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Endoscopic Evaluation of the Response to Intrauterine Irrigation with 3.3% N-Acetylcysteine in Mares

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ENDOSCOPIC EVALUATION OF THE RESPONSE TO INTRAUTERINE IRRIGATION WITH 3.3% N-ACETYLCYSTEINE IN MARES

by

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Abstract

Strangles is a contagious disease caused by the bacteria *Streptococcus equi* that has continued to affect horses through the development of long-term asymptomatic carriers. Effective identification of horses carrying *S. equi* and treatment of these horses has proven difficult. The chemical N-acetylcysteine (NAC) has mucolytic properties and has been used in the treatment of carriers; however the reports show controversial and anecdotal evidence regarding the effectiveness and inflammatory side effects. The goal of this study is to observe whether NAC is irritating to mucosal tissue with the interest of determining if NAC could be used to treat the carrier state of Strangles. Ten, healthy Standardbred mares were used in this random study which involved the endoscopic evaluation of uterine tissue in response to one of three treatment groups; no infusion, Lactated Ringer's Solution (LRS), or a 3.3% NAC solution. The collected endoscopic images were subjectively evaluated for signs of tissue irritation by a double-blinded researcher. The results from this study indicate that the uterine tissues showed minor inflammation for the treatment groups of LRS and NAC when compared to the no infusion group, and NAC showed fewer signs of inflammation when compared to LRS. These results indicate that NAC may have anti-inflammatory properties.

Acknowledgements

I send my deepest thank-you to Dr. Robert Causey for advising me throughout this project and introducing me to the field of veterinary research. I also want to thank Elizabeth Turner and other AVS students for their assistance with this project as well as the University of Maine Standardbred mares for being so cooperative with this study. Additionally, I would like to thank the members of my Thesis Committee and AVS Capstone professors for your guidance, support, and revisions throughout this project.

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1. Strangles Disease

Strangles is an upper respiratory disease of equid species that is caused by the bacterium *Streptococcus equi*. Strangles is an economically significant disease due to the extensive welfare costs and quarantine protocols that result as a consequence of this infection. The *S. equi* bacteria can be transmitted to other horses by direct contact or indirectly through fomite and environmental contamination. Strangles therefore is a very contagious disease with an infection rate of 85-100% for horses that come in contact with *S. equi* bacteria (Taylor and Wilson, 2006). Upon coming in contact with this organism, there is a 3-8 day incubation period before clinical signs develop (Taylor and Wilson, 2006). Clinical signs of strangles include a fever greater than 103 F, swelling and abscess formation of lymph nodes, mucopurulent nasal discharge, and local inflammation and edema. These signs can persist for 3-4 weeks and bacteria can be shed for an additional 4 week period after clinical signs dissipate (Taylor and Wilson, 2006; Sweeney et al., 2005).

There are many complications of strangles that can raise the mortality rate from the average of 8-10 percent (Holland et al., 2006; Sweeney et al., 2005; Newton et al., 1997) to nearly 40 percent (Sweeney et al., 2005). Approximately 20% of horses can have complications from strangles (Sweeney et al., 2005). Two major complications include; “bastard strangles” - the systemic abscessation of lymph tissues, and purpura haemorrhagica - an immune initiated hypersensitivity resulting in vasculitis. Additionally, another 10% of infected horses, and in some outbreaks this is much higher (Knowles et al., 2010; Newton et al., 2000), will become asymptomatic carriers of *S. equi*

for extended periods of time (Waller et al., 2011; Rosser et al., 2007; Taylor and Wilson, 2006; Sweeney et al., 2005).

Streptococcus equi has several virulent factors that shield the bacteria from the immune system and prevent phagocytosis and opsonization of the bacteria. The immune system responds to the presence of *S. equi* by concentrating neutrophils in the lymph tissues. This accumulation leads to the swelling of lymph nodes and can lead to abscess formation. The guttural pouch carrier state is established when the retropharyngeal lymph nodes abscess and drain into the guttural pouch (Waller et al., 2011). Once in the guttural pouch, *S. equi* and mucus can solidify into masses called chondroids and be present months later. A study by Newton et al. (1997) found that 5 out of 6 carriers of strangles harbored *S. equi* bacteria in the guttural pouch for 7 to 34 months, allowing undetected carriers to intermittently shed *S. equi* after the initial infection (Newton et al., 2000). *Streptococcus equi* has a short life span outside of the horse, and it is this prolonged carrier state that has allowed for this disease to be maintained within the equid population (Waller et al., 2011).

A horse who is displaying clinical signs of strangles should be immediately isolated from the rest of the barn and other horses should be closely monitored for signs of infection. A veterinarian should confirm the diagnosis using a PCR test, bacterial culture, or ELISA blood test. Strangles treatment options range from letting the horse naturally fight the disease, to providing supportive care or antimicrobials based on the severity of the symptoms. The most commonly used antibiotic for horses with strangles

is penicillin. A positive *S. equi* horse should remain in isolation, following strict biosecurity protocols, until all clinical signs have subsided and the horse is verified as strangles free. Biosecurity measures include halting the movement of animals onto or off of the farm, preventing cross contamination between the isolation area and the rest of the farm, following strict hygiene measures for caretakers, and properly cleaning and sanitizing the facilities and equipment. A complete biosecurity protocol can be referenced from Holland et al. (2006), Taylor and Wilson (2006), or Sweeney et al. (2005). Horses are typically deemed *S. equi* free after three consecutive negative cultures, taken at least one week apart, and a negative PCR test. The PCR tests are used in conjunction with cultures due to their higher sensitivity when compared to nasopharyngeal swab cultures or guttural pouch lavage cultures, but also because PCR tests cannot differentiate between live and dead bacteria due to their assessment of DNA (Newton et al., 1997; 2000).

Holland and many others also recommend that the guttural pouches of infected horses be cleaned out, treated, and confirmed negative for *S. equi* before an animal is reintegrated into the herd (Holland et al., 2006; Taylor and Wilson, 2006; Sweeney et al., 2005). The guttural pouches are two blind, air-filled sacs off of the auditory tube in the head of a horse. Each sac has a volume of 300-500 milliliters (Baptiste et al., 2000; Chiesa et al., 1999) and is believed to facilitate temperature regulation of brain blood flow during extensive exercise as shown by Baptiste et al. (2000). It is important to confirm that the guttural pouches are *S. equi* negative to help prevent the carrier state and thus reduce the future incidence rate of strangles. There is also a need for the production

of safe and effective vaccines to help prevent strangles infections. Although several vaccines are available, safety concerns due to adverse reactions and partial protectiveness have limited vaccine use to only at-risk horses (Waller and Jolley, 2007). A new vaccine recently tested by Guss et al. (2009) holds promise for providing effective protection against strangles. The 7-component vaccine includes two IgG endopeptidase antigens and provides 85% protection when challenged with *S. equi* (Guss et al., 2009).

2. N-acetylcysteine

N-acetylcysteine (NAC) is a drug with multiple purposes; it is a mucolytic agent, an anti-oxidant, a free radical scavenger, and can bind metals into complexes (Reinero et al., 2011; Atkuri et al., 2007; Thorne Research Inc., 2002). Routes of administration for NAC include oral, intravenously, or as an aerosol (Atkuri et al., 2007) with side effects ranging from nausea and vomiting, to bronchospasm, and to anaphylactic shock (Levine and LoVecchio, 2011; Henke and Ratjen, 2007). According to the package insert for acetylcysteine 20% solution, using this chemical as an aerosol will result in mucolytic responses which may be helpful in the treatment of the carrier state of strangles (American Regent Inc., 2005).

N-acetylcysteine is primarily used in the medical industry to treat acetaminophen overdoses. Acetaminophen is the pain and fever reducing chemical found in Tylenol, Percocet, and Vicodin (Levine and LoVecchio, 2011). Acetaminophen is metabolized by the liver into N-acetyl-p-benzoquinoneimine – a toxic compound that is usually neutralized by glutathione. Glutathione is a compound that maintains oxidative-reductive

balance in the body (Atkuri et al., 2007). Overdosing with acetaminophen leads to a deficiency of glutathione, which causes N-acetyl-p-benzoquinoneimine accumulation in the liver resulting in hepatotoxicity (Levine and LoVecchio, 2011). Acetaminophen overdoses are treated with a 5% oral NAC solution and acts to prevent hepatotoxicity through replenishing glutathione levels, or more directly through supplying cysteines - the precursor to glutathione (Atkuri et al., 2007; American Regent Inc., 2005). Treatment with NAC is most effective within 8 hours of overdose (Levine and LoVecchio, 2011; Thorne Research Inc., 2002).

Another medical use of NAC utilizes the mucolytic properties of this chemical as therapeutic treatment for patients with cystic fibrosis. Cystic fibrosis is a condition caused by the malfunction of the chloride ion transporter resulting in dehydrated respiratory secretions and mucus accumulation (Henke and Ratjen, 2007). Patients with cystic fibrosis receive NAC in an aerosol form to thin respiratory mucus. Mucus contains a disulfide bond, which is broken by the thiol group of NAC causing a reduction in viscosity of respiratory mucus (Reinero et al., 2011; Henke and Ratjen, 2007; Thorne Research, 2002). Although NAC has been proven to effectively breakdown mucus and has been shown to reduce inflammation in the lungs of patients with cystic fibrosis (Atkuri et al., 2007), it is no longer recommended for use because thinner mucus is more difficult to expel from airways and there is also concern about the risk of exposing a patient to bacteria that may be trapped in the mucus (Henke and Ratjen, 2007).

In the veterinary field, NAC is used to treat acetaminophen overdoses in cats and has been tested for other uses as well. A recent experiment by Reinero et al. (2011) has assessed the safety of using NAC as a relief for asthmatic cats. Asthma is characterized by increased airway inflammation and mucus secretion (Reinero et al., 2011). The cats were sedated, given 400 mg of a 10% NAC solution and monitored for airway resistance by a ventilator. Reinero et al. (2011) found that administration of NAC increased airway resistance.

