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Improved Detection of Streptococcus Equi Subspecies Equi in Drinking Water

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IMPROVED DETECTION OF STREPTOCOCCUS EQUI SUBSPECIES EQUI IN DRINKING WATER

by

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Abstract

Streptococcus equi subspecies *equi* (*S. equi*) is the causative agent of strangles, a contagious respiratory disease of horses. Transmission of the bacteria can occur when animals share water sources. Detection of *S. equi* in water could improve strangles surveillance and move towards eradication of the disease. The aims of this study were to determine the optimal membrane pore size for bacterial retention from an aqueous suspension, to determine the likely dispersion pattern of *S. equi* contaminated mucus in a water bucket to develop a collection technique to be used by veterinarians, and to find the sensitive range for *S. equi* detection in water. Samples from the top, middle, and bottom of a five-gallon water bucket were collected by aspiration and swabbing, and streptococci harvested by filtration. Mucus strands remained suspended at the top, middle, and bottom the bucket for over an hour. Membrane filters with pore sizes of 0.45 μm were found to retain all streptococci. After one hour, viable *S. equi* were obtained predominantly from the top and middle of the bucket. The threshold for detection lies between 10 C.F.U./mL and 0.1 C.F.U./ mL. Membrane filtration of water from the top two thirds of a bucket proved to be the most sensitive sampling technique. However, the technique requires validation in the field.

Preface

The importance of the research discussed in this thesis will ultimately depend on its impact on the equine community. This work marks the starting point of a long process of testing and developing a membrane filtration technique with the potential to aid in strangles eradication. Eradication of strangles may be possible given the correct preventative care, quick diagnosis, and effective treatment measures along with a vigorous and widespread combat effort by equine owners, managers, government organizations, and veterinarians. This combat effort can be made possible through increased awareness and collaborative work of scientists, equine practitioners, and owners. A meeting held in Orono, ME in the spring of 2014 brought together Veterinarians and specialists to discuss the importance, difficulties, and realities of membrane filtration as well as a *Streptococcus equi* rapid diagnostic test produced by Maine Biotechnology Services, Inc. The general consensus was that this concept has real world application and would prove to be a valuable tool to the equine community. A representative of Tufts University expressed interest in testing this method at the University's facilities, which would provide beneficial data collection and increased statistical significance, thus increasing acceptance of the proposed bacterial collection methods.

This project could be the beginning of something major. As an undergraduate student, even the potential honor of being able to say, "I helped make it happen", is overwhelming. I have had the wonderful opportunity to work in conjunction with such influential professionals on a project for which I have so much passion. I hope to be a

part of the continuation of this project at the University of Maine or wherever it is taken, in order see it through.

Acknowledgements

I would like to thank Dr. Robert Causey for providing guidance through the experimental process and financing the purchase of materials for this study, Maine Biotechnology Services Inc. and Nathalie Forster for supporting this work, and I would also like to thank Dr. Jim Weber for providing his expert knowledge and sample syringe filters. Also a big thank you to Dr. Samuel Hanes and Dr. Martin Stokes, as well as the three already named, for agreeing to sit on my defense committee. A collaborative thank you goes out to Dr. Martha Smith who obtained swab samples for our field study.

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Introduction

Strangles is a highly contagious bacterial disease caused by *Streptococcus equi* subspecies *equi* (*S. equi*) and is characterized by sudden onset fever, mucopurulent nasal discharge, and isolated swelling of the submandibular and retropharyngeal lymph nodes [1]. Usually, bacteria are introduced onto a farm by a carrier animal and subsequently spread via direct and indirect contact between animals. Direct contact includes normal social behaviors such as grooming and nuzzling, and can be managed by separating animals. Shared tack, feeding equipment, and water sources are indirect modes of bacterial transmission and are the more difficult to control. At sites with active cases of strangles, bacterial transmission frequently occurs via shared water sources [1, 13]. Horses that shed bacteria containing mucus from the nasal passage can easily contaminate a water trough and spread bacteria to other horses [2].

Others have looked at the persistence of *S. equi* in environmental sites other than water, but available literature on the subject is sparse [1]. Jorm [3] showed that, under laboratory conditions, *S. equi* could survive for upwards of 63 days on wood and glass surfaces with no exposure to other environmental bacterial flora. In contrast, a more recent study by Weese [4] showed that under field conditions, *S. equi* only survives outdoors for 1-3 days and is degraded by sunlight. In the same study, it was found that rain had little effect on persistence of *S. equi*, giving rise to hope that bacteria may be detected in indoor water buckets. The differences between these two studies on *S. equi* survival may be attributed to a lack of competing soil flora in the earlier study [1].

