Evaluation of a Revised Protocol for Stall Terminations in the Large Animal Hospital

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ABSTRACT

Cleaning and disinfection are critical areas of Veterinary hospital biosecurity. Without a validated biosecurity program, veterinary hospitals are in a vulnerable state. Potential outbreaks of pathogenic microorganisms could occur causing loss of: patient lives, hospital revenue and hospital prestige. Instances of pathogenic outbreaks have been recorded in small animal settings (i.e. veterinary clinics and shelters) and in large animal settings (i.e. farms and hospitals.) Veterinary hospitals, often considered the gold standard of veterinary care are not immune to biosecurity breaches. In last 5 years 82% of veterinary teaching hospitals reported nosocomial infections (Anderson, 2010). The aim of this project was to validate cleaning and sanitation procedures that are already in place in the Harry T. Peters, Jr. Large Animal Hospital located in Blacksburg, VA. This project compared two sanitation protocols and validated them using environmental sampling. Using chi-square analysis results indicated there was no significant difference between the sanitation methods.

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I. Introduction

Cleaning and disinfection are critical areas of Veterinary hospital biosecurity. Without a validated biosecurity program, veterinary hospitals are in a vulnerable state. Potential outbreaks of pathogenic microorganisms could occur causing loss of: patient lives, hospital revenue and hospital prestige. Instances of pathogenic outbreaks have been recorded in small animal settings (i.e. veterinary clinics and shelters) and in large animal settings (i.e. farms and hospitals.) Veterinary hospitals, often considered the gold standard of veterinary care are not immune to biosecurity breaches. In last 5 years 82% of veterinary teaching hospitals reported nosocomial infections (Anderson, 2010). The aim of this project is to validate cleaning and sanitation procedures that are already in place in the Harry T. Peters, Jr. Large Animal Hospital located in Blacksburg, VA. This will project compare two sanitation protocols and will validate these procedures using environmental sampling techniques.

a. Statement of Problem

The Biosecurity Plan at the Veterinary Teaching Hospital includes a cleaning/sanitation section that provides a description of products and directions for proper sanitation. However, disinfection and sanitation chemical products are often selected and used without validation. Data is needed to justify why specific sanitation products and procedures are in place (CDC,2008). Environmental sampling techniques can be used to validate whether current protocols are effective or need improvement.

b. Purpose of Study

The primary goal of this project is to validate cleaning procedures (which use ZEP products) in the Large Animal Hospital. Secondary goals are; 1) determining potential cost savings by implementing new products, 2) decreasing potential harmful chemical products into the waste stream and 3) create a product validation operating procedure.

c. Hypothesis

H_1: Using Simple Green (an eco-friendly general purpose cleaner) as the 1st step in the stall termination process will be just as effective as using the Zep 4089(bleach based foaming detergent) twice.

H_O: Using Simple Green (an eco-friendly general purpose cleaner) as the 1st step in the stall termination process will less effective than using the Zep 4089(bleach based foaming detergent) twice.

ll. Background

About Biosecurity Plans

A biosecurity plan is a detailed manual that describes safe, effective procedures for carrying out various processes including spill response, decontamination, and patient intake procedures etc. The purpose of the plan is to mitigate zoonotic risks as well as infection control. These plans are essential tools that every animal shelter, veterinary hospital, and farm should have. They need to be reviewed and updated yearly by trained veterinarians or staff. Every setting (e.g. farm or hospital) is different and presents its own set of unique risks. While there is not a one

size fits all biosecurity plan, all plans will cover the same topics and will carry out the process differently.

The National Association of State Public Health Veterinarians "NASPHV" and the Veterinary Control Committee recommends including the following topics in the biosecurity manual: 1) Cleaning and Disinfection of Equipment and Surfaces, 2) Isolation of Animals with Infectious disease, 3) Laundering, 4) Spill Response and Decontamination, 5) Medical Waste and 6) Vector Control(Williams, Scheftel, Elchos, Hopkins, & Levine, 2015). These topics are further described below:

1) Cleaning and Disinfection of Equipment and Surfaces

Arguably, cleaning and disinfection are one of the most important parts of a biosecurity plan (Boyce, 2016). Manual disinfection is the most common way of disinfecting surfaces; unfortunately this method is not always effective (Boyce, 2016). To safeguard staff, clients and patients from zoonotic infectious diseases you must determine the best product for cleaning and disinfecting all surfaces and equipment. Factors to consider when selecting a product include surface material, organisms present and contact time required (Rutala & Weber, 2014). For example, specific chemicals such bleach, i.e. sodium hypochlorite will corrode steel surfaces; so this may not be the best option for cleaning metal kennels.

