Poster Session

Novel Injectable Oxytetracycline Formulation Allows Subcutaneous Administration With Significantly Reduced Injection Site Blemishes

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This study was conceived in response to NCA's resolution to encourage biological and pharmaceutical manufacturers to provide tissue reaction data on all animal health products and to encourage development of acceptable alternative routes of administration to the problematic IM method. We compared the tissue reaction of a novel OTC formulation administered by either IM or subcutaneous (SQ) injection. The SQ and IM routes of administration with this novel oxytetracycline formulation have been found to be bioequivalent, assuring that clinical efficacy and residue depletion are the same by either route. Oxyject® 100* is an FDA-approved injectable 10% oxytetracycline product currently labeled for IM and/or IV administration to cattle and swine. Application has been made to FDA for approval of SQ use. Oxyject utilizes glycerol formal in a novel solvent/carrier system. Glycerol formal is unique in its ability to maintain low viscosity (high syringability) even at sub-zero temperatures.

Eight single-source healthy, uniform crossbred yearling calves of both sexes averaging 490 pounds were used in the study. Calves were individually identified, group-housed in outdoor pens and randomly assigned to two treatment groups. Prior to treatment, an area approximately 2 inches square was shaved to mark injection sites. All injections were given at a rate of 10 ml per injection site in accordance with label directions to facilitate injection site comparisons. Individual dosages were in the approved label range of 3-5 mg oxytetracycline base/lb. On day 0 all calves received a SC injection in the left cervical region and an IM injection in the left gluteal region. The same procedures were repeated on day 7, however all injections were given on the right side. An 18 gauge needle, 1.5 inches long was used for all injections. Group I calves were sacrificed 13 days after the last injections (day 20 of the study). Group II calves were sacrificed 20 days after the last injections (day 27 of the study). These sacrifice times resulted in examining tissue reactions at 13, 20, and 27 days post-injection. (The required pre-slaughter withdrawal period for Oxyject is 20 days.) A total of 16 injection sites by each route of administration were evaluated.

Frequency of observed injection site necrosis on post-injection days 13, 20 and 27 were 4/4, 7/8 and 4/4 for the IM group but only 3/4, 1/8 and 0/4 for the SQ group respectively. Frequency of observed injection site white fibrous scar tissue infiltration on post-injection days 13, 20 and 27 were 1/4, 4/8 and 4/4 for the IM group and 0/4, 0/8 and 0/4 for the SQ group respectively.

Mean trim-out weights at 20 days post-injection were 0.384 +/- 0.11 kg and 0.063 +/- 0.04 g for the IM and SQ groups respectively ($P \leq .05$), a reduction of 84%.

Conclusions

SQ vs IM administration of a novel injectable oxytetracycline formulation significantly reduced the incidence of injection site blemishes and associated trim-out weights. This novel formulation given by the SQ route of administration offers the beef industry a much-needed, quality-assured means to utilize a popular, economical and effective antibiotic in the management of infectious diseases of cattle.

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Bacterial Isolates and Pathological Lesions in Toe Abscesses of Feedlot Cattle

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Toe abscesses (pododermatitis septica traumatica) were diagnosed in five midwestern feedlot lameness outbreaks submitted to the Animal Disease Research and Diagnostic Laboratory during the 1992-93 winter. Affected cattle developed severe lameness from 3 days to 3 weeks after feedlot arrival. Close examination of feet revealed abnormal hoof wear, sepa-

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ration of the hoof wall from the sole, and drainage and swelling of affected feet. Lateral claws on hind feet were most commonly affected. Necropsy examinations revealed abnormal hoof wear at the apex of affected claws, separation of the hoof wall from the sole, laminitis osteomyelitis of second and third phalanges, arthritis, tendinitis, cellulitis, necrosis and abscessation of the third phalanx (P3), occasional fracture of P3, and sole abscesses. Infection spread to the proximal limb and internal organs in three cases. Bacterial isolates from three cases included *Actinomyces pyogenes* and *Bacteroides melaninogenicus* in two out of the three cases. These bacterial isolates are commonly found in suppurative processes of cattle. It is interesting to note that *Fusobacterium necrophorum*, a common isolate in footrot, was not identified. Causes of the problem include abrasive and traumatic injuries which allow bacteria to infect the foot. Hooves were softer and more prone to damage because of unusually wet weather conditions the previous summer and fall. Excitable breeds of cattle have been observed to be more commonly affected. Morbidity ranged from a few animals up to 75%. Mortality was 7% in the worst group. Affected calves often finished behind penmates, were sent for emergency slaughter, or died due to advanced disease. Treatment includes corrective foot trimming to allow drainage and antibiotic therapy. Prevention tips include handling cattle on bedded surfaces, quiet handling of excitable groups of cattle, and close observation of newly-received cattle for signs of lameness. Early treatment of affected animals is critical for recovery. More cases need to be investigated to confirm the bacterial agents involved in the lesions.