Testing water will improve acceptance and ease of strangles surveillance. Currently, there are few ways to test for *S. equi* organisms in horses, and even fewer environmental tests. Current bacteria collection methods are limited to invasive procedures, such as nasal swabs, flushes, or endoscopy, which require a training, time, and money. These bacterial collection methods have been proven to fail in providing accurate results in a large number of animals with clinical signs of strangles [5]. Testing water sources for *S. equi* will allow for many horses to be screened within a short period, eliminate the need for restraint, eliminate immediate stress on the horse, and may prove to be reliable at providing accurate results.

The concept of isolating *S. equi* bacteria from drinking water has not yet been published, but various purification and filtration methods are currently used to remove other bacterial species from water, mostly in public health applications. Species such as coliform or fecal streptococci, which are bacteria from human and animal fecal contamination, can be removed from drinking water by ultraviolet treatment, boiling, and chlorination [6]. Membrane filtration is also used to sterilize and reduce contaminants in drinking water [7]. The Center for Disease Control and Prevention states that microfiltration of water through a pore size of approximately 0.1 μm will remove bacteria such as *Salmonella* and *Escherichia coli* [8]. Membrane filtration has been successfully used to detect *Staphylococcus aureus* and *Escherichia coli* in milk samples to diagnose mastitis [9].

A filtration method to boost sensitivity may be used to detect small numbers of bacteria in water. Millipore (Billerica, Massachusetts) makes a wide array of membrane

filters with varying pore sizes to remove contaminants in aqueous solutions. Their quality testing procedures require various pore and filter diameters to be used to ensure high bacterial retention. Those filters are removed after filtration and set directly onto an agar plate for culture [10]. Durapore® PVDF (polyvinylidene fluoride) membranes have high sterility, low extractables, and the lowest protein binding of any syringe filter [11]. Hydrophilic filters are able to be wetted with any liquid and are used to filter both liquids and gasses [12]. Based on this information, we tested the hydrophilic PVDF membranes with 0.45 μm to retain *S. equi* and boost sensitivity of strangles detection.

Streptococcus equi subspecies *equi* bacteria are shed in mucus in the form of nasal discharge [1, 13]. Nasal mucus contains mucins, antiseptic enzymes, and immunoglobulins, and is secreted from goblet cells contained in mucus membranes throughout the body [14]. We predicted that humans and horses have similar mucus acting in their airways and thus have similar physical properties including viscosity, density, and solubility. Infusing human mucus with *S. equi* is a method of replicating contaminated equine mucopurulent nasal discharge. In order to test water for the presence of bacteria with a membrane filter, it was beneficial to know where the mucus and bacteria would aggregate within the water column.

Objectives

The first objective was to determine what membrane pore size would allow for complete *S. equi* retention from an aqueous solution and produce a sterile filtrate. The second objective was to determine the dispersion pattern of *S. equi* and mucus in a water

bucket in order to develop a collection technique to be used by veterinarians. The third objective was to define the sensitive range for bacterial detection in water using membrane filtration.

Hypothesis

I hypothesized that mucus inoculated with *S. equi* would sink through the water column and accumulate at the base of a plastic container. I also hypothesized that Millex Durapore® polyvinylidene fluoride membranes with 0.45 μm pores would allow detection by culture of *S. equi* bacteria in water at a concentration of at least 1.0 colony forming unit (C.F.U.) per mL.

Methods and Materials

Determining Mucus Dispersion

In order to best understand how nasal discharge disperses in water, we began with an observational trial. A mucus mixture was made with 5.0 mL type II porcine stomach mucin and 10 mL tap water in a 50 mL centrifuge tube. The centrifuge tube was held on an agitator until the mixture was free of suspended mucin, roughly 5 minutes. Human saliva was collected in a 15 mL well and set aside. Four 250 mL beakers were filled with 200 mL of tap water each, and left to sit for three minutes. Crystal violet dye was mixed with saliva in a ratio of 10:1 (500 μL saliva to 50 μL crystal violet). This same procedure and ratio mixture was repeated with the mucin mixture. Half of the 10:1 saliva solution

(275 μL) was pipetted just under the surface of the water of beaker 1. The other 275 μL of the 10:1 saliva solution was pipetted onto the surface of the water of beaker number 2. The 550 μL of the mucin and crystal violet mixture was halved and 275 μL of the mixture was pipetted atop the water of both beakers 3 and 4. Finally, crystal violet with no mucus was dropped into a beaker. Visual recordings and photographs were taken every minute for the first fifteen minutes, every fifteen minutes until an hour had elapsed, and every hour until complete dispersion was seen or three hours had elapsed. This trial was repeated with a ratio of 250 μL mucin to 75 μL crystal violet.

