**White Paper:**

**Evaluation of Vetericyn Plus™ Pinkeye Spray as an aid in corneal healing and reduction of pain and infection of the cornea following experimentally induced Bovine Keratoconjunctivitis**

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**Introduction:**

Infectious Bovine Keratoconjunctivitis (IBK), commonly called “pink eye”, is a very painful condition affecting beef and dairy cattle worldwide. The bacterium, *Moraxella bovis* is known to be responsible for this condition. It has been estimated that annual losses associated with only decreased weight gain from infected cattle exceeds 150 million dollars (Lane et al., 2006). Infectious Bovine Keratoconjunctivitis has been referred to as the most important ocular disease in cattle worldwide (Funk et al, 2014). There are a number of pathogens associated with IBK in cattle, such as Bovine Herpes Virus-1 (BHV-1) which is the causative agent of Infectious Bovine Rhinotracheitis (IBR). However, *M. bovis*, a gram negative rod shaped bacteria, has thus far been the only organism demonstrated to cause IBK in cattle (Angelos et al., 2010, Gould et al, 2013). There are other organisms which have the ability to result in severe conjunctivitis and edema of the cornea but they are not known to cause central corneal ulceration (Angelos 2010, Gould et al., 2013). Antibiotic therapy has been primarily utilized for this condition. However, there has been a strong push by the Centers for Disease Control (CDC), the Food and Drug Administration (FDA), the World Health Organization (WHO), and the American Veterinary Medical Association (AVMA) to develop and utilize products that do not predispose to antimicrobial resistance. Hence, the necessity for further evaluation of therapeutics such as Vetericyn™ Pink Eye Spray as an alternative therapy to aid in corneal healing and reduction of pain and infection due to ocular infection in cattle resulting from *M. bovis*.

**Objectives:**

The objectives of the research performed herein were to: 1) assess the ability of Vetericyn Plus™ Pink Eye Spray to decrease and/or eliminate the growth of *M. bovis* in vivo in cattle experimentally infected with *M. bovis* organisms, 2) assess the ability of Vetericyn Plus™ Pink Eye Spray to aid in corneal healing and reduction of pain of experimentally induced corneal lesions resulting from abrasion of the epithelium of the cornea and concurrent *M. bovis* infection, 3) to assess chlorine and sodium residues in the serum, plasma, and chlorine in liver, fat muscle and urine following twice daily administration Vetericyn Plus™ Pink Eye Spray in calves with corneal lesions, 4) collect and assess chlorine residues in milk in lactating dairy cattle following twice daily administration of Vetericyn Plus™ Pink Eye Spray topicaly on experimentally induced corneal lesions and epidermal lesions in dairy cows, and 5) perform a cost analysis of Vetericyn Plus™ verses, parenteral administration of Oxytetracycline, Tulathromycin, and Florfenicol.
Hypothesis:

The hypothesis of this research herein was that Vetericyn Plus™ Pinkeye Spray, when utilized in a standard therapeutic protocol, will be found to be an economically advantageous product which promotes corneal healing and aids in the reduction of pain and infection due to experimentally induced IBK and results in no detectable residues in plasma, serum, milk, urine, fat, liver, and muscle.

Materials and Methods:

Calves:

Thirty dairy, 8 Holstein and 22 Jersey, bull calves having determined to have normal ophthalmic examinations and who were culture negative for M. bovis were randomly assigned to 3 groups for a single eye block randomized blinded challenge study. Calves were housed in pairs according to their respective group in an approved isolation facility. Following topical corneal administration of proparacaine hydrochloride and a single dose of 1.1 mg/kg of Banamine® intravenously (IV), each calf in Groups 1 and 2 had a 0.6 mm corneal lesion made on the left central corneas utilizing n-heptanol. Immediately following lesion formation, 1.0 x 10^7 of Moraxella bovis (strain Epp63-300; origin: NADC) was administered topically to the left central corneas of Groups 1 and 2. The calves in Group 3 (Control group) received topical corneal administration of M. bovis to their left eyes but nothing further. In Group 1, two ml of Vetericyn Plus™ Pinkeye Spray was administered topically to each calves’ cornea twice daily for 10 days. In Group 2, two ml of 0.9% Saline was administered topically to each calves’ cornea twice daily for 10 days. Each animal was given a pain score twice daily (based on blepharospasm, ocular discharge and tearing) utilizing a scale of one to four. All eyes were cultured on day -7, 0, 1-5, and day 10. Daily fluorescein staining was performed on the eyes of all calves followed by digital photography of the lesion to assess healing of the corneas. The sizes of the lesions were assessed daily utilizing J-image software. Additionally, serum and plasma samples were drawn from all calves on days 0, 1, 10, 11, and 17 and evaluated for changes in sodium and chloride levels. Total chlorine levels were measured in Group 1 on days 0, 11, and 17 on urine, fat, liver, and muscle via DPD chlorometric assay.