Another factor to consider would be safety, you want to ensure staff can use the product daily with little health risks and it that it does not present severe aquatic toxicity. Standard cleaning techniques should include removing organic material,

“scrubbing” the surface rinsing and applying disinfectant (Steneroden, Metre, Jackson Morely, 2010). During application of disinfectant, contact time must be observed to ensure microorganisms are inactivated.

Finally, something the NASPHV did not mention is the importance of validation. Implementing sanitation procedures are a good step but, to ensure that these processes work they must be validated through environmental sampling (Dvorak, 2008). While not often mentioned in veterinary medicine other fields such as food safety use this technique has part of their preventative hazard control plan. Both the VA-MD College of Veterinary Medicine and University of South Florida use contact plates to validate cleanliness of their rodent holding rooms. By monitoring and using surveillance techniques outbreak can be recognized earlier (Steneroden, 2010).

2) Isolation of Animal with Infectious disease

Patients that are potentially or confirmed as sick with an infectious disease need to be kept separate from other animals. Isolation rooms or stalls need to be available for these patients. When in doubt patients should be treated as “guilty until proven innocent" in regards to health status (Donskey, 2013). PPE such as gowns, gloves etc. need to be worn and disposed of properly. Decontamination and sanitation need to occur regularly and in between every isolation patient.

3) Laundering

Laundry such as scrubs, lab coats, towels etc. should be washed whenever a biological spill occurs. These items need to separated, sorted and washed with standard laundry

detergent with very hot water. Another option is to use a commercial laundry service provider.

4) Spill Response and Decontamination

When biological spills occur they need to be contained and then surfaces must be cleaned and disinfected using a proper disinfectant. Contact time for the product on the surface is essential to ensure the surface is no longer contaminated.

5) Medical Waste

Materials that were used or generated during surgeries, office visits or research must be disposed of as medical waste. Sharps must be placed in biohazard sharp containers and disposed of according to State law. All staff should be trained on the "waste stream" to insure no medical waste ends up in the regular trash.

6) Vector Control

Some zoonotic diseases are transmitted by vectors (i.e. rodents, arthropods) which carry the disease and have the potential to infect other animals or humans. Veterinary hospitals that include field services are especially prone to carry vectors from field to hospital and encounter increased exposure. Protective clothing to reduce bug bites such as ticks should be worn, additionally facilities should take measures to keep their spaces tidy and entry doors/barn doors sealed.

Disinfection Product History and Importance in Veterinary Hospitals

Product selection will play a vital role in developing sanitation procedures. As mentioned in the previous section factors such surface material, safety of use, contact time, kill claims, cost and microorganisms present will be considered when selecting a product (Rutala & Weber, 2014). Historically, products such as Hi-Tor(EcoLab, St.Paul, Minnesota) and Roccal-D(Zoetis, Florham Park, New Jersey) were used as one-step products for disinfecting surfaces in the veterinary field. These name brand products are composed of quaternary ammonium compounds, more commonly referred to as "QACs". While QACs have been used in the veterinary field as well as, food prep and hospitals for many years as a broad spectrum disinfectant, new data shows that QACs should not be used in veterinary medicine due to aquatic toxicity, antibiotic resistance and relative ineffectiveness in the presence of organic material (Addie et al., 2015;Zhang et al., 2015). Recent studies indicate that due to the widespread use of QACs there is a potential for adverse health effects for both humans and animals (Melin, 2016). Many hospitals are moving away from these products and looking towards accelerated hydrogen peroxide products as their go-to chemical disinfectant. Diluted hydrogen peroxide cleaners are similar to QACs as it is a bactiericidal, virucidal, fungicidal; however it is considered more "eco-friendly", and there is less contact time required (Schmidt, Gaikowski, & Gingerich, 2006) (Rutala, Gergen & Weber, 2012). For example, RESCUE is a new brand name of hydrogen peroxide disinfectant that require lower contact times but, very similar disinfectant claims to the older QAC products(Rutala, Gergen & Weber, 2012). An important step in remember is no matter what product you are using the disinfectant will be more effective when it is sprayed on a "clean surface". Organic materials such as hay, fecal material etc. can make cleaners less effective (CDC, 2016). Thus the

idea of a one-step disinfectant is highly inappropriate and goes against the general guidelines of disinfecting, which recommend a cleaning step first followed by the use of a disinfectant product (CDC, 2008; Dvorak, 2008).

In 2017, Virox, in collaboration with AAHA, published an Infection Control and Biosecurity in Veterinary Medicine booklet where they listed the most common mistakes in biosecurity plans 9 listed had to do with disinfection protocols. This is quite concerning and even more proof of why it is so critical to ensure products are being used effectively and properly (Donskey, 2013; NASPHV, 2015).