Determining Staining Method

In order to determine the best way to accurately stain saliva in an aqueous environment, we tested and compared the adhesive ability of crystal violet and red 40. Two 250 mL beakers were filled with 200 mL tap water. Human saliva was collected in a 15 mL well. Two 500 μL samples of saliva were pipetted into two clean mixing wells. Each sample was mixed with 100 μL red 40 dye and mixed with the end of the pipette. Each entire 600 μL saliva and dye mixture was pipetted onto the water's surface in each of the beakers labeled 3 and 4. Visual recordings and photos were taken every minute for the first ten minutes and every five minutes until complete dispersion was seen, or twenty minutes.

Determining Pore Size

Millex Durapore® filters with polyvinylidene fluoride (PVDF) membranes were tested for their ability to concentrate *S. equi* from an aqueous solution and the degree to which bacteria adhered to the membranes surface. A pore size of 0.45 μm was used to determine the relative pore size to retain all *S. equi* bacteria. Swinnex polypropylene 25 mm plastic syringe filter holders from Millipore were used with corresponding 25 mm PVDF membranes.

A previously collected *S. equi* sample was used to grow colonies of bacteria on an agar plate. A sterile swab was used to agitate and collect colonies. The swab was inserted into a test tube with 1.0 mL sterile water and stirred to create a concentrated aqueous sample of streptococci, labeled A1. A first 50 μL sample from A1 was transferred via pipette to a 50 mL centrifuge tube containing 50 mL sterile water. The solution was labeled S1.

Streptococcal colony forming units were determined in S1 by serially diluting and plating on blood plates as follows: a 100 μL sample from S1 was pipetted into a test tube containing 900 μL sterile water and mixed. The resulting 1.0 mL dilution was called S1D1. A 100 μL sample from S1D1 was pipetted into another test tube containing 900 μL sterile water in order to make a diluted sample labeled S1D2. The dilution process was repeated until there were five dilutions labeled S1D1 through S1D5. A 100 μL sample from each dilution was plated on agar blood plates and incubated. Colonies were counted for each of the plates and recorded. The concentration of bacteria in S1 was calculated based on the number of C.F.U.'s counted on each blood plate.

To evaluate the efficacy of the filter, the 50 mL S1 sample was drawn up into a 50 mL syringe and a 0.45 μm filter was attached to the end of the syringe. Only 49 mL was passed through the filter, leaving 1.0 mL of unfiltered suspension. Colony forming units in the filtered and unfiltered material were determined by serial dilution as described above. Based on results from this 0.45 μm membrane trial, it was deemed unnecessary to complete a second trial using a 0.22 μm membrane.

Aspiration Technique

It was deemed necessary to test a method of filtration that would reduce bacterial adhesion to the membrane surface to yield a concentrated suspension. A new stock solution was made from previously grown streptococci. A sterile swab was used to agitate and collect colonies from the plate. The swab was inserted into a microfuge tube containing 1.0 mL sterile water and rotated vigorously to create a concentrated aqueous sample of bacteria. A 35 μL sample from this *S. zoo* stock solution was transferred, via pipette, to a 50 mL centrifuge tube containing 35 mL sterile water and labeled S1zoo. The same dilution process as previously performed was used to create five dilutions from the S1zoo solution labeled S1D1zoo-S2D5zoo. One hundred micro-liters from each dilution was plated on agar and incubated. The number of C.F.U.'s per plate was counted in order to determine the concentration of the S1zoo solution.

Following the same procedure as the previous filtration trial, a suspension of S1zoo was expelled through a 0.45 μm filter attached to a blood collection tube. The filtrate was aspirated back and fourth between the syringe and the blood tube to dislodge

bacteria bound to the membrane. Colony forming units were counted and comparisons were made between the first trial and the aspiration trial to determine the ability to create a concentrated suspension of streptococci using this aspiration technique.