The Evaluation of Injection Site Reactions in Beef Cattle, Comparing Three Multivalent Clostridial Vaccines.

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Abstract

Three groups of beef cattle, totaling approximately 250 animals were used in this study. The animals were mixed breed steers and heifers, weighing between 400 and 600 lb (180 and 270 kg, respectively) live weight at the start of the trial. The study was divided into two parts: the first part compared two multivalent clostridial vaccines: (1) Vision™ 7 with Spur® (product 1), and (2) Bar-Vac®7 (product 2) , in two separate groups of cattle. The cattle were divided by gender (group 1 = 74 steers, and group 2 = 96 heifers), and the two groups were housed at different locations. Within each group, the cattle were randomly assigned to treatment with either product 1 or 2. The second part of the study compared product 1 with a third multivalent clostridial vaccine, Alpha® 7(product 3), in a single mixed group of cattle (group 3, n=77). The cattle were randomly assigned to treatment with product 2 or 3.

The allotted vaccine was obliquely injected into the subcutaneous (SC) tissues on the side of the neck. The diameter, or width and length of the injection site swellings were measured with calipers on days 7 and 30 post-vaccination; these measurements were used to calculate the reaction area. An ultrasound machine was used to measure the SC tissue depth at the injection site at 7 and 30 days post-vaccination.

In the first part of the study, the cattle treated with product 1 had a significantly smaller mean reaction area on days 7 and 30, when compared with those treated with product 2. Between days 7 and 30 the swellings decreased in size in the cattle treated with product 1, whereas they were not significantly changed during this period in the cattle treated with product 2. On day 30 the mean SC depth was significantly smaller in the cattle treated with product 1, when compared with those treated with product 2.

The recommended volume for product 2 was 5 ml, and for product 1, it was 2 ml. Because the difference in the injected volume may have contributed to the observed differences in mean reaction area and SC depth, the study was repeated in a different group of cattle (group 3), substituting product 3 (recommended volume 2 ml) for product 2.

The findings of the second part of the study were similar to those of the first part: treatment with product 1 resulted in a significantly smaller mean reaction area and SC depth on days 7 and 30, when compared with product 3. The mean reaction area decreased in size between days 7 and 30 in the cattle treated with product 1, whereas it remained unchanged in the cattle treated with product 3. During this period the cattle treated with product 3 showed a significant increase in mean SC depth.

The main conclusion of this study was that Vision™7 with Spur® (product 1) caused smaller injection site reactions, which regressed more quickly than those cause by the other two multivalent clostridial vaccines, Bar-Vac® 7 (product 2) and Alpha® 7 (product 3).

Bovine Immunodeficiency Virus Associated with Encephalitis, Mastitis, Footrot and other Secondary Infections in Cattle.

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Abstract

Over 30 adult cattle from a single dairy herd died during 1990-91. The cattle had various disease problems such as: lethargy, chronic pododermatitis, mastitis, pneumonia, mycotic abomasitis, lymphadenopathy, lymphosarcoma, and injection site myositis with abscessation. Bacterial septicemia was confirmed in several cattle and cultures for Chlamydia were negative. Brain lesions were detected in many of these cattle and were characterized by slight to moderate lymphocytic meningitis and lymphocytic perivascular cuffing with occasional large cells which contained amorphous acidophilic intracytoplasmic material. In several cattle, there were focal mononuclear cell accumulations in areas of neuronal degeneration. In an occasional brain, there was focal lymphocytic infiltration of the neuropil associated with enlarged astrocytes, other areas of neuropil had diffuse microglial activation. Lymph node reactions were considered related to the secondary infections; however, there was a lack of cortical follicular development. Spleens had depletion of periarteriolar lymphoid white pulp tissue. Infection with Bovine Immunodeficiency Virus and Bovine Leukemia Virus was documented in many animals in the herd. The development of brain lesions was considered an effect of BIV as these lesions are not associated with BLV infections. The potential effect of BIV on the development of intractable mastitis and pododermatitis will be the focus of additional research efforts.

Antibody Interference of Modified Live Vaccines

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Maternal antibody interference with modified live vaccines (MLV) in puppies has been known for nearly 50 years. Small animal practitioners give a series of vaccinations anticipating that one of the injections of antigen will not be neutralized by antibody because the titer of antibody will have fallen below a given level. Unneutralized vaccine virus can then reach target cells, replicate into large numbers stimulating immune cells to produce significant protection.