Additionally, NAC has been tested in the uterus of the horse as a treatment for reducing post-breeding endometritus. Many mares suffer low fertility as a result from post-breeding inflammation of the uterine lining and delayed uterine clearance (Melkus et al., 2011). A previous University of Maine Honors College student measured inflammatory responses of the uterus to the treatment of NAC or saline using cytology and biopsy methods (McGhie - unpublished). Although these results were inconclusive, a more recent study by Melkus et al. (2011), which also measured inflammatory responses with cytology and biopsy procedures, has shown NAC to be beneficial. Melkus et al. (2011) irrigated the uteri of horses with either a 5% NAC solution or saline and evaluated for inflammatory responses at either 24 or 72 hours after irrigation. This study found that irrigations of 5% NAC did not cause negative epithelial tissue effects in the uterus and was anti-inflammatory when compared to tissue responses from saline treatment (Melkus et al., 2011).

In equine medicine, the use of NAC has been reported in the treatment of guttural pouch diseases that result in empyema - the accumulation of pus (Sweeney et al., 2005; Perkins et al., 2003; Carmalt, 2002). Strangles is one of the diseases where the use of NAC has been noted. It is controversial whether there are additional benefits to including NAC into a guttural pouch lavage solution, or if the physical flushing provides the greatest benefit (Perkins et al., 2003). Perkins et al. (2003) discourages the use of NAC in the guttural pouches due to "harmful and serious adverse effects." Conversely, Bentz et al. (1996) successfully treated a *S. equi* infected horse using NAC without negative side effects. In this case study, the right guttural pouch was filled with thick purulent fluid which responded well to saline lavages. The left guttural pouch however contained a chondroid and did not dissolve after five saline lavages. In an attempt to avoid surgery, a lavage of 20% NAC was used to dissolve the chondroid. After four lavages of 20% NAC, the chondroid was completely dissolved as verified by endoscopy. The horse involved in the case study by Bentz et al. (1996) experienced no negative side effects from NAC. The common side effects noted by other authors in association with the administration of NAC in horses are dysphagia- difficulty swallowing, and erythema- redness and inflammation (Sweeney et al., 2005; Perkins et al., 2003).

3. Objective

In this study, endoscopy will be used to visually describe and compare tissue responses between three treatment groups; no infusion, 3.3% NAC solution, and Lactated Ringer's Solution (LRS). Due to the possibility of negative side effects and the physiology of the guttural pouch, the uterus will be used as the site of irrigation. The

epithelial tissue of the guttural pouch is similar to that of the uterus – both are mucosal tissues composed of columnar ciliated cells intermixed with mucus secreting goblet cells (Aughey and Frye, 2001; Causey et al., 2000). The major difference is that the guttural pouch is a pseudo-stratified tissue, while the uterus is a single layer (Perkins et al., 2003; Aughey and Frye, 2001). One of the keys to reducing strangles disease is treating the carrier state, and NAC has the potential to effectively cleanse the guttural pouch of *S. Equi* after strangles infections due to its mucolytic properties. This study will use an endoscope to look for negative side effects of using a diluted NAC solution in the uterus. Several other studies have assessed the inflammatory response of uterine tissues to NAC using cytology and biopsy methods (Melkus et al., 2011; McGhie – unpublished); however, these studies have not employed the use of an endoscope for visual assessment.

4. Materials and Methods

4.1 Randomization of Horses

Ten Standardbred mares from the J. F. Witter Teaching and Research Center were randomly assigned to three treatment groups; no infusion (4), LRS (3), and NAC (3). These horses were first ordered alphabetically and then given a random, three digit number generated by an online database (www.random.org). The horses were then re-ordered numerically and systematically assigned to the treatment group. The table used for this randomization process has been duplicated on the next page – in which columns one and four were filled first, followed by column two and column three (see Table 1).

Table 1: Process of Randomization

Names - Alphabetical	Randomly Assigned #	*Name – by Number	Treatment Group
Andy	281	Whiteout	No infusion
**Annie	229	Ford	LRS
Blisstex	898	Annie	NAC
Dewey	528	Andy	No infusion
Ford	119	Dewey	LRS
Holly	942	Honor	NAC
Honor	665	Jill	No infusion
Jill	755	Wicky	LRS
Whiteout	111	Blisstex	NAC
Wicky	834	Holly	No infusion

*This column was filled in last.
**Upon arrival of Annie, her name was changed to Laney; Laney will be used for the rest of this paper.

4.2 Preparation of Infusions

The infusions were prepared within four hours prior to irrigation. The LRS infusions contained 180 mL of Lactated Ringer's Solution (Hospira Inc.; each 100 mL contains 600 mg sodium chloride, 310 mg sodium lactate-anhydrous, 30 mg potassium chloride and 20 mg calcium chloride-dihydrate). The NAC infusions were made using 150 mL LRS and 30 mL of 20% Acetylcysteine (American Regents Inc.) to make a 3.3% NAC solution. These solutions were measured and stored in 250 mL LRS bags for easier transport to the barn.

4.3 Irrigation Procedure

Prior to irrigation, each horse was trans-rectally palpated and the uterus was viewed by ultrasound. This was done to ensure that the uterus did not have any pre-existing fluid accumulation as well as to determine the stage in the heat cycle. Horses that displayed anxious behaviors in the stocks were mildly sedated with 5 or 10 mg of

detomidine hydrochloride (Dormosedan, Pfizer). The tail of the horse was wrapped using a non-sterile palpation sleeve and bandage tape, and tied around the neck to give clear access to the vulva. The vulva was then repeatedly washed using cotton soaked in warm water and an iodine scrub (Prepodyne Scrub, West Agro). An insemination pipette was then vaginally inserted through the cervix to the uterus using a sterile palpation sleeve and non-spermicidal sterile lube (Priority Care). The uterus was then irrigated with either LRS or NAC depending on the assigned treatment group. Horses in the no infusion treatment group only underwent the endoscopy procedure detailed below.

4.4 Endoscopy Procedure

The endoscopy procedure was performed on all horses from the no infusion treatment group and on all horses in the LRS and NAC groups 48 hours after irrigation. Prior to endoscopy, the tail of each horse was wrapped and the vulva was washed as described in section 4.3. Horses that displayed anxious behaviors were mildly sedated with 5 or 10 mg of detomidine hydrochloride (Dormosedan, Pfizer). A 9mm diameter flexible endoscope (Fujinon 4400) was then inserted into the uterus through the vagina and cervix. A sterile palpation sleeve and non-spermicidal sterile lube (Priority Care) were used for this process. A video and several still frame images of the uterus were collected for each horse.

5. Results

The still frame endoscopic images collected during this study were reviewed and subjectively scored for the presence (given a score of one) or absence (score of zero) of

several traits that are indicative of inflammation. The traits assessed included; hyperemia, edema, patchy inflammation, petechial hemorrhage, exudate, and transudate. The scoring was completed by a researcher blinded to both the treatment group and image origin. After scoring, a cumulative score was summed and the treatment group and horse name were revealed. The image scores for each horse are recorded in Table 2 and correlated to the treatment group in Table 3. One of the assessed images from each horse follows to support the score given to each uterus (Figures 1-10).

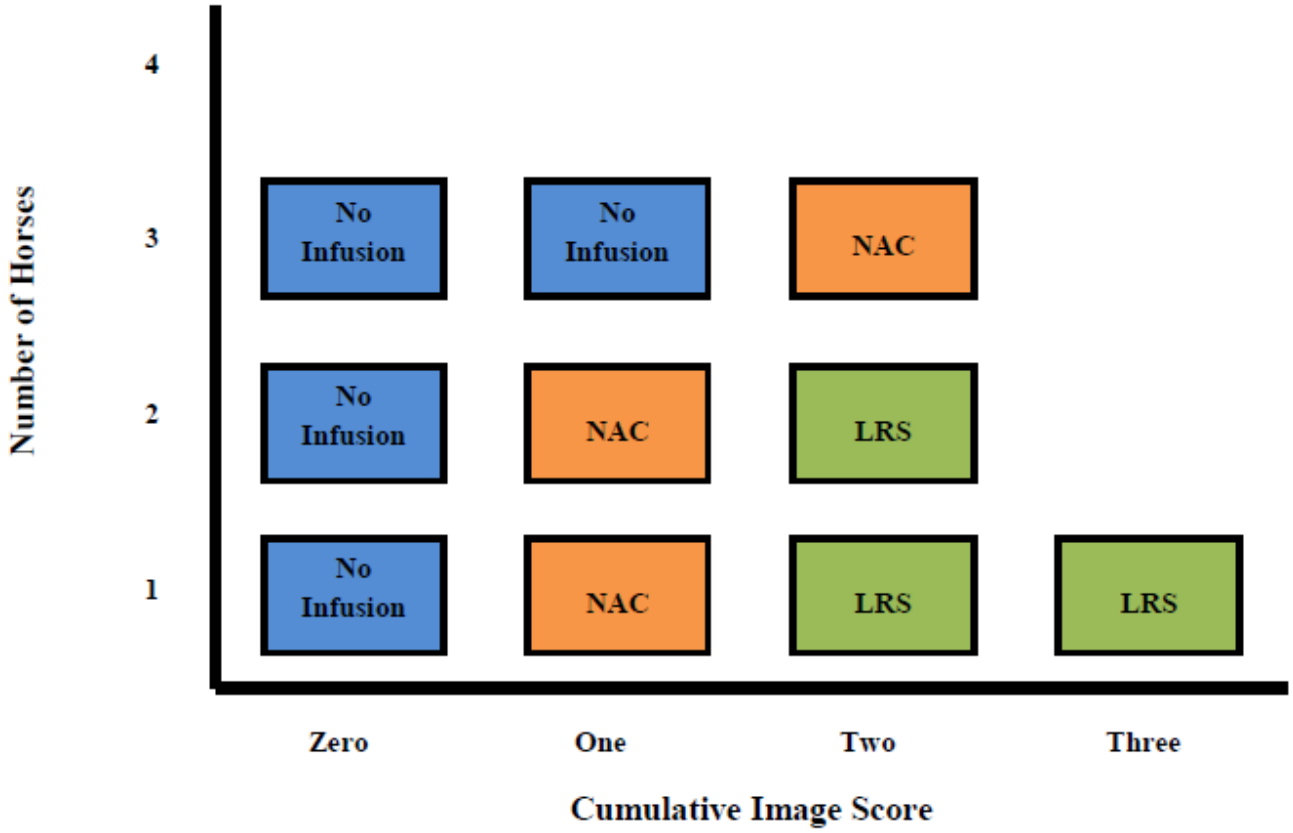
Horses 1, 2, 3 and 10 belonged to the no infusion treatment group and had cumulative scores of zero, zero, one, and zero respectively. Horses 4, 7 and 9 belonged to the NAC treatment group and had scores of two, one, and one respectively. All of these uteri exhibited signs of hyperemia, and the uterus of Horse 4 had several areas with increased redness – classified as patchy inflammation. Horses 5, 6 and 8 were infused with LRS and had uterine scores of three, two, and two respectively. All of these uteri also exhibited signs of hyperemia. Additionally, higher degrees of irritation were noted for the horses that received the LRS infusion. The inflammatory signs noted included: two uteri with edema, one with petechial hemorrhage, and one with transudate. These results indicate that irrigation of either NAC or LRS will cause increased tissue irritation and inflammation when compared to horses in the no infusion group. Additionally, horses in the LRS group demonstrated greater degrees of inflammation in comparison to the horses from the NAC group.