Lactating Cows:

An additional group of 10 adult lactating Holstein dairy cows were utilized for residue testing on milk samples. A single eye design was also utilized in this portion of the study. Following topical corneal administration of proparacaine hydrochloride and a single dose of 1.1 mg/kg of Banamine® IV, each cow had a 0.6 mm corneal lesion made on the left central corneas utilizing n-heptanol. The cows did not receive topical corneal administration of M. bovis to their left eye. Also two 0.6 mm skin lesions were made in the middle of the left side of each of the cows in the study to maximize absorption of Vetericyn Plus™ Pinkeye Spray. Two ml of Vetericyn Plus™ Pinkeye Spray was administered topically to both corneas of each cow and to one of the skin lesions twice daily for 10 days. Pain scores for each cow were recorded twice daily. Milk samples were collected on day 0, 11, and 17 and analyzed for chlorine via the DPD colorimetric method.

Statistical evaluation was performed utilizing SAS® software. The data were natural log transformed and a Kenwood-Roger correction was utilized. The covariance structure was autoregressive or Toeplitz.

Results:

All lactating cows and all calves in group 1 and 2 developed lesions in the left eye as determined by fluorescein staining. All calves in group 2 developed lesions consistent with IBK in the left eyes. Calves in group 2 only were determined to be culture positive for M. bovis during the study period. Between Days 1 and 2, Group 1 had significantly, P < 0.05, decreased pain scores when compared to controls. On
average there was a reduction in pain score by 79.1\% by day 2 and an 83.7\% reduction in pain by day 10 when compared to controls. Group 2 had an average reduction in pain score of 18.3\%, and 67.9\% by day 2 and by day 10, respectively, when compared to controls. Following 24, 36, 48, and 60 hr of twice daily topical corneal administration of Vetericyn Plus™ Pinkeye Spray to the cows, it was found that 50\%, 60\%, 70\%, and 100\% of cows had no signs of pain and upon examination no evidence of corneal lesions. None of the cows developed IBK clinical signs.

The average of the Days to cure for Group 1 was 2.2 days and Group 2 was 5.5 days, respectively. It was found that the Days to cure was significantly different between Group 1 and Group 2 (P = 0.0161). As far as lesion sizes: Lesion circumference, treatments were significantly different (P = 0.0375), and days were significantly different (P = <0.0001) but interaction of treatment and day was not significantly different (P = 0.329); Lesion width, treatments were significantly different (P = 0.0147) and days were significantly different (P = <0.0001) but interaction of treatment and day was not significantly different (P = 0.329); Lesion height, treatments were not significantly different (P = 0.108) but days were significantly different (P = <0.0001) and interaction of treatment and day was not significantly different (P = 0.244); Lesion area, treatments trended towards significance (P = 0.0829), days were significantly different (P = <0.0001) but interaction of treatment and day was not significantly different (P = 0.158). All samples of plasma and serum from the cows and calves fell within the normal reference ranges for sodium and chloride and there was no appreciable difference in the amount of sodium and chloride in the plasma and serum among all calves and cows at any of the sampling time points. Additionally, there were no differences in the amount of chlorine in the milk, urine, fat, liver and muscle at any of the time points sampled in any of the cow sampled.

In a cost comparison of Vetericyn Plus™ Pinkeye Spray verses that of Oxytetracycline, Tulathromycin, and Florenfenicol, there was found to be a total drug cost savings of $34.84, $58.13, and $108.02 when utilizing a 40 ml regimen of Vetericyn Plus™ Pinkeye Spray and $32.42, $55.71 and $105.60 when utilizing an 80 ml regimen, respectively. The milk loss would make utilization of Vetericyn Plus™ Pinkeye Spray that much more economically advantageous due to no milk withdrawal with use of this product. There is a savings of income from milk at $100 for a cow that is producing 50 lbs per head per day and a savings of $160 for a cow that is producing 80 lbs of milk per head per day at a milk price of $20/cwt of milk. The total cost savings of Vetericyn Plus™ Pinkeye Spray verses that of Oxytetracycline in a 1200 lb lactating cow is $137.84 for a cow that is producing 50 lbs per head per day and a difference of $197.84 for a cow that is producing 80 lbs of milk per head per day when adding drug, equipment and milk loss. Tulathromycin, and Florenfenicol are not approved for use in lactating dairy cattle. Labor costs are not calculated in this analysis.

**Conclusions:**

The results of this study indicates that Vetericyn Plus™ Pinkeye Spray can be utilized as an aid in corneal healing and in the reduction of pain and infection due to IBK in cattle with no detectable residues in plasma, serum, milk, urine, liver, fat or muscle. Additionally, results of the drug cost analysis indicate that Vetericyn Plus™ Pinkeye Spray is an economically advantageous therapy when compared to Oxytetracycline, Tulathromycin, and Florenfenicol.

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