used as control blanks; 0.2mL was added to a second 96 well microplate. The DEF cells were incubated at 37°C in an atmosphere of 5% CO_2 for 24 hours.

To examine the effects of the disinfectants, the media in each row of wells was then replaced with 0.15mL of either 0, 2, 10, or 20 fold dilution of labeled strength disinfectant with 1:1 saline/media, 10% bovine calf serum DMEM. The cells were incubated at 37°C in an atmosphere of 5% CO_2 for a further 120 hours.

After 120 hours of incubation, the plate was sent off for quantitative reverse transcriptase polymerase chain reaction (qRT-PCR testing) to determine how the disinfectants affected the cells.

Validation of disinfectant neutralization

Viral levels at 10^{-1} to 10^{-4} dilutions

For viral levels at 10^{-1} to 10^{-4} dilutions, three 0.2mL vials of ABV infected DEF cells were quick thawed. 0.1mL of the virus solution was added to 0.9mL DMEM 2%, creating a 10^{-1} solution. One well was infected with 0.5mL of this virus/media. Then 0.1mL of the 10^{-1} solution was added to 0.9mL DMEM 2%, creating a 10^{-2} solution. 0.5mL of this virus/media was used to infect another well. This serial dilution was continued until a 10^{-4} dilution was reached. Three independent replicates using 3 individual aliquots of virus were created.

Disinfection neutralization- using two different amounts of FBS (methods A & B)

Method A: 0.2mL of saline was added to 1.8mL disinfectant. 0.2mL of this solution was added to 1.8mL FBS then vortexed and held for 5 minutes. 0.2mL disinfectant/FBS was added to 1.8mL DMEM 10% then vortexed and held for 5 minutes. 0.02mL of virus (10^{-1} concentrated

virus solution from previous section) was added to the solution then vortexed and held for 10 minutes. Two wells were each infected with 0.5mL of disinfectant/FBS/DMEM solution.

Method B: 0.2mL of saline was added to 1.8mL disinfectant. 0.2mL of this solution was added to 3.8mL FBS then vortexed and held for 5 minutes. 0.2mL disinfectant/FBS was added to 1.8mL DMEM 10% then vortexed and held for 5 minutes. 0.02mL of virus (10^{-1} concentrated virus solution from previous section) was added to the solution then vortexed and held for 10 minutes. Two wells were each infected with 0.5mL of disinfectant/FBS/DMEM solution.

Infecting 24 well plate

0.5mL virus/disinfectant/media solution was added to each well then incubated and rotated every 15 minutes for 4 rotations. The media was removed and 1.0mL of fresh media 2% was added to each well. The plate was incubated for 4 days and then frozen at -80ºC then submitted for PCR analysis.

Acidic and alkaline environments

To determine the survivability of ABV in different pH environments, solutions of pH values 2-11 were created using PBS, hydrochloric acid, and sodium hydroxide. Each pH solution was then microfiltered using a 0.2µm filter and syringe to prevent any contamination. For each pH value, 0.5mL of ABV was added to the pH saline solution. After 5 minutes, 0.35mL of virus/pH saline was added to 3.15mL DMEM with 5% FBS. 1.0mL of each of the 12 solutions was plated on 24 well plates, incubated at 37°C for 11 days, and then sent off for qRT-PCR testing to determine

effects of pH on the virus. After 3 days of incubation, the media was changed to DMEM with 2%FBS.

Viral survivability with drying

Scotch Brite pads

Very fine aluminum silicate abrasive mineral adhered to woven synthetic fiber was used. The pads were autoclaved in a beaker before use.

Drying method

A 5.0mL aliquot of ABV-4 was quick thawed then added to 10mL PBS and mixed. 0.3mL of the virus/PBS mixture was placed onto each of 21 pads split into 3 large petri dishes and covered with the lids about halfway. The time zero pads were immediately resuspended in 5mL of DMEM FBS 5% media in 3 separate 15mL conical tubes. The remaining pads were placed in an incubator at 26°C and 21% humidity. The pads were removed at various time points and each time a set of 3 pads was placed in 3 more 15mL conical tubes with 15mL DMEM FBS 5% media. Once the pads were resuspended in media, the tubes were placed in a -80°C freezer.

Growing virus

After freezing, the pads in solution were thawed out. The media/virus mixture was then aliquoted out onto a 24 well plate that was growing DEF cells. The plate was then incubated.

Analyzing viral growth

In order to see how drying time affected the virus, the 24 well plate was sent off for qRT-PCR) testing. Each time period was plated twice, graphed twice, and done in triplicate by qRT-PCR.