Sensitivity of Various Field Sampling Techniques

A clean five-gallon equine water bucket was washed with a 95% ethanol solution and rinsed three times with sterile water. The bucket was then filled with three gallons of distilled water. Human saliva was collected in a weigh boat and 1.0 mL of human saliva was transferred to an uncapped blood collection tube containing 200 µL of a *S. zoo* suspension. The tube was agitated and the saliva mixture was gently poured directly in the center of the five-gallon bucket. A fresh 100 µL sample of un-inoculated saliva was plated out and the C.F.U's of the original bacterial suspension was determined by serial dilution.

Over five trials, inoculates were prepared to achieve streptococcal concentrations above and below the hypothesized 1.0 C.F.U/mL detection threshold. Three liquid gallons contains 11,355 mL, so using 200 µL of a suspension containing 10,000 C.F.U./µL would give the bucket an overall bacterial concentration of 3.5 C.F.U./ mL. Based on colony counts of serial dilutions, the streptococcal concentration in the bucket over the five trials was estimated as 352, 17.6, 1.0, 0.176, and 0.004 C.F.U./mL.

Following inoculation, the bucket was left to sit for an hour and 20 minutes to allow for complete saliva dispersion. Six locations were chosen from which to collect samples: from the top, middle, and bottom, both around the circumference and down the

center of the transverse plane of the bucket (Figure 1).

A 100 μL water sample, directly off the surface of the water in the center of the bucket, was plated directly on blood agar. A 50 mL sample from the top, middle, and bottom areas in the center of the bucket, was collected using a sterile equine insemination pipette attached to a sterile plastic 60 mL plunger syringe. Once the liquid sample was collected, the insemination pipette was removed and a microfuge tube was filled with 1.0 mL of the pre-filtered solution from which five serial dilutions were made and plated out. The Swinnex filter holder, loaded with a 0.45 μm membrane filter, was then attached to the syringe and the rest of the sample was passed through the filter. The membrane filter was removed from the holder and placed contaminated-side down on an agar plate for incubation.

Paired sterile cotton swabs were used to sample the circumference of the bucket at the three depths listed above. The two swabs were held simultaneously and used to swab the entire circumference of the inside edge of the bucket at the three depths. (A palpation sleeve was worn to prevent contamination while reaching in to swab the bucket). One swab was streaked out on an agar plate for incubation and its paired swab was inserted into a centrifuge tube containing 50 mL sterile water. This second swab was rotated vigorously to release bacteria and seed the 50 mL sterile water. A 100 μL sample of the seeded 50 mL was poured into a microfuge tube from which five serial dilutions would be made and plated on agar. The remaining seeded water was drawn up into a 60 mL plunger syringe with an 18-gauge needle. The contents of the syringe were passed thought a 0.45 μm filter. The filter holder was opened and sterile tongs were used to

remove the membrane filter. The filter was plated, contaminated side down, on an agar plate.

Results and Discussion

When surface tension was not broken, saliva remained suspended on the surface of water for as long as 11 minutes. After this suspension phase, saliva slowly sank through the water column to the bottom of a container, leaving strands of mucus and dye through the water column (Figure 2). Crystal violet remained bound to saliva, with minimal dye leaching, allowing for identification of its location in the beaker for up to three hours. The dispersion pattern of the saliva was altered when the surface tension was broken in beaker 1. Saliva was observed to sink quickly to the base of the beaker with no suspension through the water column. Minimal dye leeching was recorded and the location of the mucus could be identified for three hours, until the dye had completely dispersed.

The mucin mixture lacked the cohesive properties that naturally occurring saliva and mucus possesses. It was quicker to disperse and significant dye leeching was seen, making it difficult to identify the location of mucin in the beaker. Complete dispersion was recorded after only 11 minutes. When the ratio of mucin to crystal violet was increased from to 50 μL to 75 μL, noticeable dye leeching was still seen and complete dye dispersion took 10 minutes. Red 40 dye was tested in addition to crystal violet in order to see if the mucin mixture could be identified in the beaker for longer when stained

with another dye. Immediate and vigorous dye dispersion was observed when 500 µL of mucin and 100 µL Red 40 were pipetted onto the water's surface. The dye created a bio-film over the water's surface within fifteen seconds. Saliva was stained for a short amount of time but noticeable leeching into the water was seen within 4 minutes. Strands of mucin could be identified sinking through the water column during this time. Full dye dispersion was seen by 10 minutes, after which there was no visible sign of the location of saliva in the beaker. The mucin mixtures' quick dispersion of was attributed to its hydrophilic properties and not to the inability of a dye to stain it.