Table 2: Subjective Evaluation of Endoscopic Images of the Uterus

Uterus Trait	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10
Hyperemia	0	0	1	1	1	1	1	1	1	0
Edema	0	0	0	0	1	0	0	1	0	0
Patchy Inflammation	0	0	0	1	0	0	0	0	0	0
Petechial Hemorrhage	0	0	0	0	1	0	0	0	0	0
Exudate	0	0	0	0	0	0	0	0	0	0
Transudate	0	0	0	0	0	1	0	0	0	0
Total	0	0	1	2	3	2	1	2	1	0
***Group Condition**	No Infusion	No Infusion	No Infusion	NAC	LRS	LRS	NAC	LRS	NAC	No Infusion
***Horse Name**	Holly	Whiteout	Andy	Honor	Dewey	Wicky	Laney	Ford	Blisstex	Jill

Zero indicates absence of trait.
One indicates the presence of a trait.
*The information in these rows was not made available until after all images were scored.

Table 3: The Relationship between Uterine Image Scores and Treatment Group



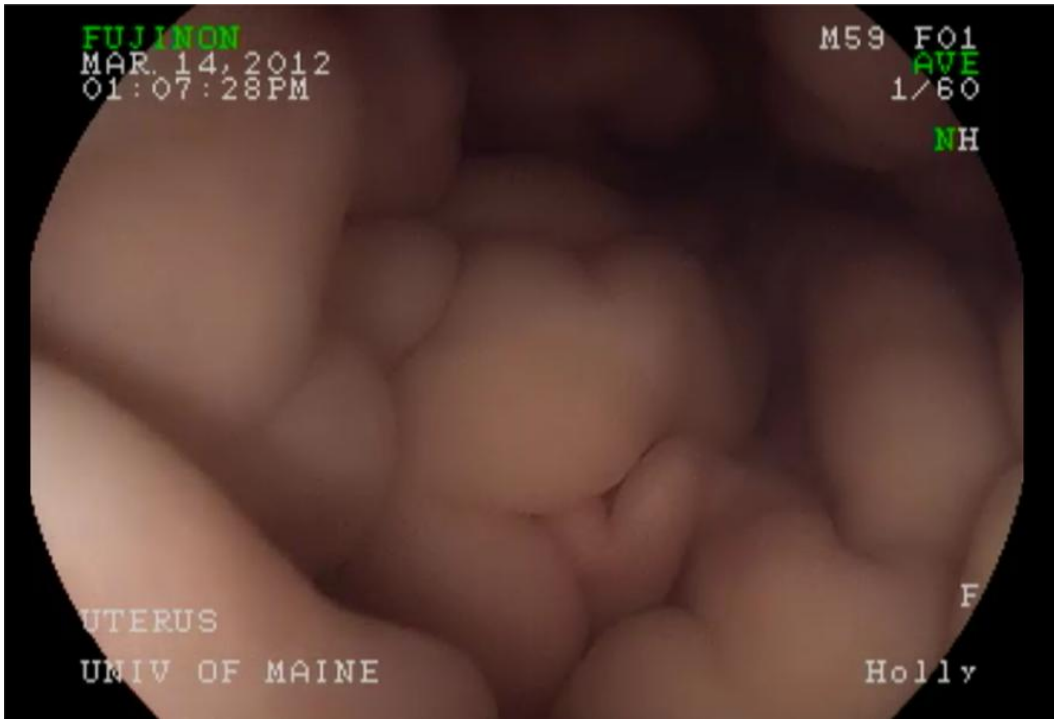
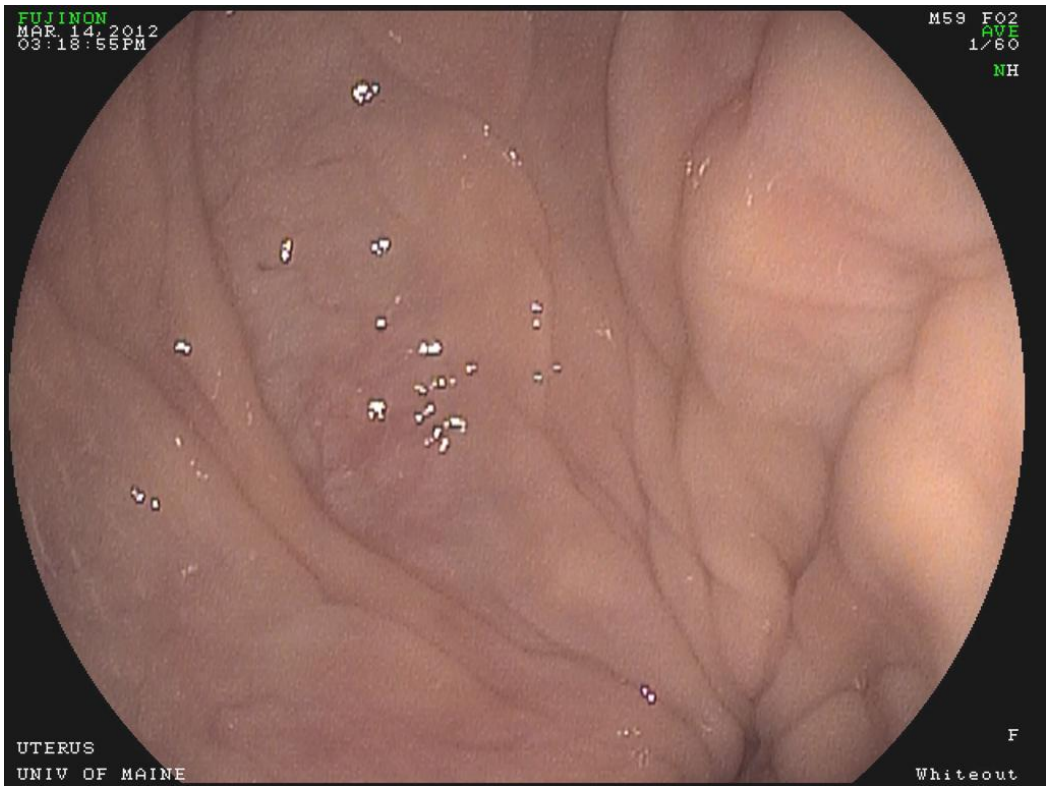


Figure 1 - **Horse #1** (above): Holly – score of zero.

Figure 2 - **Horse #2** (below): Whiteout – score of zero.