The pilot study described below would be an excellent reference tool to use when designing and verifying sanitation procedures and products are being used effectively.

lll. Research Design and Methods

a. Random Assignment

For this pilot study, a total 50 stalls were cleaned and disinfected using two different cleaning procedures. There are 34 stalls in the Large Animal Hospital barns. Stalls are divided between barns A, B and C. Stalls that were used in this study were based on client use thus, not all 34 stalls were sampled. Certain stalls were used multiple times and thus sampled several times. Other stalls were not used and thus not sampled. Using a coin flip cleaning methods 1 and 2 were randomly assigned; heads was even and tails was odd. Using a random number generator we created a number table from 1-50. All even numbers were assigned method 1 and all odd numbers were assigned method 2.

b. Design

The study was designed to validate the current cleaning method, which is referred to as Cleaning Method 1 (CM-1), and to validate a proposed cleaning method referred to as Cleaning Method 2 (CM-2). Both cleaning methods involve several steps that include: 1) removal of shavings, 2) rinse, 3) cleaning product application, 4) rinse, 5) disinfectant application and 6) a final rinse (Table 1). In the current protocol, Zep 4089 used for step 3 and 5. Zep 4089(Zep Inc, Emerson, GA) is corrosive as it contains bleach-like products, detergents and surfactants (Zep SDS, 2017). While CM-2 is similar, the use of an environmental friendly cleaner i.e. Simple Green is being substituted for step 3. Simple Green (Sunshine Makers Inc, Huntington, CA) non-toxic, biodegradable, non-corrosive and has the ability to cut through grease, grime and heavily soiled items (Simple Green SDS, 2018). Simple Green and Zep 4089 ingredients are found in Table 2.

The products were applied using a "foamer" which hold 64 ounces of product. Dilutions are set using the nozzle. For the Simple Green and ZEP 4089 we used a dilution that would distribute 4oz per gallon.

To ensure stalls were cleaned appropriately a chart was created and placed in the large animal husbandry area (Appendix D). This chart was referenced each time a husbandry technician cleaned a stall.

Table 1. Description of cleaning methods.

Cleaning Method 1(CM-1) Current	Cleaning Method 2 (CM-2) Proposed
Remove Shavings	Remove Shavings
Rinse	Rinse
Application of 4089 (10 Min)	Simple Green
Rinse	Rinse
Application of 4089 (10 Min)	Application of 4089 (10 Min)
Rinse	Rinse

Table 2. Cleaning product ingredients.

Zep 4089	Simple Green
Heavy Duty, High Foaming, Chlorinated Detergent	Powerful cleaner and degreaser designed for effective and environmentally safer use
POTASSIUM HYDROXIDE; caustic potash; lye	Water
SODIUM HYDROXIDE; caustic soda; soda lye	C9-11 Alcohols Ethoxylated, Sodium Citrate, Sodium Carbonate
SODIUM HYPOCHLORITE; hypochlorous acid, sodium salt; bleach	Tetrasodium Glutamate Diacetate, Citric Acid, etc.

c. Sampling

Contact sampling proved as an effective means of sampling in similar studies (McMillan, 2004; Hogan et.al, 2015). Thus, for this project I conducted environmental sampling using BD Neutralizing Agar contact plates also called RODAC plates(which stands for Replicate Organism

Detection And Counting). The plate is made with typical growth medium (peptone, yeast, dextrose) and five neutralizers (sodium bisulfate, sodium thioglycollate, sodium thiosulfate, lecithin, polysorbate 80) which will inactivate any antimicrobial residues left behind by chemical cleaners when the plate makes contact with the surface (Becton, Dickson and Company, 2009). Use of this type of plate allowed me to see the "bactericidal activity" on the surface and ensure the cleaning products are actually working effectively (BD,2009). One plate was used in each of three areas of the horse stalls: A) back of stall door, B) near the drain and C) back inside perimeter of stall (images and schematic in Appendix A and Appendix B). Sampling occurred after extermination of a stall (discharge of patient). Daily stall extermination occurs between 4:00pm and 9:00pm. Once the stall was cleaned a, "do not enter" sign was hung on the stall door. We (Kira and I) plated the following morning at 7am. Plates were incubated at 37 degrees Celsius and checked for growth at 24hrs and 48hrs. Data was recorded in a data collection log then transferred to an excel sheet and Prism for analysis (Appendix E).