From these findings, we chose human saliva over porcine mucin as a substitute for equine nasal discharge in our bucket trials due to its structural integrity in water. In addition, we reasoned that human saliva would introduce other bacteria to the bucket, thus providing the opportunity to collect results under field-like conditions where bacterial contamination is a factor. It was also decided that bucket samples would be taken after at least 1 hour and 20 minutes to allow for significant dispersion of bacteria and saliva.

It was found that a 0.45 µm membrane was sufficient at collecting *S. equi* and producing a sterile filtrate. There was no bacteria found in the filtrate and the filter itself gave a strong positive. However, the 1.0 mL aqueous solution left in the syringe contained no more bacteria than the original pre-filtered suspension (Figure 3). These findings show that bacteria were strongly bound to the filter and did not remain in solution. Since the 0.45 µm membrane was able to produce a sterile filtrate, we reasoned that the *S. equi* bacteria were larger than 0.45 µm and there was no need to test a 0.22 µm

membrane filter.

Aspiration proved to be ineffective after results showed bacterial contamination of the filtrate. Concentrations of the 50 mL stock solution and the 1.0 mL aqueous solution left in the syringe were indistinguishable from one another, meaning that bacteria were not washed off of the filter and back into solution as was desired (Figure 4). The filter, again, provided a strong positive result but the filtrate produced was not sterile. We predict that the numerous aspirations compromised the connection between the filter holder and the membrane and allowed for bacterial liquid to bypass the membrane and end up in the filtrate (Table 1). From these results, we ruled out the aspiration technique as being beneficial for bacterial detection. It was determined that the most promising method for positive bacterial detection would be the direct plating of the membrane since the liquid could not be concentrated.

The results of all bucket trials can be found in Table 2. The critical range of bacterial detection for each of the detection method, direct swab, filtered swab solution, and filtered liquid sample, are outlined. Our hypothesis that bacteria could be detected at a concentration as low as 1.0 C.F.U/mL cannot be disproved based on these findings. We calculated the number of C.F.U.'s needed in order to attain 1.0 C.F.U/mL in the bucket and selected bacterial dilutions that would provide us with roughly 20,000 C.F.U. per inoculate or 10,000 C.F.U./μL in the selected dilution. The concentration obtained for trial 3 and trial 4 were .176 C.F.U/ mL and 17.6 C.F.U/ mL respectively, which supported the hypothesized threshold. The pilot trial of the bucket sampling, T0, provided results that were consistent with what we would expect to find at roughly 1.0 C.F.U./mL**.** The

results of T0 are highlighted in blue in Figure 5 to show the progression of detection as the bacterial concentration decreases.

We defined the range of sensitivity for bacterial collection as being between 17.6 C.F.U /mL and .176 C.F.U./ mL. The most sensitive technique is to filter 50 mL of water from the top two thirds, directly in the center of a water bucket. Validation of the results by a field study is warranted.

Conclusion

Based on the findings of this study, it appears that filtering water through a 0.45 μm membrane will improve detection of *S. equi* from drinking water. My hypothesis cannot currently be disproved and further trials would narrow the range of sensitivity for bacterial detection to a concentration of less than 1.0 C.F.U./mL.

Other Considerations and Future Work

The most important consideration for future studies is time. It would be beneficial to run lab trials at varying times after inoculation to reveal how time affects the dispersion of bacteria. Bacteria may collect in different areas of a bucket if given more time for dispersion. Other variables such as bacterial competition should be studied in order to gain a wider understanding of how *S. equi* survives in the presence of other bacterial species. This experiment should be conducted again with different types of membrane filters. There may be another membrane that would better prevent bacteria from binding to its surface, thus concentrating bacteria in an aqueous solution and

eliminating the need to directly plate filters, as well as increase sensitivity.

Future work for expanding the acceptance of the findings reported in this paper includes field-testing and increased numbers of lab trials for greater significance of results. A correlation study between positive water buckets and positive cases of strangles must also be conducted. A field study was conducted in order to gather preliminary on-farm data, this information can be found in the Appendix.