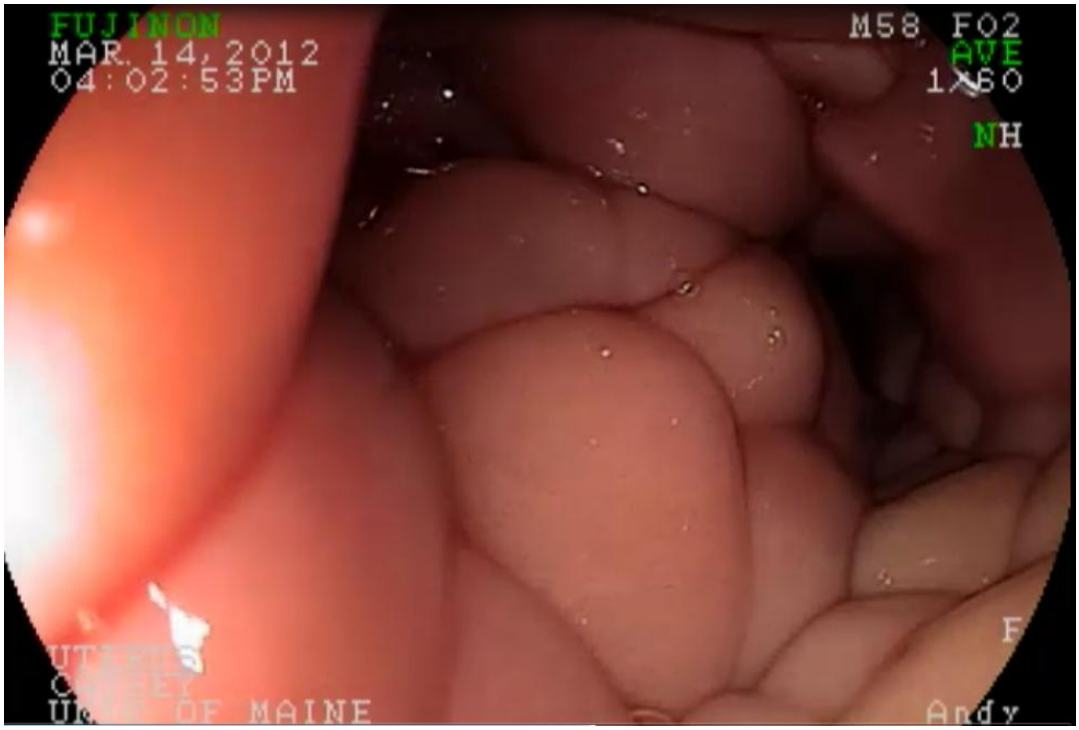
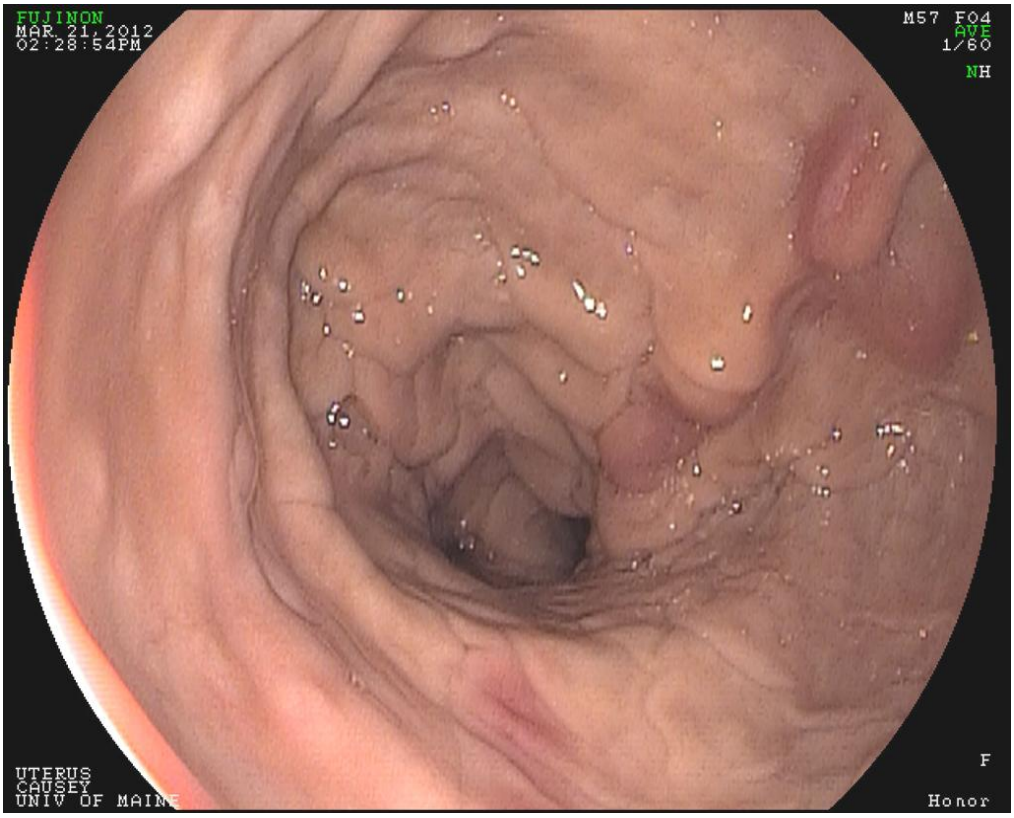


Figure 3 - Horse #3 (above): Andy – score of one; hyperemia.

Figure 4 - Horse #4 (below): Honor – score of two; hyperemia & patchy inflammation.



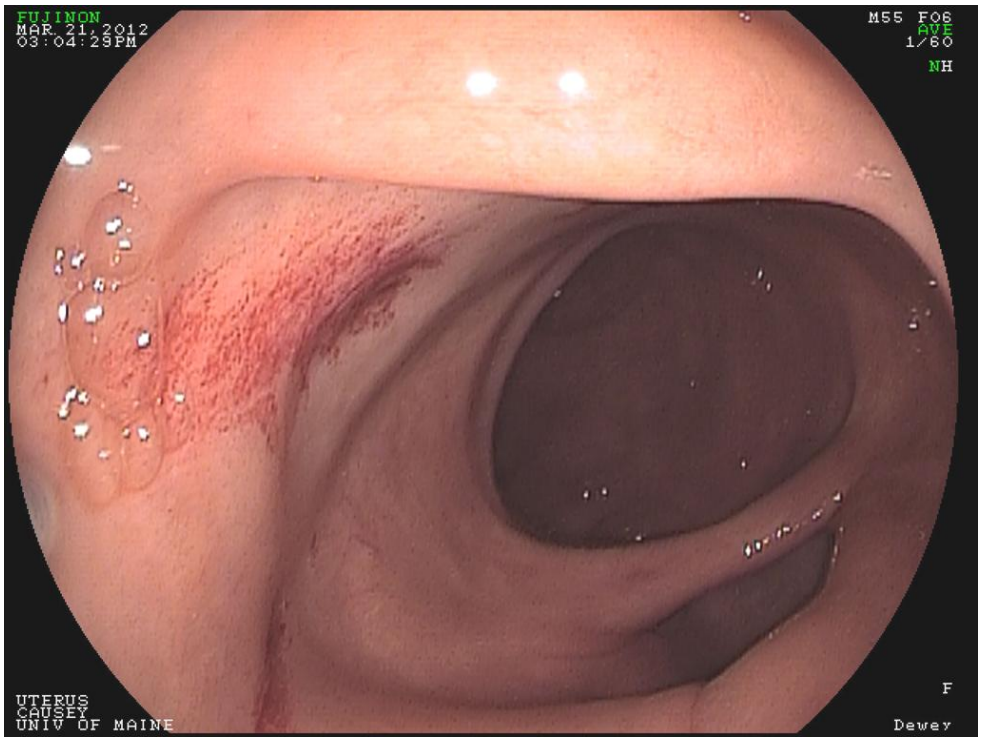
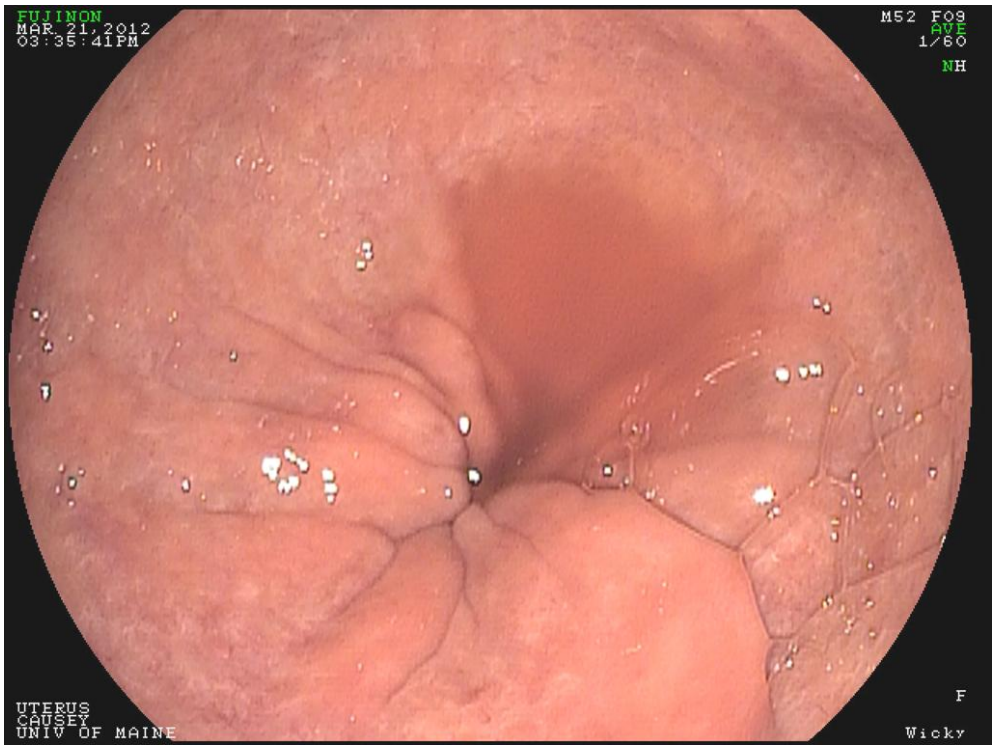


Figure 5 - Horse #5 (above): Dewey – score of 3; hyperemia, edema & petechial hemorrhage.

Figure 6 - Horse #6 (below): Wicky – score of 2; hyperemia & transudate.