This concept has now been transferred into vaccination programs for cattle. Many veterinarians and producers use injectable modified live vaccines in young calves. The efficacy of these vaccines is dependent upon low antibody titer in the calf when vaccinated. Calves whose dams carry high antibody titers to specific infectious agents will also have high antibody titers if passive transfer through the colostrum is successful. In the past, the majority of breeding herds have carried relatively low titers of antibody for the respiratory viruses. Gradually this has been changing. With the recognition of Type 2 BVDV and the prevalence of reproductive disease associated with BHV-1 and BVDV, producers will be encouraged to develop high levels of protective immunity in their cow herds. This means that in herds where modified live vaccines were successfully used in calves in the past, they will no longer produce calf immunity in the future because of high levels of maternal antibody.

Another antibody interference problem associated with MLV vaccination occurs when boosting immunity (anamnestic response) with modified live vaccines. The same principle is involved. If sufficient antibody is present at the time of vaccination, the dose of modified live virus will be neutralized before it can reach its target cells and replicate. Under these circumstances the antibody titer will drop rather than increase. The drop is (in part) due to the using up of antibody while neutralizing the vaccine virus.

In general, modified live vaccines are much more vulnerable to antibody interference than their killed counter parts because of their low antigenic mass. In addition, certain oil based adjuvants will protect vaccine antigens from antibody. This means that a product containing an antigen-protecting adjuvant and a high antigenic mass will be much more reliable in producing protective immunity than typical modified live products.

The challenge to practitioners is to develop a vaccination program that will optimize and maintain immune memory and protection in the breeding herd together with a vaccination program in calves that will establish lymphocytic memory and immune protection despite the presence of significant levels of maternal antibody.

Preliminary research has been completed supporting these basic immunological concepts. Researchers in food animal immunology are encouraged to include this model of antibody interference in their future research.

Clinical Efficacy of a Dosage Regime of 0.5 mg of Ceftiofur/Pound of Body Weight Administered Once Daily for Three Consecutive Days as Therapy for Naturally Occurring Acute Bovine Foot Rot Compared to Similarly Treated Sterile Water Placebo Controls.

Results of a Pivotal Well-Controlled Field Study.

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Objective

The primary objective of this well-controlled pivotal field efficacy study was to evaluate the efficacy of a dosage regime of 0.5 mg of ceftiofur per pound body weight administered intramuscularly once daily for three consecutive days as therapy for naturally occurring acute bovine foot rot compared to similarly treated sterile water placebo controls. The study was initiated April 1993 and completed in October 1994.

Materials and Methods

The study was blinded to the extent that the veterinarian conducting the clinical evaluations did not know which treatment regime was administered to the animals evaluated. Drug administrators were not aware of how the animals were clinically evaluated. Eight (8) investigators at 11 locations (5 dairies, 6 feedlots) completed the study. Beef (feedlot) and dairy cows enrolled had acute interdigital necrobacillosis (foot rot, pododermatitis) in one foot.

Eighty-eight (88) cattle (47 beef cattle - age ~15 months, weight range 800-900 lb; 41 dairy cows - age ~56 months, weight range 1100-1800 lb) were included in the data analysis. At least 8 animals were enrolled at each of the 11 locations. Treatment regimes (ceftiofur or sterile water) were randomly assigned to animals at enrollment using prenumbered case report forms. No concurrent antibiotics, antiseptics, paring or topicals were used and animals were not treated for foot rot 30 days prior to enrollment. Ear tag and neck chain numbers were used for identifying the animals.

The foot rot lesion and swelling were scored by the veterinarian on day 1 (enrollment), day 4 (1 day posttreatment) and day 7 (3 days posttreatment). The lesion was scored using a pre-established 0-4 scoring system (0=no lesion to 4=most severe) and the swelling scored using a similar 0-3 system (0=no swelling to 3=severe). In addition, lameness was scored using a 0=3 system (0=normal gait to 3=severe abnormality). The combined lesion, swelling and lameness scores were used as an aid for evaluating treatment regimes on days 4 and 7.

Definition of a Cure: A reduction in lameness of scores by 2 points, none to moderate swelling and healed or healing lesions observed in the affected foot on day 7.

Results

Percent cure was calculated as number of animals evaluated as "cured"/total number of animals evaluated. The following table illustrates the calculated cure rates for the ceftiofur and sterile water treated animals.

Table 1.

Type of Operation	Ceftiofur Regime* % (n)	Sterile Water Regime§ % (n)
Beef (6 sites)	69.6 (16/23)	16.7 (4/24)
Dairy (5 sites)	54.6 (12/22)	10.5 (2/19)
Combined (11 sites)	62.2 (28/45)	14.0 (6/43)

*=0.5 mg of ceftiofur (as ceftiofur sodium) per pound body weight administered intramuscularly once daily for 3 consecutive days.
§=Sterile water placebo administered intramuscularly once daily for 3 consecutive days.

One feedlot animal that received the ceftiofur treatment regime relapsed. No adverse reactions were observed.