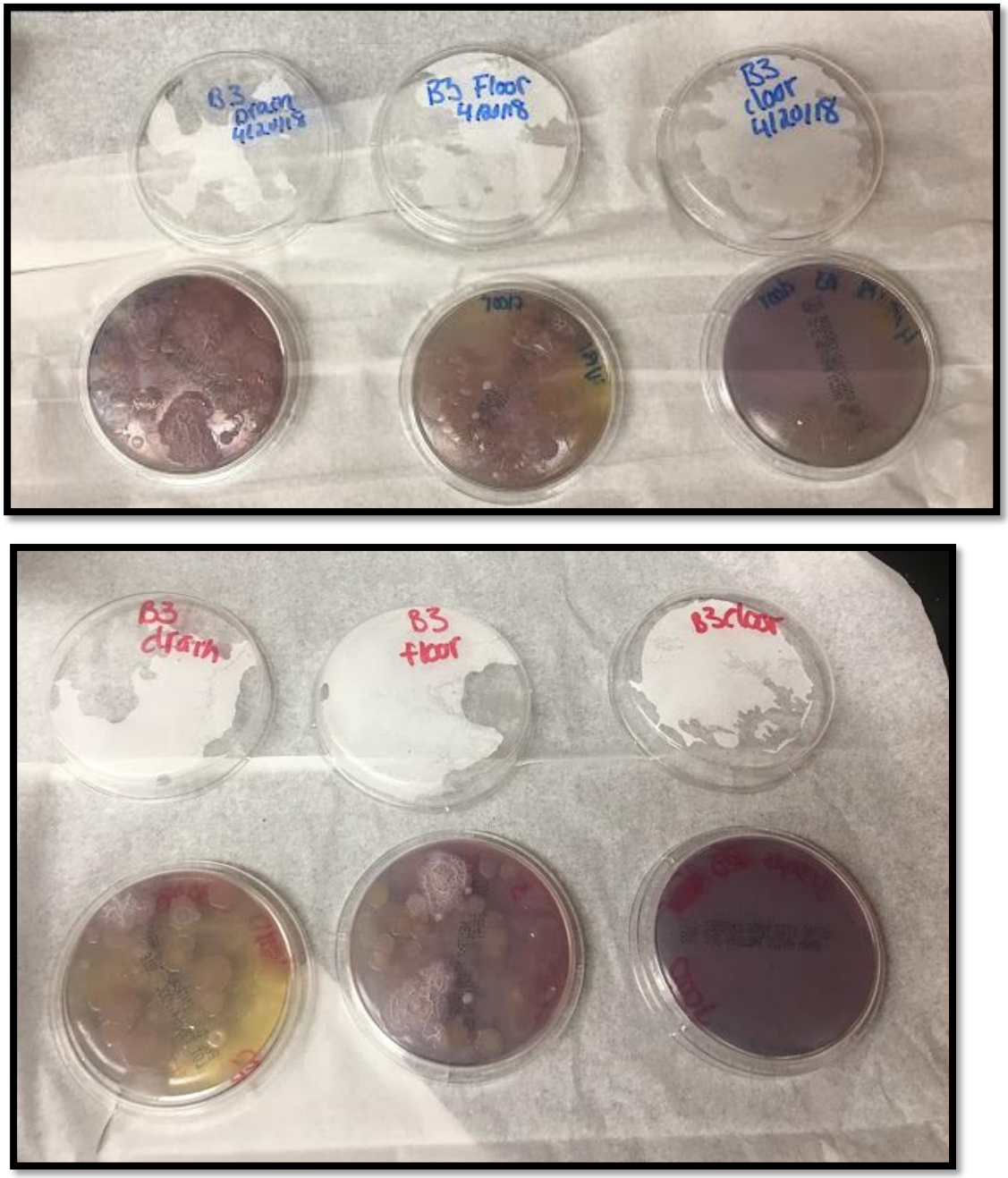
i. Sampling Schedule

Plate sampling was performed by myself and Kira from the large animal team. Samples were collected daily after stall terminations Monday-Friday.