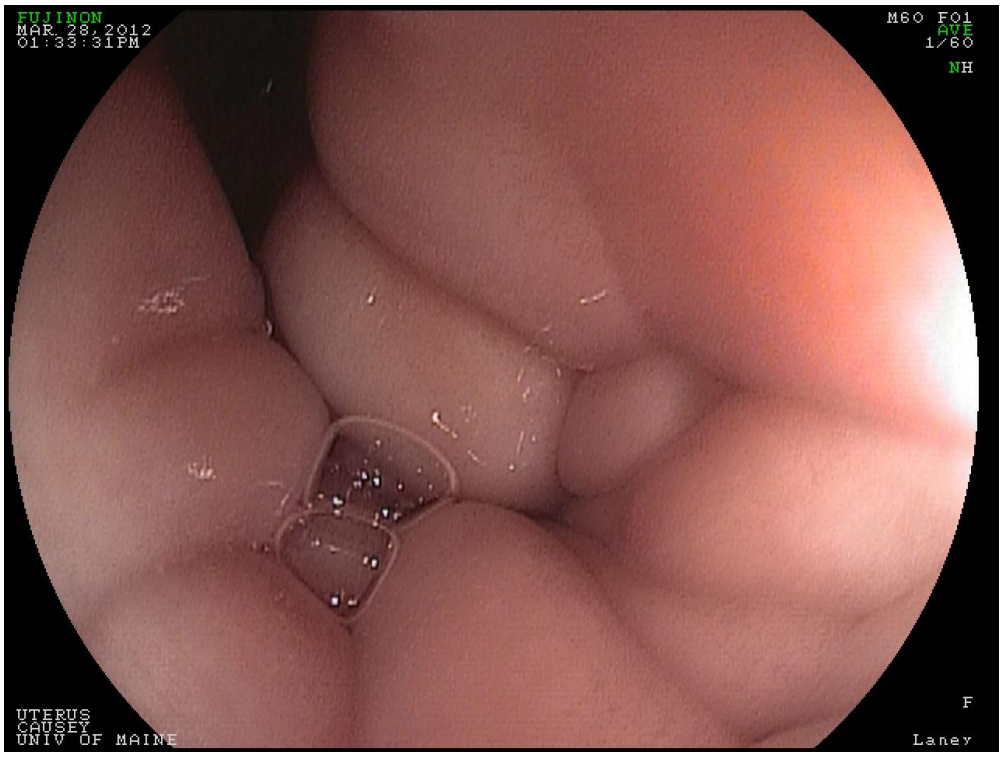
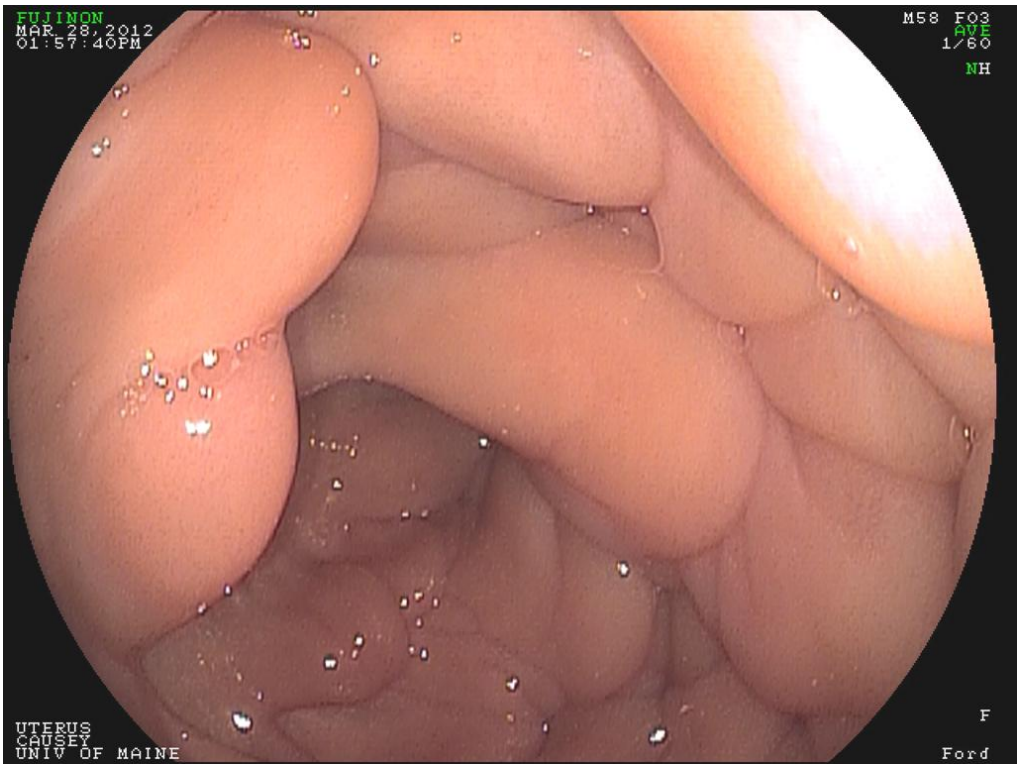


Figure 7 - Horse #7 (above): Laney – score of one; hyperemia.

Figure 8 - Horse #8 (below): Ford – score of two; hyperemia & edema.



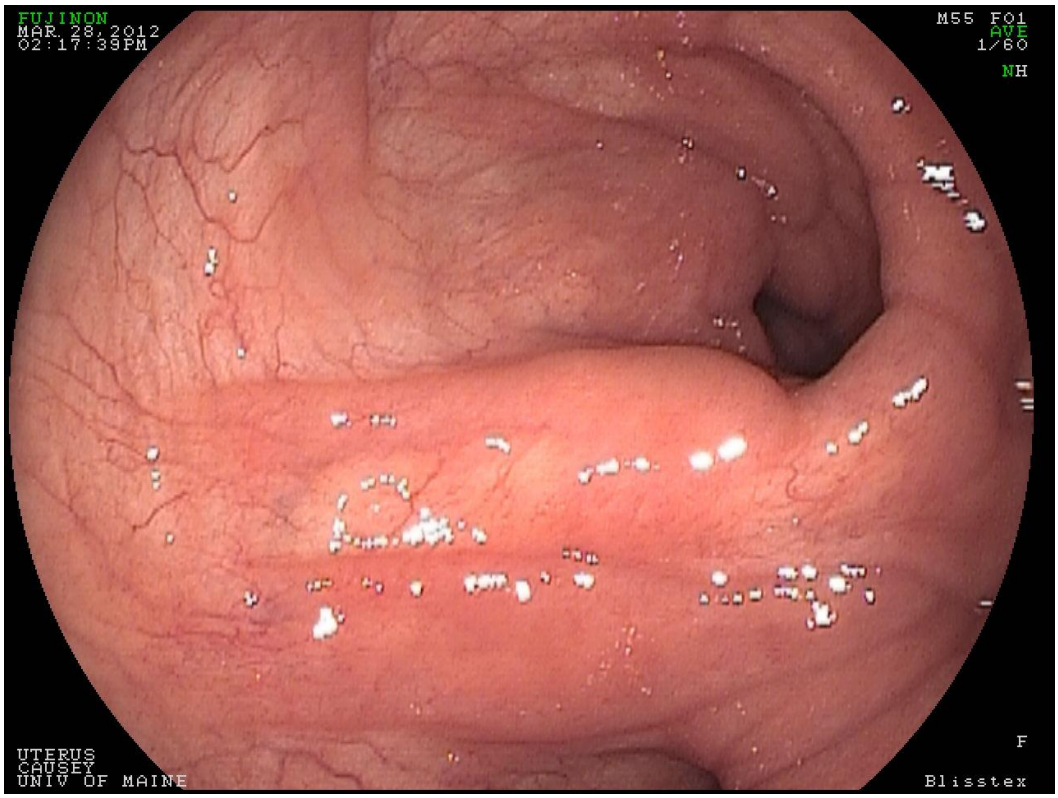
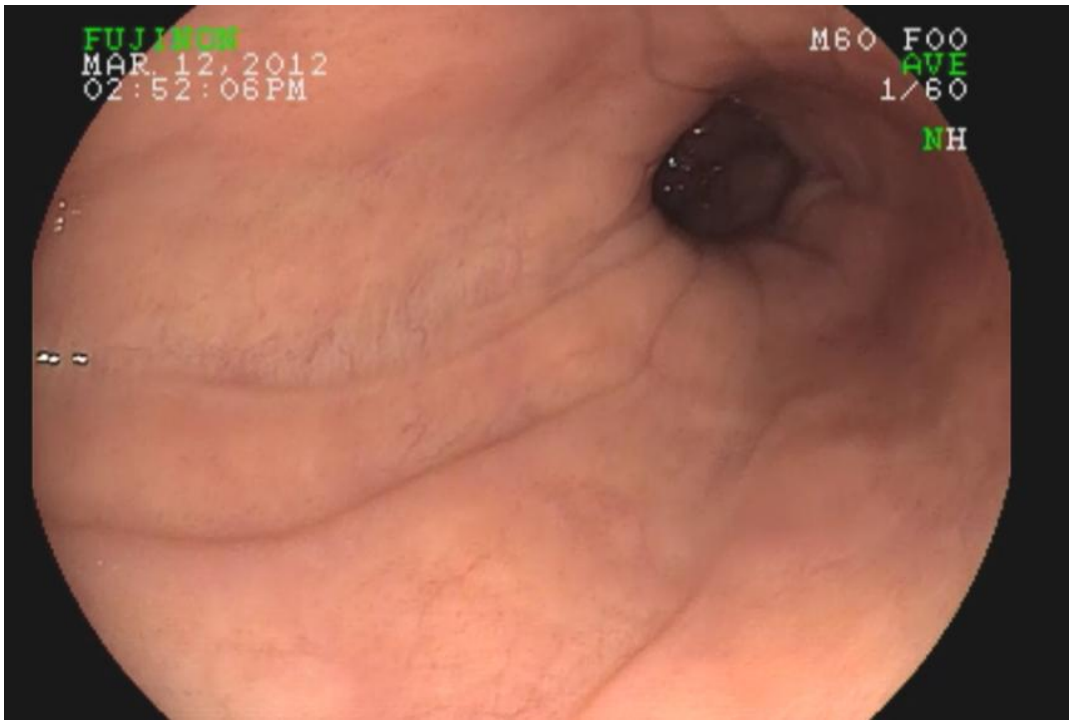


Figure 9 - Horse #9 (above): Blisstex – score of one; hyperemia.

Figure 10 - Horse #10 (below): Jill – score of zero.



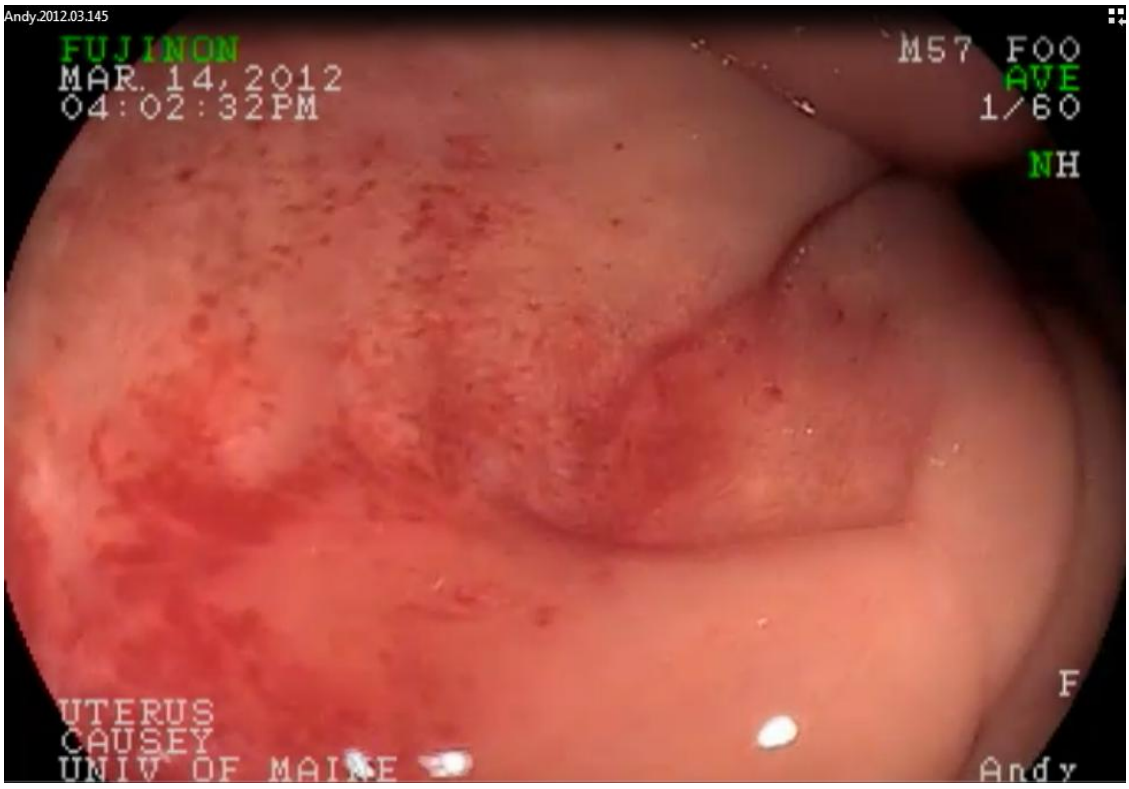


Figure 11 – Image Captured from Video of Horse #3 (above): This video segment revealed petechial hemorrhage in Andy, a horse from the no infusion treatment group.