Tables and Figures

Figure 1: Sampling Sites in Bucket Trials

Figure 2: Saliva Dispersion in Water Over Time

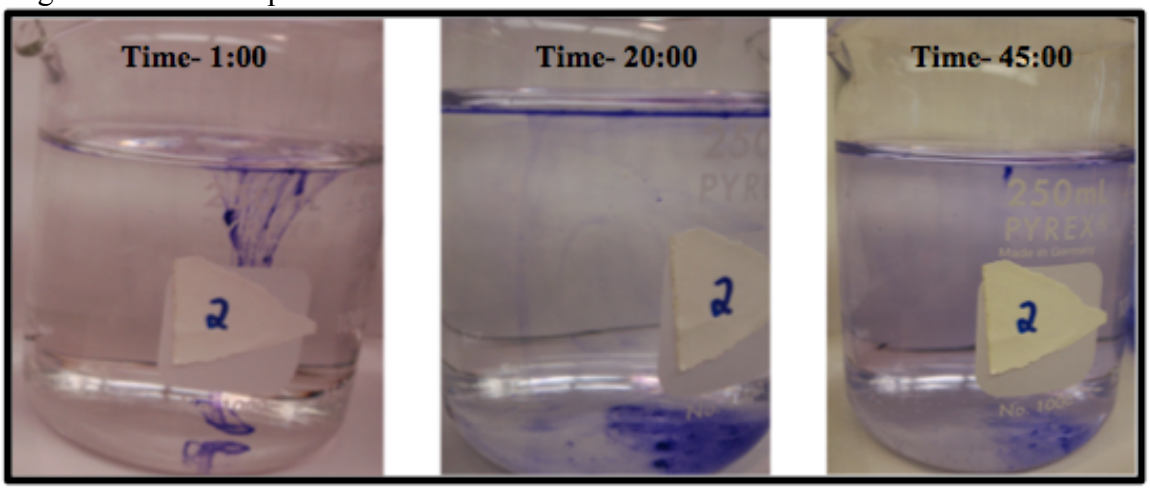

Figure 3: Comparison of S1 Filtered and Non-Filtered Bacterial Solutions

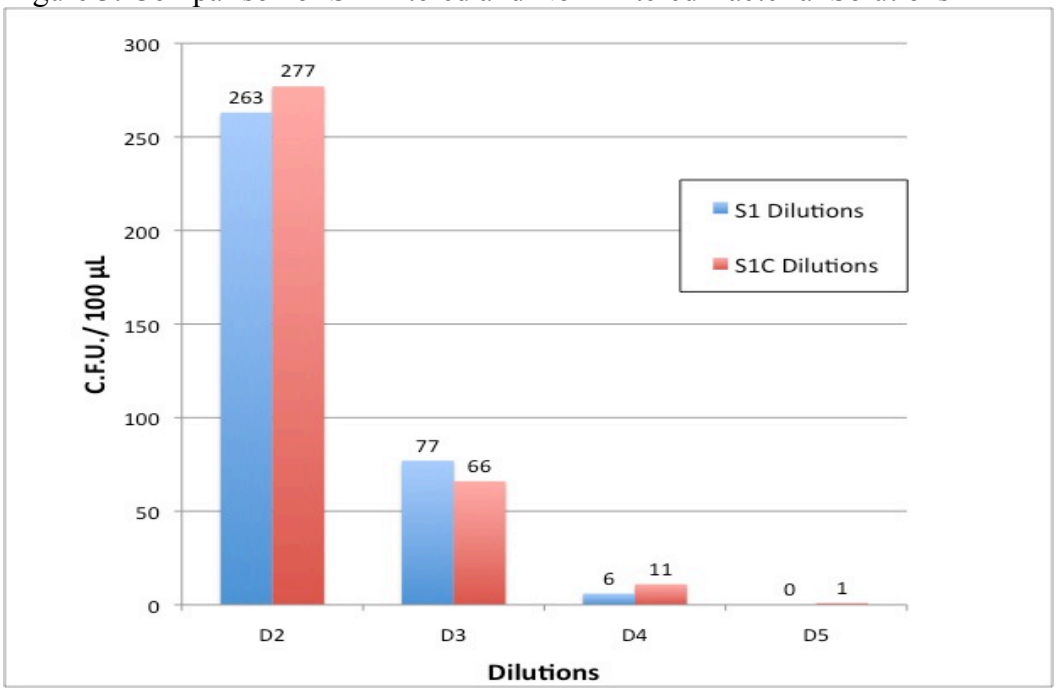

Figure 4: Comparison of Filtered and Non- Filtered Bacterial Solutions for Aspiration Trial