IV. Results

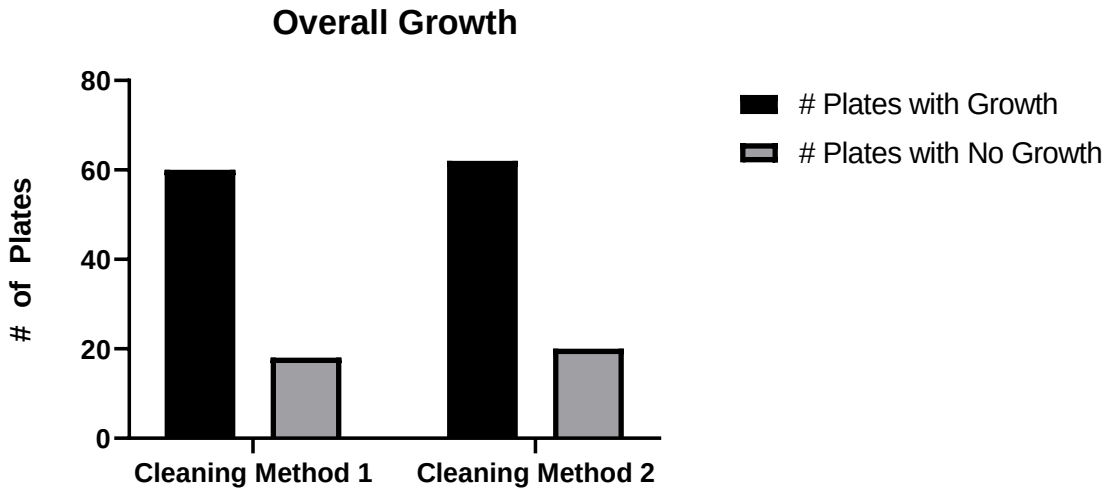
All data sampling was recorded by hand then transferred to an excel document for data analysis. To determine general growth counts any plates that showed colony growth was counted a positive growth plate.

Figure 1 Examples of contact plates from Stall B3 on different dates showing varying levels of growth. The color of marker used for plate labeling was a visual indication of which method was used: Red is CM-2 and Blue is CM-1).



If there was any type of colony present it was considered positive for plate growth. Plates with no colonies present were considered negative for growth.There were 60 positive plates from stalls cleaned using Method 1 and 62 plates had growth from stalls cleaned by Method 2. Based on the overall growth data, there was no difference between the 2 cleaning methods (χ^2 test, $p>0.05$) (Fig. 2). When looking at total growth regardless of method the data showed that out of 160 plates, 122 samples had plate media growth (76.2 %) and 38 plates showed no growth (23.1%). Only one stall that was sampled had no growth on for all three plate samples.

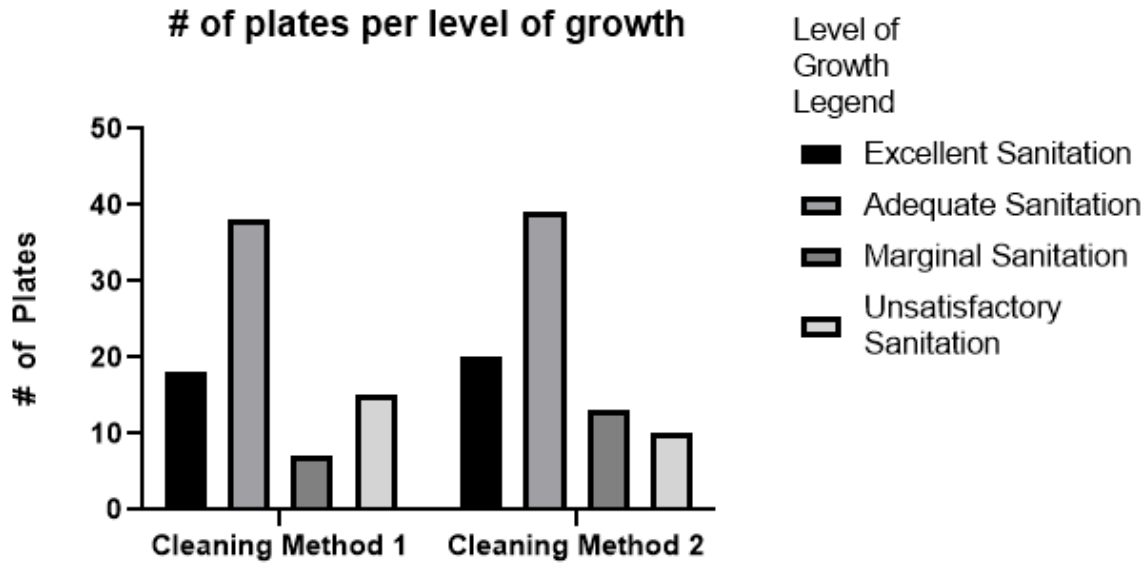
Figure 2. Results of growth on RODAC plates for each cleaning method.