6. Discussion

In general, the irrigation and endoscopy procedures were well tolerated by the mares involved in this study, and only mild and localized signs of tissue irritation were observed. No sedatives were administered during irrigation. Five horses received sedation for the endoscopy procedure, however; two of these horses were also involved in dental procedures during the same time period and may not have needed sedation solely for the endoscopy procedure.

Andy (horse #3) was the only horse in the no infusion treatment group that showed evidence of inflammation upon endoscopy. The still frame images showed evidence of hyperemia and the uterus was given the score of one. This can be partially explained because the initial endoscopy video collected for this mare did not record. Two days later, this horse underwent a second endoscopy procedure to replace the data. The presence of hyperemia therefore may be associated with cervical penetration. If this is the case, horses infused with NAC or LRS may show hyperemia in response to penetration and this indication of inflammation might not be in direct response to the LRS or NAC solutions. Additionally, an area containing petechial hemorrhage was discovered upon review of the video for Andy (see Figure 11). Video findings were not evaluated in this study and Table 2 does not reflect this finding. This incidence of petechial hemorrhage is caused by mechanical damage from the endoscope and cannot be attributed to an infusion since this mare was in the no infusion treatment group.

Transudate and petechial hemorrhage were two greater degrees of tissue irritation seen for the LRS treatment group. The petechial hemorrhage seen in the uterus of Dewey (horse #5) could have resulted from the insemination pipette or the endoscope scraping the uterine tissues as was similarly found in Andy (horse #3). Additionally, the reddish-brown fluid labeled as transudate found in Wicky (horse #6) was not sampled for testing and can therefore not be identified with certainty. Both of these findings could be a result from pre-existing uterine damage or mechanical damage as a result of this study, and may not be directly caused by the LRS solution. Future studies should consider collecting

initial endoscopic images before irrigation which may help distinguish the direct cause of these two types of inflammatory responses.

There is a clear relationship between the cumulative uterine score and the treatment group (see Table 3). Horses in the no infusion group showed no or limited inflammation, and horses in the NAC treatment group showed less signs of tissue irritation when compared to horses from the LRS treatment group. Since the 3.3% NAC solution was made by diluting 20% acetylcysteine in Lactated Ringer's Solution, these results indicate that NAC has an anti-inflammatory effect. A few other recent studies have similarly found that NAC is anti-inflammatory when compared to saline treatments (Melkus et al., 2011; LaBlanc, 2010). LaBlanc (2010) states; "Data indicated that NAC was not harmful to the endometrium and that it may counteract the irritating effect of saline."

Future studies should continue to evaluate the anti-inflammatory properties of NAC through blinded, scientific studies. Additionally, varying concentrations of NAC should be tested and other time intervals should be used to verify that NAC is only mildly irritating to mucosal tissue. Testing in the uterus has provided many benefits to this study, and although continuing studies should use this organ, tissue response to NAC irrigations applied to the guttural pouch also need to be evaluated. It is recommended that further studies evaluate the safety of infusing a 3.3% NAC solution into the guttural pouch as well as test NAC for efficacy as a mucolytic agent in the treatment of horses carrying *S. equi* after strangles outbreaks.

It must be noted that the techniques of irrigation and endoscopy may limit the practicality of infusing the guttural pouch with NAC as a treatment for strangles. These techniques may not be easily adaptable to field work and may be better suited for use in hospital settings. Irrigation of the guttural pouch requires the use on an endoscope, and this equipment is too bulky and expensive to be used widely by practitioners in the field.

7. Conclusions and Implications

The results of this observational study show that intrauterine infusions of NAC and LRS cause increased irritation to epithelial tissue when compared to horses from the no infusion treatment group. Additionally, the uteri infused with NAC showed less inflammatory response when compared to those infused with LRS indicating that NAC could have anti-inflammatory affects on mucosal tissues. All inflammatory responses were mild and localized to the uterus. Evidence from this study suggests that NAC does have the potential to be a low-risk treatment option for the carrier state of strangles. Further testing will be needed to verify the anti-inflammatory properties of NAC as well as to evaluate the efficacy of cleansing the guttural pouch of horses involved in strangles outbreaks with NAC.

8. References

American Regent, Inc., 2005. Acetylcysteine Solution 20% [package insert]. Shirley, NY.

Atkuri, K.R., Mantovani, J.J., Herzenberg, L.A., Herzenberg, L.A., 2007. N-acetylcysteine – a safe antidote for cysteine/glutathione deficiency. Current Opinion in Pharmacology 7, 355-59.

Aughey, E., Frye, F.L., 2001. Comparative veterinary histology with clinical correlates [electronic version]. London, Manson Publishing Ltd.

Baptiste, K.E., Naylor, J.M., Bailey, J., Barber, E.M., Post, K., Thornhill, J., 2000. Physiology: a function for guttural pouches in the horse. Nature 403, 382-83.

Bentz, B.G., Dowd, A.L., Freeman, D.E., 1996. Treatment of guttural pouch empyema with acetylcysteine irrigation. Equine Practice 18, 33-35.

Carmalt, J., 2002. Guttural pouch diseases in the horse. Large Animal Veterinary Rounds 2(2).

Causey, R.C., Ginn, P.S., Katz, B.P., Hall, B.J., Anderson, K.J., LeBlanc, M.M., 2000. Mucus production by endometrium of reproductively healthy mares and mares with delayed uterine clearance. Journal of Reproduction and Fertility 56(Suppl.), 333-339.

Chiesa, O.A., Vidal, D., Domingo, M., Cuenca, R., 1999. Cytological and bacteriological findings in guttural pouch lavages of clinically normal horses. Veterinary Record 144, 346-49.

Guss, B., Flock, M., Frykberg, L., Waller, A.S., Robinson, C., Smith, K.C., Flock, J., 2009. Getting to grips with strangles: an effective multi-component recombinant vaccine for the protection of horses from *Streptococcus equi* infection. PLoS Pathogens 5(9).

Henke, M.O., Ratjen, F., 2007. Mucolytics in cystic fibrosis. Paediatric Respiratory Reviews 8; 24-29.

Holland, R.E., Harris, D.G., Monge, A., 2006. How to control strangles infections on the endemic farm. AAEP Proceedings 52, 78-80.

Knowles, E.J., Mair, T.S., Butcher, N., Waller, A.S., Wood, J.L.N., 2010. Use of a novel serological test for exposure to *Streptococcus equi* subspecies *equi* in hospitalized horses. Veterinary Record 166, 294-297.

LeBlanc, M.M., 2010. Advances in the diagnosis and treatment of chronic infectious and post–mating-induced endometritis in the mare. Reproduction in Domestic Animals 45(Suppl. 2), 21–27.

Levine, M., LoVecchio, F., 2011. Acetaminophen toxicity. Primary Care Report 17, 13-22.

McGhie, N.C., 2010. The evaluation of N-acetylcysteine for reduction of post-breeding uterine inflammation in mares. University of Maine Honors Thesis [unpublished].

Melkus, E., Witte, T., Walter, I., Heuwieser, W., Aurich, C., 2011. Investigations on the endometrial response to intrauterine administration of N-acetylcysteine in oestrous mares [In Press]. Reproduction in Domestic Animals, manuscript ID: RDA-OA-Oct-2011-0487.

Newton, J.R., Wood, J.L.N., Dunn, K.A., DeBrauwere, M.N., Chanter, N., 1997. Naturally occurring persistent and asymptomatic infection of the guttural pouches of horses with *Streptococcus equi*. Veterinary Record 140, 84-90.

Newton, J.R., Verheyen, K., Talbot, N.C., Timoney, J.F., Wood, J.L.N., Lakhani, K.H., Chanter, N., 2000. Control of strangles outbreaks by isolation of guttural pouch carriers identified using PCR and culture of *Streptococcus equi*. Equine Veterinary Journal 32, 515-526.

Perkins, G.A., Pease, A., Crotty, E., Fubini, S.L., 2003. Diagnosing guttural pouch disorders and managing guttural pouch empyema in adult horses. Compendium 25, 966-983.

Reinero, C.R., Lee-Fowler, T.M., Dodam, J.R., Cohn, L.A., DeClue, A.E., Guntur, V.P., 2011. Endotracheal nebulization of N-acetylcysteine increases airway resistance in cats with experimental asthma. Journal of Feline Medicine and Surgery 13, 69-73.

Rosser, J.M., Waller, A.S., Traub-Dargatz, J.L., Salman, M.D., Magnuson, R., Triantis, J., Newton, J.R., 2007. Identification of a *Streptococcus equi* strain responsible for four outbreaks of strangles in Colorado. Journal of Equine Veterinary Science 27, 395-97.

Sweeney, C.R., Timoney, J.F., Newton, R., Hines, M.T., 2005. ACVIM consensus statement; *Streptococcus equi* infections in horses: guidelines for treatment, control and prevention of strangles. Journal of Veterinary Internal Medicine 19, 123-134.