Prior to administration of therapy, cattle from 5 locations (4 feedlot, 1 dairy) had samples from foot rot lesions cultured and anaerobic bacteria isolated. Twenty-eight (28) *Bacteroides melaninogenicus* and 21 *Fusobacterium necrophorum* were isolated from the lesions of 33 cattle. The *B. melaninogenicus* and *F. necrophorum* combination was isolated from 61% (20/33) of the lesions.

Conclusions

Under the conditions of this well-controlled pivotal field efficacy study, cattle with acute interdigital necrobacillosis (foot rot, pododermatitis) administered 0.5 mg of ceftiofur per pound body weight intramuscularly once daily for 3 consecutive days had a cure rate which was significantly greater ($p < 0.003$) than those cattle administered the sterile water treatment regimen.

Determination of Lactose and Xylose Malabsorption in Preruminant Diarrheic Calves.

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Intestinal malabsorption is believed to occur in many types of bovine diarrhea, especially those associated with villous atrophy caused by viral infection. Some studies have shown a decreased lactase activity associated with diarrhea. Continued milk feeding could aggravate diarrhea by providing additional substrate for the development of intestinal bacterial overgrowth or by increasing the movement of fluids into the lumen after fermentation of undigested lactose in the large bowel. This has led to recommendations that calves with diarrhea be deprived of milk for 48 to 96 h and fed a comparable volume of an electrolyte solution. Breath hydrogen analysis has been used in the evaluation of lactose malabsorption in humans. The purpose of the present study was to determine the frequency and severity of lactose malabsorption in calves using breath hydrogen analysis and carbohydrate absorption tests. This information should be useful in deciding whether or not to withhold milk from diarrheic calves.

In preliminary studies feeding the poorly absorbed carbohydrate sorbitol at 2.3 g/kg body weight as an indication of maximal fermentative capacity failed to produce the expected large increase in breath hydrogen excretion but did produce a transient diarrhea in five out of six control calves. Twelve healthy control and eighteen diarrheic calves were fed lactose or D-xylose on consecutive days at 1.15 g/kg body weight and a concentration of 46 g/L. Breath and blood samples were collected at 1 h intervals from 0 to 7 h. After administration of lactose, there was a significant increase in breath hydrogen excretion in diarrheic versus control calves ($p<0.05$). The increase in plasma glucose concentrations was delayed in diarrheic calves but the area under the absorption curve was similar in control and diarrheic calves. After administration of D-xylose, breath hydrogen excretion did not increase significantly but plasma D-xylose concentrations were significantly reduced in diarrheic calves. The pathogens commonly isolated from the feces were *Cryptosporidium* species, rotavirus and coronavirus. The number of pathogens and the severity of the calves' acid base deficit were not related to the severity of carbohydrate malabsorption.

The clinical application of breath hydrogen analysis has limitations in young calves. One problem with breath hydrogen estimation was the high coefficient of variation for baseline values in control calves (mean 23.%). This was probably due to changes in respiratory rate over the collection period (3 min intervals over 30 min). In diarrheic calves, pre-admission treatment with oral antibiotics did not alter breath hydrogen production. This may be due to antimicrobial resistance, the time lag between antibiotic administration on the farm and the absorption test in the clinic, or may reflect the absence of appropriate fermentative bacteria. The significant reduction in plasma xylose absorption without an overall effect on lactose absorption in our study suggests that the small intestine has greater capacity for lactose absorption. The lower peak glucose concentration and the more prolonged period of absorption following lactose administration could be explained by proximal small intestinal damage and compensation by lower areas of the small intestine. Decreased absorption of lactose and D-xylose may be the result of intestinal villous atrophy caused by viral or parasite infection.

Some farmers and veterinarians deprive diarrheic calves of milk and feed "high energy" oral electrolyte solutions instead. These "high energy" products, which actually contain less energy than milk, derive their name form the high glucose content when compared to regular oral electrolyte solutions. This practice is based on the idea that diarrheic calves may not be able to properly digest milk and that providing glucose which requires no breakdown by digestive enzymes can compensate for this. However, our comparison of lactose (which requires digestion) and xylose (which is directly absorbed) indicates that their is no benefit to feeding simple sugars.

It was concluded that carbohydrate malabsorption rather than a specific lactose maldigestion is a significant problem in diarrheic calves. Diarrheic calves appear to absorb glucose from dietary lactose more gradually than control calves, but the overall glycemia was similar in the two groups. The amount of lactose offered in this study was equivalent to that present in 1 L of milk and it is possible that larger amounts would have overwhelmed the digestive capacity of diarrheic calves. Our studies suggest that diarrheic calves can be fed small volumes of milk.