CHAPTER III

RESULTS

Disinfectants

In our attempt to inactivate ABV using four different disinfectants, a few approaches were taken to try and find the best way to kill the virus but not the cells. The first attempt involved the dilution of the disinfectants, however the presence of the disinfectants was too strong and resulted in death of the DEF cell in culture.. Next, we tried adding a protein to protect the cells. Initially, FBS was used and the process was successful. However, FBS was not a cost-effective protein to use to complete our project. With the knowledge that a protein will indeed work however, we have begun to look at using milk proteins from powdered milk.

pH

With our pH experiment, we continually experienced fungal and bacterial growth in our cell plates. We eventually cited our pH probe to be the issue. Unlike the rest of our procedures that took place in the hood, our pH probe was used on the lab bench. This probe was used to measure each of our solutions from pH 2-11, however was found to be transmitting the contaminants to our solutions which were then put on our cells. We have since microfiltered the pH solutions before placing them on the cells, and currently have good growth in those plates with no contamination.

Drying

A standard curve for the correlation of P gene mRNA and amount of infectious ABV was generated by serial 10-fold dilutions of the initial viral inoculum (Fig 1). Aliquots of virus that were subjected to drying showed a decrease in infectious virus; after 8 and 24 hours of drying the amount of infectious virus had decreased by 48 and 86%, respectively (Fig 1).

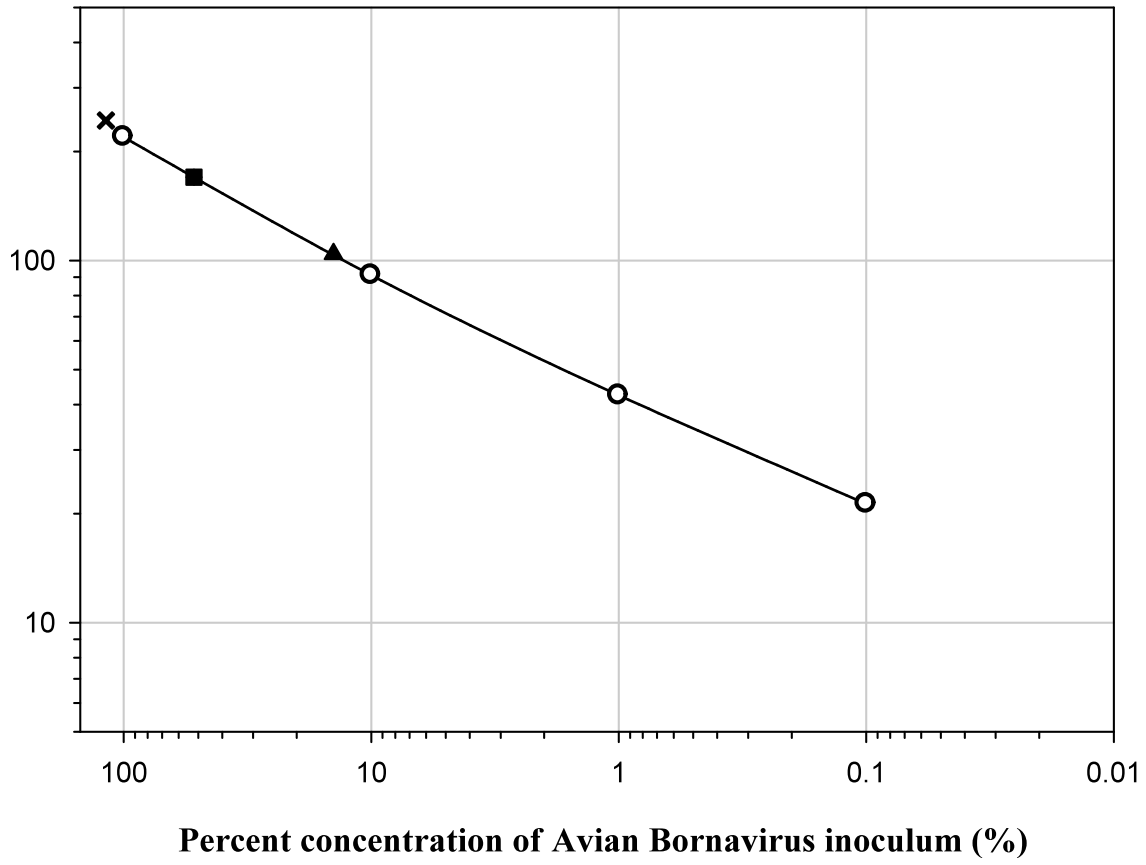

Figure 1. Standard curve for inoculum dilutions tested at 100, 10, 1, and 0.1% infectious virus present (solid line and open circles) and the virus aliquots after drying for 0 hours (**X**), 8 hours (■), and 24 hours (▲). Each data point represents duplicate samples tested in triplicate.

CHAPTER IV

CONCLUSIONS

Through this study, we were able to gain important information on how drying affects ABV. This is useful information when applied to a large aviary setting with a high volume of birds. In order to prevent spread of the fecal-oral transmitted virus, bird caretakers and handlers need to know as much as possible about how different environments affect its survivability. With drying, we were able to see that the longer the virus was allowed to dry, the greater the decrease in viral load. This information is useful for knowing how long to allow a cage or room to remain vacant and clean before reintroducing birds. Our continuing research on the effects of different disinfectants and varying pH values will also aid in determining the presence of virus in aviaries and bird homes. All of this information can then be used to take the best possible approach when cleaning aviaries in order to drastically reduced the spread of ABV.

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