Taylor, S.D., Wilson, W.D., 2006. *Streptococcus equi* subsp. *equi* (strangles) infection. Clinical Techniques in Equine Practice 5, 211-17.

Thorne Research, Inc., 2002. N-acetylcysteine. Alternative Medicine Review Monographs, 281-286.

Waller, A.S., Jolley, K.A., 2007. Getting a grip on strangles: recent progress towards improved diagnostics and vaccines. The Veterinary Journal 173, 492-501.

Waller, A.S., Paillot, R., Timoney, J.F., 2011. *Streptococcus equi*: a pathogen restricted to one host. Journal of Medical Microbiology 60, 1231-40.

Appendix 1 – IACUC Approval

PROTOCOL NUMBER: **August 2010 version**

UNIVERSITY OF MAINE
PROTOCOL REVIEW FORM
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

GENERAL INSTRUCTIONS: The Institutional Animal Care and Use Committee (IACUC) consists of scientists from several disciplines as well as non-scientists, members of the University community, and persons who have no other affiliation with the University than as members of the Committee. The protocol should therefore be described in terms understandable by an audience of educated nonspecialists. **Please return the completed protocol to the Institutional Animal Care and Use Committee, c/o Office of the Vice President for Research, 114 Alumni Hall**. **The form is due TWO weeks prior to a scheduled IACUC meeting. The meeting dates are posted at: http://www.umaine.edu/research/research-compliance/institutional-animal-care-and-use-committee-iacuc/meeting-schedule-and-protocol-due-dates/. Protocols received late will be held until the next month's meeting. Please call Gayle Jones at 1-1498 if you have questions.**

The Principal Investigator or Instructor must justify the ethical costs of using live animals by demonstrating a reasonable expectation that such usage will contribute to the advancement of knowledge which may eventually benefit humankind and/or animals. The Principal Investigator or Instructor must further demonstrate that he or she has applied the concepts of "alternatives" in designing the protocol. The term "alternatives" includes three components: replacement (using organisms that are phylogenetically lower, cell cultures, tissues from slaughter or autopsy, or nonanimal systems); reduction (in the number of animals used); and refinement (of design and methods to reduce pain and stress to animals used as well as ensuring that the number of animals used is optimal for the analysis proposed).

1. Principal Investigator, Co-Investigator(s), or Instructor(s), with **campus address, office phone, and lab phone** of PI (NOTE: The Principal Investigator or Instructor must be a faculty member or professional staff):

   PI: Robert Causey Associate Professor, Animal and Veterinary Sciences, Hitchner Hall Tel: 581-2782

   CI: Christine Kissinger (AVS Capstone/Honors student)

   Title and number of course/Title of project:

   Endoscopic evaluation of the response to intrauterine irrigation with 3.3 % N Acetyl Cysteine

Project/Course Start Date: **February 1, 2012**

Number of years project is planned to continue: **Three**
(IACUC approval is granted for three years)
If this is a renewal application, please enter the exact project or course title and previous protocol code. Be sure to identify any changes from the previously approved protocol.

Funding agency for project, if applicable. Include title of proposal if different from this protocol.

Maine Technology Institute/Maine Technology Asset Fund: Commercialization of New Technologies for Animal Disease Surveillance

2. Describe the (check appropriate category) __X__research, __X__teaching, or ____production objectives **(not procedures)** that involve use of animals. Explain these objectives in non-technical language. Do not paste in sections of grant proposals.

To detect and describe by endoscopic examination of the equine uterus the occurrence of irritation caused by intrauterine infusion of N - acetyl cysteine.

3. Describe how this use of animals contributes to the advancement of knowledge, which may eventually benefit humankind and/or animals.

Infertility in the mare is a serious cause of economic loss to horse owners. In addition, infertile mares quickly lose their value and run the risk of being sold, discarded or abandoned. Methods to treat infertility in mares therefore benefit both horse owners and maintain the value of breeding stock.

Recently 3.3% N-acetyl cysteine (NAC) in lactated ringers solution (LRS) has shown promise as an anti-inflammatory and mucolytic agent when infused in the equine uterus, and has been associated with improved conception rates. In contrast, 20 % N-acetyl cysteine administered in the guttural pouch has been associated with mucosal irritation as detected by endoscopy. We therefore wish to determine if N-acetyl cysteine, used at 3.3% strength, shows signs of irritation in the uterus, which may be a contraindication for its use.

Because this is the first time an endoscopic evaluation of the uterus has been performed post NAC infusion, it is necessary to classify this as a pilot-study in which variation to infusion may be estimated, preparatory to possible follow-up studies.

4. Identify the animals to be used (genus and species) and number (for the entire project).

Equus caballus 12 total, 4 treatment animals (3.3% NAC in LRS), 4 animals infused with LRS only and 4 uninfused controls.

5. State the rationale for use of this/these species. Address the issue of replacement by explaining why educational or research objectives cannot be met by the use of phylogenetically lower organisms, cell or tissue cultures, or non-animal systems. (Please note: the IACUC does not consider "hands-on experience" to be in and of itself an adequate educational objective, unless the course serves students whose anticipated educational and professional futures will require the skills imparted through such hands-on experience. If that is true in this instance, please describe the student population that typically enrolls in the course.)

There is no substitute for the live horse in this study because the horse tends to be idiosyncratic in its response to uterine infusions. Consequently there is no in-vitro or computerized model which we can use to confidently predict response to infusion with NAC, nor could results in a phylogenetically lower organism be extended with confidence to the horse.

6. Justify the number of animals:

a. Explain how the number of animals required fits your experimental design or teaching or production/breeding objectives.

We are planning a descriptive pilot - study involving 4 animals treated with NAC, 4 treated with LRS, and 4 un-infused animals. Assessment of acute endometrial irritation is based on visual inspection of endometrial hyperemia (redness due to increased arterial blood flow), hemorrhage, edema and exudate. The internal medicine literature describing irritation of the equine guttural pouch infused with 20 % NAC raises sufficient questions about the potential irritation caused by NAC to justify this study.

b. Give the rationale for the number in terms of the statistical methods to be used. Address the issue of reduction by explaining why the proposed number is sufficient, but not excessive. (A simple statement that the number proposed is required for statistical significance is not an adequate response.) See How to do a Power Analysis. For research in which the number of animals is limited not by statistical power, but by the number of animals that can be captured, maintained, or sampled, a power analysis will provide an indication of the statistical power of the proposed tests based on the variance measured by the researcher, or others, in previous studies. For studies not amenable to a Power Analysis (e.g., no data on variability available, only descriptive data available), provide a justification for why a power analysis is not appropriate for your project.

The number requested is adequate because this is a pilot study and no formal statistical analysis is proposed.

7. PROCEDURES

The Committee does not wish to receive copies of research proposals or laboratory manuals. The Principal Investigator or Instructor is asked to address succinctly the following questions, as applicable. Special care should be taken to justify any procedures generally discouraged by the University's code of ethics and policy.

a. Major categories of procedures. Please check the appropriate box for **each category**.

Any "yes" responses must be described in sections b. (nonsurgical procedures) and/or c. (surgical procedures) that follow. Please attach the category headings (from 1-16 below) to the description of procedures in sections 7b and c.

Yes	No		
☐	X	1.	collection or capture
☐	X	2.	kill and harvest tissue
☐	X	3.	immunization for antibody production: describe antigen, adjuvant used, route of immunization, method of obtaining blood
X	☐	4.	physiologic measurements
☐	X	5.	dietary manipulations

X	☐	6.	pharmacology/toxicology: material used, route of administration, etc. **3.3 % NAC in lactated ringers solution administered intrauterinely**
☐	X	7.	behavior studies
☐	X	8.	environmental stress, e.g., temperature, restraint, forced exercise
☐	X	9.	irradiation: type, facility to be used
☐	X	10.	hazardous materials, e.g., carcinogens, radioactive materials
☐	X	11.	biohazardous or infectious agents (use of Class 2 or higher agents requires the approval of the University's Biosafety Committee)
☐	X	12.	experimental trauma
☐	X	13.	nonsurvival surgical procedure
☐	X	14.	survival surgical procedure (animal is allowed to recover for any length of time)
☐	X	15.	multiple major operative procedures from which animal is allowed to recover
X	☐	16.	other, specify: **Uterine infusion and uterine endoscopy**

b. Nonsurgical Procedures:

Describe all nonsurgical manipulations or procedures, if any, involving the animal, e.g., drug administration, blood collection, diet change, collection, capture. Specify the drug(s), dose, route of administration, or other methods used. Specify duration of procedures. If an adjuvant will be used, state the number of injection sites per dosage and the number of doses.

Using aseptic technique a sterile solution of 30 mL of a 20% solution of NAC combined with 150 mL of sterile lactated ringers solution will be introduced into the uterus of 4 mares. An additional 4 mares will receive 180 mL lactated ringers solution only. Twenty four hours following infusion the perineal region will be aseptically prepared and a chemically sterilized, 9mm diameter flexible endoscope introduced per vaginam into the uterus, and the endometrium visualized and recorded digitally. Following endoscopy of all mares images of the uteri will be examined as a group and endometria indicating irritation will be identified. Assessment of irritation will be made by an individual (the PI) blinded to whether the horse received an infusion of NAC or saline.

Uterine endoscopy and uterine infusion are routine procedures in the mare which may be performed without sedation. However, sedation will be administered if the mare appears anxious (detomidine hydrochloride - 5 or 10 mg IV). This is a routinely used method of sedation well tolerated by horses. Sedated horses will be allowed time to regain full consciousness in their stall, hay and water having been removed to prevent choking hazards, and then turned back out onto pasture when fully alert.

Examinations will not exceed 10 minutes, and will be curtailed if evidence of irriation associated with endoscopy is detected.

Will the animals be killed? If so, what method of euthanasia will be used? (Include dosages if applicable.) See the 2007 Report of the AVMA Panel on

Euthanasia for assistance (http://www.avma.org/issues/animal_welfare/euthanasia.pdf). If not, what final disposition of the animals is planned?

NO. Animals will remain part of the UMaine Standardbred herd at the Witter Center.