Efficacy of Decoquinate on Ovine Coccidiosis

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In a double-blind study, a daily dose of decoquinate (0.5 mg/kg BW) prevented clinical signs of coccidiosis and greatly reduced oocyst shedding in lambs experimentally infected with field isolates of *Eimeria ovinodalis, E. ahsata, E. bakuensis, E. crandallis* and *E. parva.* Lambs were hand-raised from birth, not shedding coccidial oocysts at the time of experimental infection and were randomly assigned to three groups, housed in three isolated pens. Six lambs in drug treatment group began receiving daily oral dose of decoquinate 3 days prior to the single infection with field isolates of coccidia. Daily treatments continued for 28 days after infection when the trial terminated. The six lambs in the placebo group were dosed and infected the same way, but received cornmeal instead of decoquinate. Two negative control lambs were not infected with coccidia and received only the cornmeal placebo treatment. Clinical signs were monitored daily and regular fecal examinations were done to enumerate each oocyst species shed. Twelve days after infection, lambs in the placebo group began showing signs of depression, diarrhea and inappetence. Clinical signs worsened and within 2 weeks after initial signs, all the placebo lambs had either died or were euthanized. Large numbers of oocysts in the feces accompanied the clinical signs. In contrast, the decoquinate treated lambs showed no clinical signs referable to coccidiosis, although they did shed a small fraction of the number of oocyst shed by the placebo group. The negative control lambs showed no clinical signs and no oocysts were identified in their feces during the trial.

Timing of Acid-base and Calcium Responses after Feeding Anionic Salts to Dairy Cows.

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Clinical hypocalcemic parturient paresis annually affects up to 9% of U.S. dairy cows. It is caused by the inability of some cows to respond to calcium demands at parturition. Feeding a prepartum diet with a negative cation-anion difference (DCAD), expressed as the difference between the cations Na and K and the anions Cl and S [meq $(Na^+ + K^+) - (Cl^- + S^=)/100$ g DM], is achieved by adding anionic salts to the prepartum ration. Anionic salts induce a subclinical metabolic acidosis that can mediate the release of calcium from bone and possibly enhance calcium absorption. Studies have shown that feeding anionic salts prepartum reduces milk fever and other periparturient disorders. The rapidity of acid-base and Ca responses after commencement of feeding anionic salts has not been completely characterized. Therefore, the objective of this study was to characterize temporal blood and urine pH and blood Ca responses to differing DCAD concentrations. The first experiment was a continuous randomized design in which four non lactating Holstein cows were fed an alfalfa hay-based diet (+35 DCAD) for 3 d. On d 4, one cow remained on the control diet while the remaining three were switched to diets supplemented with anionic salts ($CaSO_4$, NH_4Cl, and $MgSO_4$) to reduce DCAD values to -15, -20, or -25. Blood and urine samples were collected daily at 4 h post feeding for 4 d after the diet change. Cows fed negative DCAD diets all had lower blood pH ($P < .05$) by d 4. Decreasing DCAD also lowered urine pH ($P < .01$). Blood Ca concentrations were not different ($P > .1$) over this period. Results from this experiment demonstrate that acid-base changes in response to feeding negative DCAD diets occur within four days postfeeding. In experiment two, six pregnant dry cows, 4 to 6 wk prepartum and entering their second lactation, were assigned in a continuous randomized design and fed a control diet (+17 DCAD) for 4 d. Two cows per treatment continued on the control diet or were fed diets supplemented with anionic salts ($CaSO_4$, $CaCl_2$, and $MgSO_4$) for an additional 4 d. Anionic salt supplemented diets had DCAD values of -11 and -26 in this experiment. Two days after diets were changed, cows fed the negative DCAD diets had lower urine pH than control cows (treatment effect; $P < .05$; treatment by time interaction effect; $P < .001$) and tended to have higher blood ionized Ca (treatment by time interaction effect; $P = .07$). Differences in blood pH were not evident ($P > .1$). Results from this experiment also demonstrate that responses to feeding negative DCAD diets occur within 2 to 4 d. Changes in urinary pH are very prominent and can be detected within 2 d. Feeding anionic salts caused rapid changes in acid-base and calcium status of dairy cows.

The Cow-Side Use of Glutaraldehyde Coagulation to Determine Elevation of Inflammatory Proteins in Cattle

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A research project was undertaken to determine normal total protein (g/dl), IgG (mg/dl) and fibrinogen (mg/dl) values in dairy and beef type cattle and to determine if elevated total protein, fibrinogen and IgG values could be determined by a glutaraldehyde coagulation test (GCT).