To further analyze the data, a chart from the University of Florida, Division of Comparative Medicine was referenced (USF, 2003). The sanitation chart categorizes plate growth into four sanitation levels; Excellent Sanitation= 0 growth, Adequate Sanitation= 1-10, Marginal Sanitation 11-19, Unsatisfactory Sanitation= > 20. When examining the sample plates from CM-

1 stalls, 18 plates showed 0 CFUs, and 38 plates showed 1-10 CFUs, 7 plates showed 11-19 CFUs and 15 plates showed growth greater than 20 CFUs. When looking at the sample plates from CM-2 stalls, 20 plates showed 0 CFUs, 39 plates showed 1-10 CFUs per plate, 13 plates showed 11-19 CFU and 10 of the plates showed greater than 20 CFUs(Figure 3).Based on sanitation level, there was no difference between cleaning methods (χ^2 test, $p>0.05$) with 72% of the plates showing excellent to adequate sanitation for either method .

Figure 3. Level of sanitation based on colony growth on RODAC plates for each cleaning method.



Next, sample location was considered. A mixed model two-way ANOVA revealed that there was no significant difference in number of colonies counted at 24 hours between barns A, B, or C. Problem areas such as the floor and drain were noted as they had high numbers of positive growth plates regardless of cleaning method used (Table 3). The door had lower number of positive plates likely due to the surface material being easier to clean.

Table 3. Results of RODAC plate growth per area sampled for each cleaning method. Problem areas noted are the drain and floor.

Count of General -/+	Method 1	Method 2	Grand Total
door	**26**	**27**	**53**
-	12	15	27
+	14	12	26
drain	**26**	**28**	**54**
-	2	2	4
+	24	26	50
floor	**26**	**27**	**53**
-	4	3	7
+	22	24	46
Grand Total	**78**	**82**	**160**

As part of the study a cost analysis between Simple Green and Zep 4089 showed that Simple Green is more cost efficient. A 55-gallon drum of ZEP 4089 from ZEP Sales is $654.25 while a 55 gallon drum of Simple Green ranges from $415.70-$492.92 depending on the retailer. Each foamer holds ½ gallon of product. To fill a foamer of Zep 4089 will cost $5.80, while filling a foamer of Simple Green will cost $4.13.

V. Discussion

The primary purpose of the pilot study was to validate our cleaning procedures. The study showed that the about half of the stalls tested 48.4% are meeting adequate sanitation levels. I was surprised to find that 15.7% of the stalls were found at unsatisfactory sanitation levels. I presumed that all stalls would be found at adequate or excellent sanitation levels. On a few of the plates growth was too numerous to count. This may be due to the presence of biofilms which must be removed using a scrubbing technique. Currently the Large Animal Hospital does

not including “scrubbing” in their sanitation procedures. Inclusion of this may lead to higher sanitation levels. Another variable that could alter sanitation levels would be which specific husbandry technician is carrying out the procedure e.g. veteran technician vs. new technician. Finally, the number of stalls tested may pose as limitation to the study. Sampling 100 or more could lead to different results. This study is considered a pilot study and would be an excellent tool to use in a long-terms study.

Our secondary goals were to determining potential cost savings by switching to a new eco-friendly product, to decrease potentially harmful chemical products from our water stream and to create a procedure that could be used to validate new cleaning/disinfectant products in the future. I also calculated that there would be no financial losses in switching to a more environmental friendly product in fact there would be cost savings. Additionally, it was determined there was no significant difference in using an environmentally friendly product in place of a chemical for the first “cleaning step” thus allowing us to reduce the amount of chemical waste in our local water systems.

By accomplishing the purposes and goals of our study the hypothesis (H_1: Using a general purpose foaming cleaner as the 1st step in the cleaning process will be just as effective as using the current 1-step product twice) was proven true thus we reject the null hypothesis (H_o: Using a general purpose foaming cleaner as the 1st step in the cleaning process will be less effective as using the current 1-step product twice).

VI. Implications and Future Projects

This pilot study could help lead to future improvements of the Biosecurity Program at the Veterinary Teaching Hospital. By implementing a product like Simple Green as the first cleaning step, new second step disinfectants can also be tested such as Rescue or Virkon-S which contain hydrogenated peroxide. The benefits of testing Rescue or Virkon-S include the fact that they are less hazardous to users, require shorter contact time and have validated kill claims. Another interesting study would be to use scrubbing techniques in the stall termination process to see if that reduces the buildup of biofilm. Ideally once a biosecurity program is set in place and verified in the Large Animal Hospital, a standardized program for the entirety of the Veterinary Animal Hospital can be created. This program/plan should be reviewed yearly. Future projects could also include photo analysis of all sample plates, more stalls in the sample size, and identification of colonies present.

VII. Acknowledgments

I'd like to especially thank several people who have encouraged me throughout my journey as a graduate student and helped make this pilot study a successful project.

My Committee, Dr. Sally Paulson, Dr. Bill Pierson, and Dr. Eifert, who provided guidance and feedback throughout this project. Special thanks to Dr.Pierson who provided the funding for the project.