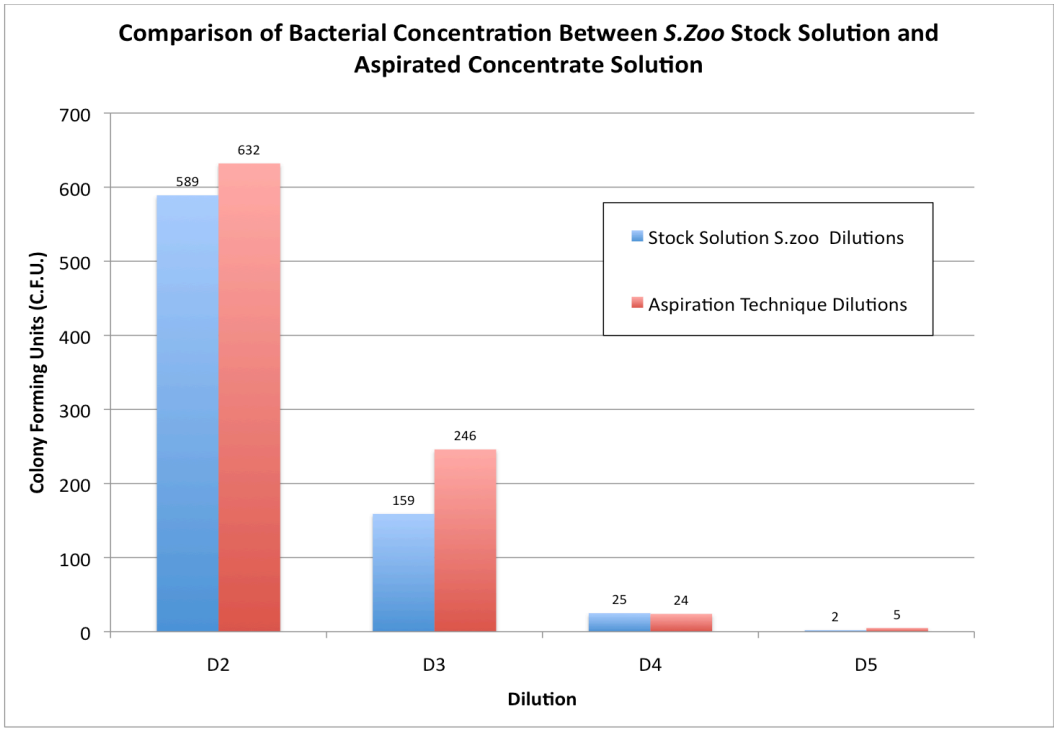

Table 1: Ability of 0.45 µm Membranes to Concentrate and Filter *S.zoo*

	Un-Filtered Bacterial Solution		Filtered Bacterial Solution		Filter	Filtrate	
Bacterial Solution	+/-	C.F.U./mL	+/-	C.F.U./ mL	+/-	+/-	C.F.U./ mL
S1	+	$5x10^5$	+	$5x10^5$	+	-	0.0
S2	+	$5x10^5$	+	$5x10^5$	+	-	0.0
Aspiration Solution	+	$2x10^5$	+	$2x10^5$	+	+	$2x10^5$

Table 2: *S. zoo* Detection Results for Five Bucket Trials.

	Bacterial Concentration in Bucket (C.F.U. /mL)									
	352		17.6		1.0		.176		0.004	
Area of Bucket	+/-	C.F.U.	+/-	C.F.U.	+/-	C.F.U.	+/-	C.F.U.	+/-	C.F.U.
10 cc off top	+	16	+	4	–		–		–	
Top third, center, filter	+	4	+	14	+	1	–		–	
Middle third, center, filter	+	3	+	12	–		–		–	
Bottom third, center, filter	–		+	8	+	1	–		–	
Swab Solution, top third, circumference	+	4	+	1	+		–		–	
Swab Solution, middle third, circumference	+	9	–		+		–		–	
Swab Solution, bottom third, circumference	+	6	+	1	+	2	–		–	
Top third, direct swab circumference	+	34	+	2	+	4	–		–	
Middle third, direct swab circumference	+	46	+	1	+	5	–		–	
Bottom third, direct swab circumference	+	15	+	1	+	23	–		–	

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Appendix

FIELD APPLICATION TO DETECT STRANGLES IN EQUINE DRINKING WATER

Introduction

Based on results obtained to date, direct swab, filtration of swab solution, and liquid filtration show promise as methods of sensitive detection of *S. equi* in drinking water. A preliminary field test was therefore deemed necessary to validate these in-vitro results and to tests the practicality and acceptability of the methods in the field. Therefore, the method was tested in a setting where *S. equi* was likely to be detected. Recently, a nearby farm suffered an outbreak of strangles, and had several newly convalescent animals. This farm was therefore selected as a site from which to collect sample that would likely yield positive results.