Glutaraldehyde is a bifunctional aldehyde that cross links with amino groups on certain protein molecules to form a gel polymer. IgG and fibrinogen are the primary blood proteins that are polymerized by glutaraldehyde. Test groups included a mixed milking herd of 89 Jersey and Holstein dairy cattle, 122 Brahma-Angus cross feed lot steers, and 74 cull dairy cows at a slaughterhouse facility. All animals were tail bled and 1.0 ml of whole blood was immediately added to 1.0 ml of a 1.2% solution of specially stabilized glutaraldehyde (now available as Gelmate - Veterinary Dynamics Inc.). Tube contents were gently mixed and the reaction timed until a solid clot formed. Cattle were divided into 3 groups based on their GCT reaction times: **group I** clotted in > 9 min or did not clot, **group II** clotted in < 5 min, and **group III** clotted in 5-9 min. Approximately 5 ml of whole blood was also added to EDTA tubes. Plasma fibrinogen levels were determined by heat precipitation, total plasma protein by refractometer and IgG by radial immunodiffusion.

Mean combined IgG-fibrinogen differs among the three groups for all three test herds ($p < 0.001$, ANOVA). Additionally, within the dairy cattle as well as the cull dairy cattle, the mean IgG-fibrinogen levels for group II was found to differ significantly from both group I and III (Tukey's *post hoc* multiple comparison, 0.05 level of significance). Within the feedlot cattle the pattern was reversed: the group I IgG-fibrinogen mean was found to differ significantly from both groups II and III ($p=0.05$).

Classification trees were grown for the data using group membership as the response. Based on total protein, fibrinogen, IgG and the combined IgG-fibrinogen, cattle were found to have been misclassified by the glutaraldehyde gel test 14.86%, 15.57% and 15.73 % of the time for cull, feedlot and dairy cattle respectively.

Total protein, fibrinogen and IgG values were within normal ranges for group I cattle, i.e. those whose blood clotted in > 9 min or did not clot. Clot times of < 5 min correlated most consistently with elevations of inflammatory proteins. The majority of dairy cattle that fell into groups II and III had mastitis. As combined IgG-fibrinogen values were not consistently significantly elevated, it is probable that unidentified acute phase proteins polymerized with glutaraldehyde to cause gel formation.

It is often difficult to distinguish metabolic from nonmetabolic inflammatory diseases in cattle. However, this distinction is important especially with tightening regulations and public perceptions of the misuse of antibiotics. Fibrinogen, total plasma protein and IgG levels are often elevated in acute and chronic infection or inflammation in cattle. Diseases causing such elevations include reticulo-peritonitis, liver abscesses, pleuritis, mastitis and tuberculosis. Measuring the levels of these parameters, especially IgG can be time consuming and expensive. The GCT was found to be a quick reliable cow-side screening test for elevation of inflammatory proteins in the whole blood of cattle. This information is useful for both diagnosis and prognosis.

In Vitro Production of Bovine Embryos in the Presence of Noncytopathic Bovine Viral Diarrhea Virus (BVDV)

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The extent to which *in vitro* derived embryos will be used for commercial calf production is uncertain; however, the technology is now available. There is some concern about introduction of BVDV, since serum, cells and tissues used for *in vitro* embryo production are potential sources of the virus. Objectives of this study were (1) to determine if the presence of BVDV during fertilization of ova and culture of presumptive zygotes would affect rates of cleavage and development, and (2) to determine if degenerated ova or embryos produced in the presence of BVDV have virus associated with them after washing. Oocytes (n = 645) collected from slaughterhouse ovaries were matured and inseminated *in vitro*, and presumptive zygotes were cultured for seven days. Cultures of bovine oviductal epithelial cells for use during fertilization and culture were divided into two groups. One group of cell cultures was infected with noncytopathic BVDV while the other group was not exposed to the virus. Approximately equal groups of oocytes were inseminated and the resulting presumptive zygotes were cocultured with infected or uninfected oviductal cells. Care was taken to insure that no anti-BVDV antibody was present in any media. After two days, cultures were examined for evidence of virus infection and to assess cleavage. After seven days in culture, zona pellucida-intact morulae, blastocysts and degenerated-ova were washed twelve times and assayed for infectious virus. Rates of cleavage and development did not differ between virus-infected and uninfected (control) cultures. Further, there were no other observable differences in the virus-infected cultures. After washing, BVDV was isolated from 79% and 37% of virus-exposed groups of degenerated ova and individual morulae/blastocysts, respectively. No virus was isolated from degenerated ova or morula/blastocysts from unexposed controls.

The study was designed to test a "worst-case scenario" in which virus was present and specific antibody was not present. It is concluded that under these circumstances washing of *in vitro*-derived, zona pellucida-intact embryos would not provide absolute assurance that embryos were free of BVDV.

An Antigen Capture ELISA Utilizing Monoclonal Antibodies for Detecting Bovine Coronavirus in Diagnostic and Epidemiologic Investigations.