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Special thanks to Dr. Caswell and Jimmy Budnick who looked at my plates and helped with identification.

Last but, certainly not least, thank you to my husband and family who provided non-stop encouragement throughout the project.

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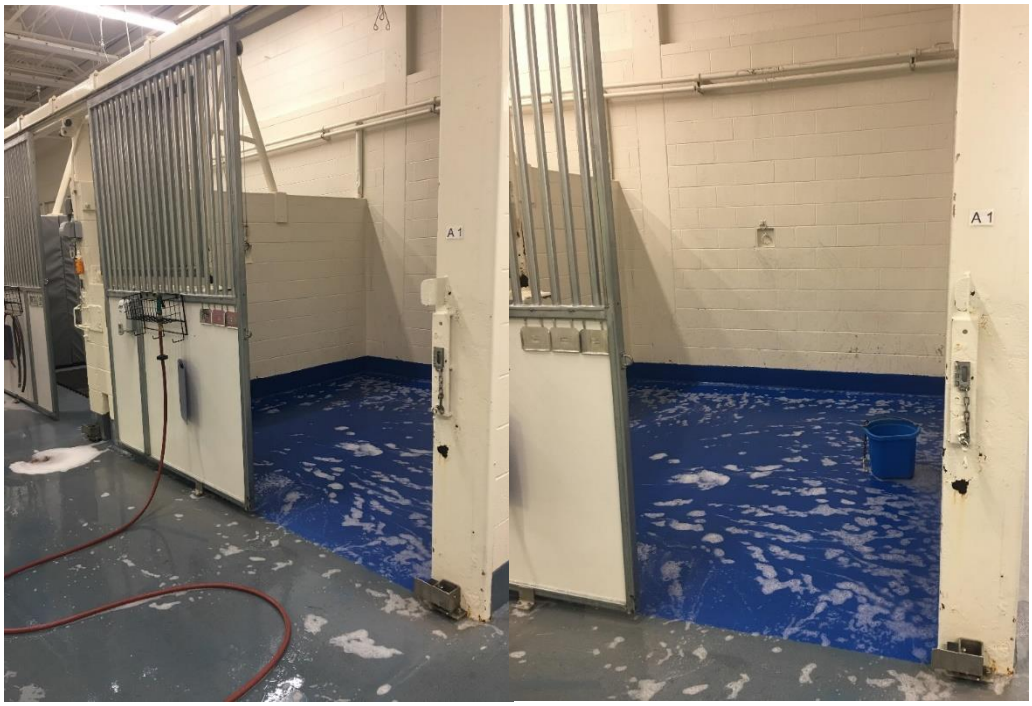
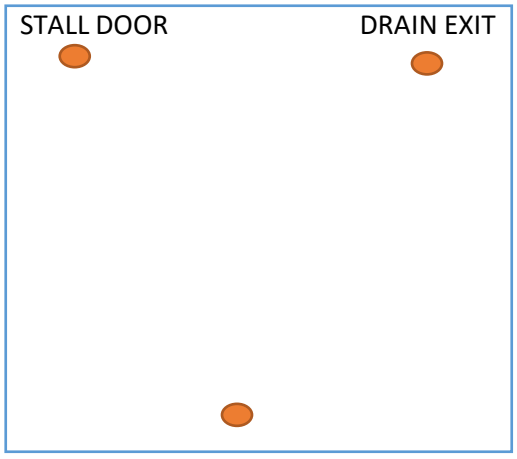
VIII. Appendix

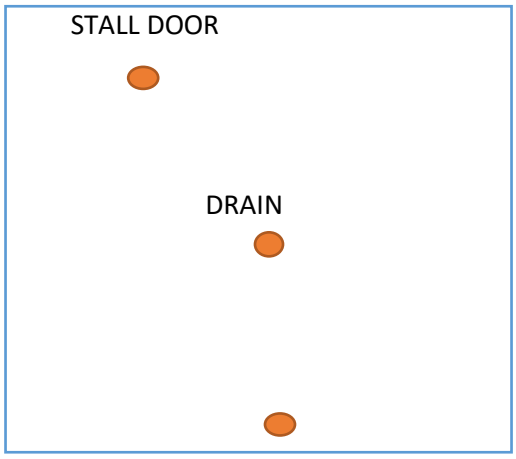
Appendix A: Horse Stall Graphics: (3 samples per stall, 1 inside perimeter, 1 near the drain, 1 on back of stall door)



Appendix B: Stall Schematic and Barn Map (Orange Dot indicate where Sampling will occur)