If euthanasia becomes necessary, due to unplanned injury or illness to the animal(s), how will it be accomplished (include dosages if applicable)? See the 2007 Report of the AVMA Panel on Euthanasia for assistance (http://www.avma.org/issues/animal_welfare/euthanasia.pdf). NOTE: question must be answered.

If necessary, euthanasia will be performed by rapid intravenous administration of 100 ml of 390 mg/mL Sodium pentobarbital with 50 mg/mL sodium phenytoin (euthanasia solution) by one of the attending veterinarians from Foxcroft Veterinary Services, Dover-Foxcroft, ME.

c. Surgical Procedures:

Describe briefly any surgical procedures. Describe the anaesthetic method, including all drugs, dosages, routes of administration, and supplementation schedules. Describe the postsurgical monitoring and care procedures, including what response(s) you will look for to indicate recovery, and the method of euthanasia (if it becomes necessary due to unplanned injury or illness).

N/A

Is animal allowed to recover for any length of time? Yes/No If yes, how long will animal survive surgery?

N/A

If there is potential for discomfort or pain as a result of the procedures, describe their nature and duration. Explain what will be done to relieve them, including drugs and dosages, nursing care, mechanical devices, etc.

N/A

d. Search for Alternatives: If the proposed procedures cause more than momentary or slight pain or distress to the animal(s), federal regulations require that you search for alternatives. The PI must provide a written narrative description **(see NOTE below)** of the sources used to determine that less painful alternatives are not available. To fulfill this requirement, bibliographical searches may be performed through the Animal Welfare Information Center (AWIC) of the National Agriculture Library (http://awic.nal.usda.gov/nal_display/index.php?info_center=3&tax_level=1&tax_subject=184).

Will the proposed procedures cause more than momentary or slight pain or distress to the animal(s)?

X No. (Examples of procedures for which this response is appropriate include observation of behavior under conditions of little or no distress, dietary manipulations, and injections or blood sampling by qualified personnel.)

☐ Yes. (Examples of procedures for which this response is appropriate include survival surgery, nonsurvival surgery, electrofishing, and procedures producing pain or distress unrelieved by analgesics such as toxicity studies, microbial virulence testing, radiation sickness, and research on stress, shock, or pain.) The required narrative is attached.

→ **NOTE: When preparing the narrative, the instructions in the USDA policy MUST be followed. For a description of this policy, see: http://www.aphis.usda.gov/animal_welfare/downloads/policy/policy12.pdf**.

→ **NOTE**: When proposed procedures will cause more than momentary or slight pain or distress to animal(s), **a consultation with the attending veterinarian must occur in the planning stages of those procedures prior to submitting this protocol for review**. Please email Dr. James Weber, james.weber@umit.maine.edu, with a description of the proposed procedures and your approach to minimizing pain/distress.

8. Animal Sources and Housing

a. Please indicate source of animals. Note: The IACUC will approve animal purchases from a licensed pet store provided the researcher/instructor informs the pet store (in writing) that the purchased animals will be used for research/teaching.
UMaine Standardbred Herd

b. If the animals are caught in the wild:
N/A
Where and how will the animals be trapped?
N/A
How often will the traps be checked?
N/A
What other steps will be taken to protect the animals from exposure or other danger?
N/A
Indicate if Federal/State permits are required and whether they have been obtained.
N/A
If the animals will be brought to the campus, what precautions will be taken to prevent zoonotic diseases?
N/A
Please include your plans for removal of traps or barriers (e.g., pitfall traps).
N/A

c. Where will the animals be housed? Describe the housing (See the Guide for the Care and Use of Lab Animals

(**http://www.nap.edu/readingroom/books/labrats/**) pages 22-36) for information on Physical Environment, including space requirements. If your housing will not meet the Guide requirements, include a request for an exception to the Guide. If your animals are not listed in the Guide, housing should meet the recommendations of the appropriate guidelines for the proposed species. Include dimensions of cage, tank, etc.

J F Witter Teaching and Research Center

d. Identify the room or facility in which the procedures will be conducted.

Animal Handling Room

9. List all person(s) (including PI) who will handle animals (e.g., carry out the procedure(s), animal care, etc.) or provide training of personnel. For each person named below, describe his/her experience in performing proposed procedures; if none, explain how training will be obtained.

Name/Title/Experience

Robert Causey DVM, PhD, Diplomate American College of Theriogenologists. Has experience of uterine infusion and endoscopy in teaching, clinical and research settings.

Christine Kissinger: Senior student. This project is her capstone/honors thesis and all her work will be carried out under the supervision of the PI.

10. If animals will be housed, please list the name, phone number, and email of the person who should be contacted to accompany the IACUC during facility inspections:

Robert Causey 581-2782 email on first class

11. Have all personnel named above been certified by the IACUC for Responsible Care and Use of Animals?
X Yes ☐ No A web-based tutorial for this certification is available at: http://umaine.edu/research/research-compliance/institutional-animal-care-and-use-committee-iacuc/web-based-training/. (Note: protocols will not be processed until all personnel have been certified.)

12. Risk Assessment (risks to researchers):

In compliance with our Public Health Service Animal Welfare Assurance, we have implemented an Occupational Health/Medical Surveillance Program. The first step will be for investigators to identify potential hazards with tasks involved with the study, so the IACUC veterinarian and Safety and Environmental Management (SEM) can assess the risks to determine if further information will be required from everyone named in the protocol (i.e., a health questionnaire).

Please complete the following for your proposed protocol.

a) Provide a brief description of the protocol (cut and paste response from question 2 of the protocol). (NOTE: Only this page, not the whole protocol, goes to SEM and the Occupational Health Physician, thus the request for duplication of the answer to question 2.)

To detect and describe by endoscopic examination of the equine uterus the occurrence of irritation caused by intrauterine infusion of N- acetyl cysteine.

b) List the tasks required.

1. **Uterine Endoscopy**
2. **Uterine infusion**
3.

c) For each of the tasks described in b) above, list the associated hazards.

1. **Horse resisting procedures leading to injury of people**
2.
3. etc.

d) For each of the hazards described in c) above list how the hazards will be managed.

1. **Chemical Restraint (sedation) if necessary**
2. **Physical Restraint (stocks)**
3. **Animal handlers trained by and under supervision of PI.**

NOTE: In evaluating this risk assessment statement, we will be looking for animal care tasks that increase the risk of illness (such as a zoonotic disease), physical injury (such as animal bites), and/or allergic reactions to those handling the animals.

After this risk assessment is reviewed, everyone named in the protocol may be required to complete a health questionnaire. The health questionnaire may require review by the Occupational Health Physician. If so, there is a charge for this review, and you will be required to provide an account number. (Currently the charge is $45; we do not know the cost of a physical exam if warranted after review of the questionnaire, but this page will be updated when that information is known.)

If you have any questions regarding the completion of this page, please contact James Patrick, Safety and Environmental Management, 1-4055.

ASSURANCES FOR THE HUMANE CARE AND USE OF ANIMALS

As the Principal Investigator on this protocol, I assure that…

1) I have provided an accurate description of the animal care and use protocol to be followed in the proposed project/course.

2) the activities proposed do not unnecessarily duplicate previous experiments.

3) all individuals named in this application who are at risk will be registered in the Occupational Health and Safety Program.

4) all individuals performing animal procedures described in this application are technically competent and have been (or will be) properly trained in the procedures to ensure that no unnecessary pain or distress will be caused as a result of the procedures.

5) I will obtain approval from the IACUC before initiating any changes to this protocol.

6) I am familiar with and will comply with the *University of Maine's Policies and Procedures for the Humane Care and Use of Animals*, and I assume responsibility for compliance by all personnel involved with this protocol.

7) I have read and will follow the appropriate guidelines for the proposed species.

8) if using laboratory animals, all personnel handling the animals have had a tetanus shot within the past ten years.

9) all applicable rules and regulations regarding radiation protection, biosafety, recombinant issues, hazardous chemicals, etc., have been addressed in the preparation of this application and the appropriate reviews have been initiated.

10) animals will be purchased only from licensed, reputable vendors. If animals are purchased form a pet store, the pet store has been informed (in writing) that the animals will be used for research or teaching purposes.

11) I will maintain appropriate animal records (e.g., census, health, veterinary care, euthanasia, surgery, diagnostic, anesthesia, etc.)

12) **I will report at once to the IACUC any unanticipated harm to animals.**

Signature of Principal Investigator/Instructor — **Date** 1/17/12

I hereby confirm that I have read this protocol and my signature denotes departmental approval of this project.

Signature of Department Head/School Director — **Date** 1/17/12

PROTOCOL NUMBER: A2012-01-05

Course/Project Title: Endoscopic Evaluation of the Response to Intrauterine Irrigation with 3.3% N Acetyl Cysteine

PI:

Causey Robert

For Committee Use Only:

Date of IACUC Receipt: 1/17/2012 Date of IACUC Review: 1/24/2012

Committee Action:

____ 1. Approved until ____________
Species and # of animals approved: ____________

✓ 2. Modifications required.
Modifications accepted for approval: 2/3/2012 Approved until: 2/6/2015
Species and # of animals approved: 12 mares

____ 3. Disapproved. See attached statement.

____ 4. Reviewed and determined not to fall under the Policies and Procedures for the Humane Care and Use of Animals (explanation) ____________

Full IACUC Review: ✓ Designated Member Review: ✓ 2/3/2012 by J. Weber + M. Kinnison

IACUC Signatures:

Alan M. Parsons [signature] [signature]

[signature] Susan E. Pearson [signature]

Deborah A. Bouchard

PROTOCOL.DOC August 2010

Biography of the Author

Christine T. Kissinger was born and raised in Rhode Island. Her high school passion for agriculture and participation in FFA lead her to study at the University of Maine in the department of Animal Sciences. Christine graduated in May 2012 with a Bachelor's Degree in Animal Science with a concentration in Pre-Veterinary Medicine and a Minor in Chemistry. Christine worked as a Resident Assistant for the University of Maine for 3 years and as a Maine Learning Assistant for the Physics department for 1 semester. Upon graduation, Christine plans to marry her long time fiancé and will be attending Tufts Cummings School of Veterinary Medicine to earn her DVM degree.