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Bovine Coronavirus (BCV) is an important causative risk factor for neonatal calf diarrhea. Investigators worldwide have suggested an association of BCV with the syndrome of winter dysentery (WD) in adult dairy cattle. To conduct an epidemiological investigation into the role of BCV in winter dysentery, it is necessary to develop a rapid, reliable assay, applicable to large numbers of samples. An antigen capture ELISA using a monoclonal antibody (MAb) pool for antigen capture (MACELISA) has been developed and evaluated. The assay detected virus adapted to cell culture from two strains of calf BCV and seven strains of BCV from WD outbreaks. Fecal samples of known BCV infection status (calf and WD BCV strains) from 17 gnotobiotic and 43 field case calves were tested to evaluate the assay. With these 60 known calf samples, the MACELISA had a sensitivity of 97.2% and a specificity of over 99%. The Kappa value, comparing agreement of the assay to electron microscopy (EM) and immunoelectron microscopy (IEM) results, was 0.96 for the MACELISA. Because of the excellent agreement to EM and IEM results, and the rapidity by which results may be obtained from large sample sizes, the MACELISA may be a useful diagnostic and epidemiologic tool for investigating the role of BCV infection in neonatal calf diarrhea and winter dysentery outbreaks. Preliminary data from an epidemiological investigation of winter dysentery using the MACELISA will be presented.

Effects of Antibiotics on Developmental Capacity of Bovine Embryos

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Media for recovery, washing, culture, and transfer of bovine embryos are usually supplemented with antibiotics to suppress the growth of contaminating microorganisms and to prevent the spread of pathogens. The effects of these antibiotics on the viability and developmental capacity of embryos are not known. In preliminary studies, a cell line established from a bovine embryo was used to examine toxicities of antibiotics (penicillin G, streptomycin, amphotericin B) that have been utilized in media for handling bovine embryos.

Monolayers of bovine embryonic cells were cultured in 96-well plates in media [Ham's F10 plus fetal bovine serum (FBS)] supplemented with no antibiotic (control), the concentration of antibiotic recommended for cell culture, 10 times, and 100 times the recommended concentration at 37C in 5% CO_2 to establish an LD_{50}. Subgroups of each treatment were either cultured with antibiotic for 6 h (to simulate commercial procedures for transient handling of fresh embryos) followed by 72 h of incubation without antibiotic or continuously for 72 h (simulating long term culture of embryos). After the incubation period, the total number of viable cells in triplicate cultures were determined by absolute counts of stained cells (Diff Quick). Means for treatments were compared using Duncan's Multiple Range Test. Bovine embryonic cells showed no detrimental effects when treated for 6h at the recommended cell culture level or 10 times that level. Decreases in cell numbers were seen at 10 times and 100 times the concentrations of amphotericin B when exposures were for 72 h. Based on these preliminary results, similar treatments were applied to Day 7 embryos collected from superovulated cows in PBS supplemented with 2% fetal bovine serum and no antibiotics. Good and excellent quality embryos that were determined to be zona pellucida-intact by examination over all surfaces at 50X magnification were divided into groups of ten for each treatment. Embryos were washed 10 times in PBS with 2% FBS without antibiotics and assigned to treatment media and times used in cell culture assays except the concentrations were 0, 1 time, and 10 times the normal concentration. Also, an additional antibiotic treatment (the combination of penicillin G, streptomycin, and amphotericin B) was used. Developmental potential after treatment was assessed by comparing the time for treated and control embryos (no antibiotic) to hatch when cultured for 72 h at 37C in 5% CO_2. Toxicity assays using Day 7 embryos revealed no delay in development with 1 time or 10 times the recommended concentration of penicillin G or streptomycin at either the 6 or 72 h exposure. However, 10 times the recommended concentration of amphotericin B alone or in the combination of Penicillin G/streptomycin/amphotericin B did prevent normal development when the antibiotics were in the medium for 72 h of culture. The embryonic cell line was predictive of toxicity of antibiotics to embryos. Short term exposure to even high concentrations of certain antibiotics does not result in apparent toxicity and may allow treatment to insure freedom from specific pathogens or contaminants.

Field Evaluation of the Immunologic Response to *Pasteurella haemolytica* A1 Leukotoxoid in Colostrum Fed Dairy Calves.

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Pasteurella haemolytica biotype A, serotype 1 is the organism most frequently isolated from the lungs of calves with severe, fibrinopurulent pneumonia. The ability of colostrum fed dairy calves to respond to vaccination with a subunit vaccine which incorporates the surface antigens and leukotoxin from *P. haemolytica* A1 was evaluated. Holstein heifer calves (n=97) from 3 commercial dairies were left unvaccinated (group 1), or vaccinated at 4 and 7 weeks of age (group 2) or at 7 and 10 weeks of age (group 3) with 2 ml of Presponse (Langford Laboratories, Inc.) by intramuscular injection. Serum samples were obtained from each calf at 1, 4, 7, 10, 13, and 16 weeks of age and serum antibody titers to the bacterial surface antigens and leukotoxin were determined by enzyme-linked immunosorbent assay. Due to significant herd effects on immunologic response, data from each herd were analyzed separately. Antibody titers varied considerably among calves within each herd, but did not differ significantly between treatment groups. In control calves, 27% had an active immune response to the leukotoxin and 20% responded to the surface antigens. In calves vaccinated at 4 and 7 weeks of age, 43% responded to the leukotoxin and 26% responded to the surface antigens. In calves vaccinated at 7 and 10 weeks of age, 41% had an active response to the leukotoxin and 21% responded to the surface antigens. Mean antibody titers to the surface antigens and leukotoxin decreased for the first 4 or 5 sampling periods, followed by slight increases during the subsequent sampling period, and did not differ between the treatment groups. These data indicate that vaccination with Presponse at 4 and 7 weeks or 7 and 10 weeks of age does not stimulate a serologic response in all colostrum fed dairy calves. However, it may be beneficial in providing protection to those calves that do respond actively to the vaccine.