Methods and Materials

An equine breeding farm in southern Maine had an outbreak of strangles in November of 2013 and though most horses were convalescent, a few still showed clinical signs. Automatic waterers were used as the primary water delivery system on the farm, and no free water was present at most sites. Because of this, the swab solution method was chosen for sampling. Culture transport swabs were therefore taken from waterers in eight individual box stalls and two group paddocks. Swab samples were stored at 4 C and processed 24 hours after collection. Each swab was rotated vigorously in 50 mL sterile water, to make a suspension, and each suspension was run through a 0.45 μm filter.

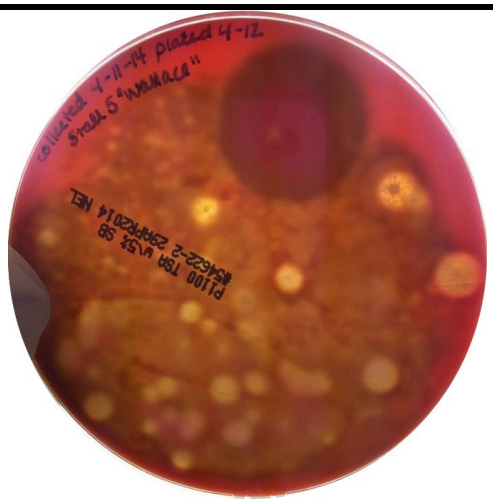
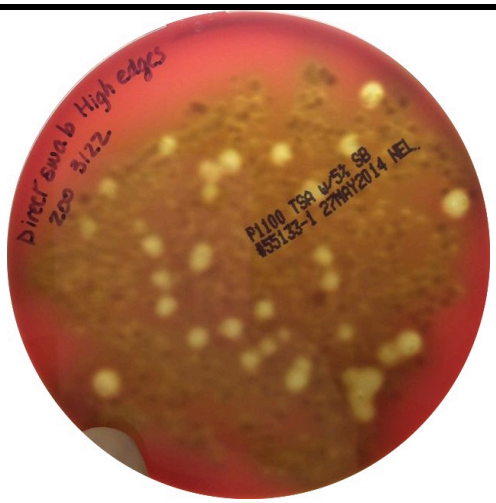
Filters were streaked out over the surface of individual agar plates and incubated at 37 C. Colonies were counted after 48 hours of incubation and results were recorded. Hemolysis of the blood plates constituted a positive result.

Results

Appendix Table 1: Swab Solution Results for Field Study

Source	Horse Name	C.F.U.	+/-
Outside Paddock	Geldings	12	+
Outside Paddock	Male Yearlings	15	+
Stall	George	11	+
Stall	Rico	8	+
Stall	Emily	11	+
Stall	Baker	17	+
Stall	Molly	26	+
Stall	Ella	23	+
Stall	Wallace	27	+
Stall	Nadine	0	-

Appendix Figure 1: Comparison of Field and Lab Blood Plates

Field Results for "Wallace"	Lab Results
	

Discussion

Each of the horses or groups of horses, whose waterers were sampled, was currently showing, or had recently shown clinical signs of strangles. Nadine showed clinical signs, however, her test results were negative. The number of C.F.U.'s counted from the individual stalls was greater than the C.F.U.'s counted during lab trials using the same swab solution method. This suggests that the method is sufficiently sensitive to detect beta hemolytic bacteria in drinking water of infected animals from sick animals.

The plate comparison (Appendix Figure 1) shows the similarity of the results collected in the lab and those collected in the field. The next step of validation for a field method is to determine the specific bacteria that cause hemolysis, as well as test the other collection techniques for their practicality and efficacy.

Conclusion

We can conclude that the swab solution method is sensitive enough to detect hemolytic bacteria from water of animals with positive cases of strangles.

Author's Biography

Lily Anne McLaughlin was born in Oak Park, Illinois on April 5, 1992. She was raised in Greenville, Maine and graduated from Greenville High School in 2010. Majoring in Animal and Veterinary Science, Lily also has a minor in Equine Studies. She is a member of Phi Kappa Phi National Honor Society, Golden Key Honor Society, and participates in equine extracurricular groups such as the Drill Team and the Equestrian Team. After graduation, Lily plans to further her education by attending Graduate School and hopes to someday work as an equine therapist specializing in eating disorder therapy.