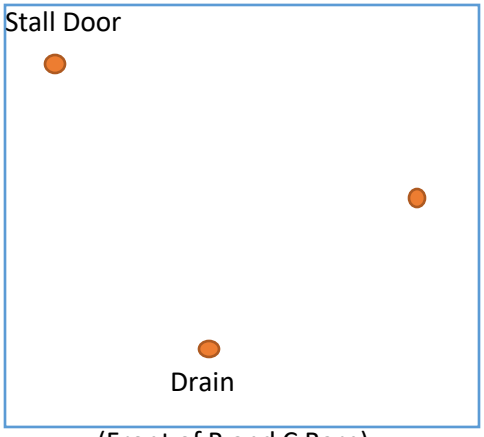


(Front of A Barn)

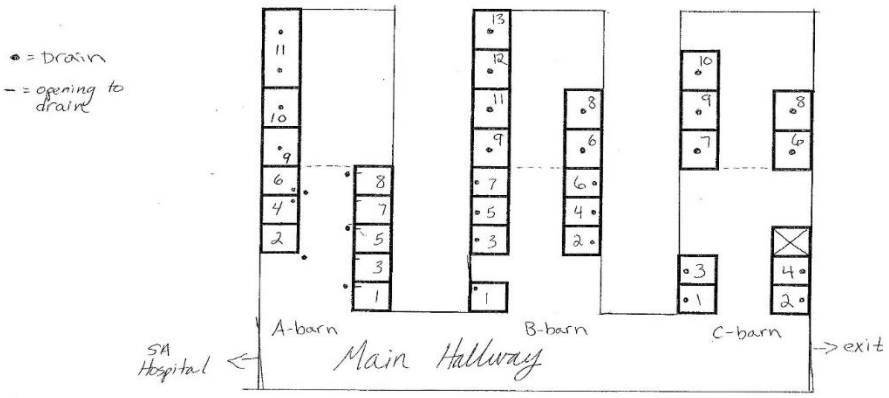


(Back of A, B, C Barns)

Appendix B: Stall Schematic and Barn Map continued.



(Front of B and C Barn)



Appendix C: Sample Size-Random Assignment (All even numbers were assigned method 1, or odd numbers assigned method 2)

Stall Order	Random Number Generated	Corresponding Cleaning Method
Stall 1	50	Even Cleaning Method 1
Stall 2	21	Odd Cleaning Method 2
Stall 3	26	Even Cleaning Method 1
Stall 4	7	Odd Cleaning Method 2
Stall 5	3	Odd Cleaning Method 2
Stall 6	23	Odd Cleaning Method 2
Stall 7	19	Odd Cleaning Method 2
Stall 8	25	Odd Cleaning Method 2
Stall 9	37	Odd Cleaning Method 2
Stall 10	12	Even Cleaning Method 1
Stall 11	15	Odd Cleaning Method 2
Stall 12	43	Odd Cleaning Method 2
Stall 13	29	Odd Cleaning Method 2
Stall 14	27	Odd Cleaning Method 2
Stall 15	18	Even Cleaning Method 1
Stall 16	44	Even Cleaning Method 1
Stall 17	36	Even Cleaning Method 1
Stall 18	8	Even Cleaning Method 1
Stall 19	1	Odd Cleaning Method 2
Stall 20	48	Even Cleaning Method 1
Stall 21	20	Even Cleaning Method 1
Stall 22	10	Even Cleaning Method 1

Appendix D: Example of Chart that will be in Large Animal (this will allow staff to track how many stalls have been cleaned and how to clean them)

Stall Repition	Cleaning Method	Date	Stall Identification #	Check Box when Complete		
Stall 1	Current					
Stall 2	New					
Stall 3	Current					
Stall 4	New					
Stall 5	New					
Stall 6	New					
Stall 7	New					
Stall 8	New					
Stall 9	New					
Stall 10	Current					
Stall 11	New					
Stall 12	New					
Stall 13	New					
Stall 14	New					
Stall 15	Current					
Stall 16	Current					
Stall 17	Current					
Stall 18	Current					

Current Method	New Method
Remove Shavings	Remove Shavings
Rinse	Rinse
4089	Simple Green
Rinse	Rinse
4089	4089
Rinse	Rinse
Check Box When Termination is Complete	
* Hang Do Not Enter Sign*	
Stall Dry Overnight	
Stalls Sampled in AM by Kira & Allie	

Appendix E: Data Collection and Plate Labeling

Data Log

Date	Time of Collection(am/pm)	Stall Ide.#	Method	Area	Incubation Time(am/pm)	24 hours	48 hours	Comments

Key	
Method 1	Current
Method 2	New
DOOR	Back of Stall Door
DRAIN	Near Drain
FLOOR	Inside Perimiter