BVD Virus: Doctor's Dilemma - Bovine Bane

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Are modified live BVD vaccines as safe and efficacious as purported? Are all killed vaccines as ineffective as most experts claim? Here is a new look at BVDV prophylaxis.

Type 2 BVD virus, recently identified as genomically and antigenically different from BVDV Type 1, is now believed to have been around for some time, perhaps decades. The sporadic episodes of infection by especially virulent BVD type 2 virus has caused a new flurry of research into BVDV. Recommendations for the producer derived from this research include 1) a strong vaccination program against BVDV; 2) maintaining a closed herd as much as possible; 3) quarantine and vaccinate new arrivals; 4) identify and remove persistently infected cattle from the herd; 5) minimize mechanical vector transmission of the virus by strict sanitary procedures and limit traffic of outsiders into cattle areas.

Most experts in BVDV research recommend that a modified live vaccine against BVDV is more likely to produce effective, long-lasting immunity to BVD than killed vaccines. They acknowledge that while all ML vaccines contain only Type 1 BVDV, vaccine manufacturers report that there appears to be some cross-protection against Type 2 BVDV. However, this may be less efficient and may not protect against any of the reproductive problems caused by BVDV.

This strong recommendation for the use of modified live BVD vaccines must be carefully examined for the following reasons:

1) Since BVDV characteristically has a high mutation rate, might not ML viruses mutate or commingle genes with field strains of BVD and, on occasion, produce a new virulent strain of BVDV?

2) Since modified live vaccines commonly produce longer lasting immunity than killed vaccines, is it possible that they do so by sequestering vaccine virus somewhere in the host and from there continually stimulate the immune response? If this is the case for BVDV, then its highly mutagenic propensity would compound the hazards associated with a live vaccine virus replicating within the host over long periods of time.

3) Since the low antigenic mass of modified live BVDV vaccines is highly vulnerable to antibody, a single dose of modified live vaccine may produce an effective immune response in only a small percentage of cattle in a herd having significant titers of maternal or acquired antibody.

Consideration of these factors should prompt veterinarians to reexamine their BVD vaccination program.

If modified live BVD vaccines are hazardous and in many situations not effective, what are the other options open to the practitioner? The ideal product would have the following properties:

- A killed vaccine that is safe.
- Antigenic diversity including BVDV Types 1 and 2.
- An adjuvant that would:
  1) stimulate strong lymphocytic memory
  2) stimulate high titers of protective antibody
  3) stimulate strong cell-mediated immunity
  4) protect vaccine antigens from antibody thus permitting a strong immune response in the presence of high antibody titers.

When will such a vaccine be available? Right Now! Grand Laboratories' Virashield line contains both cytopathic Type 1 BVDV and noncytopathic Type 2 BVDV together with Grand's XTEND III adjuvant.

Megapixel Camera Identification of the Major Proteins of Bovine Seminal Plasma Following Two-Dimensional Polyacrylamide Gel Electrophoresis of 50µg Samples*

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In recent years the interactions between the constituents of bovine seminal plasma (BSP) and the spermatozoa following ejaculation, and during their passage through the female tract, has been the focus of several research groups. Killian *et al* have identified four fertility-associated proteins which may prove useful in predicting bull fertility.[1] Manjunath and co-workers have shown that several proteins of seminal vesicle origin bind to the spermatozoan membrane after ejaculation.[2,3] These proteins may facilitate sperm capacitation within the female tract by modifying the plasma membrane. Ax and co-workers have investigated the acrosome reaction and the binding of glycosaminoglycans from the female tract. The binding proteins originate in the male accessory sex glands.[4,5]

One of the problems encountered with two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) is the streaking of proteins such that individual spot identification is compromised. This streaking is evident in the gel images that have been published.[1,2] The principal aim of this study was to determine whether a lower loading dose (50µg) of protein would permit identification of more discrete protein spots using megapixel camera technology.

Two ejaculates were obtained from each of six bulls housed at a commercial artificial insemination center (Select Sires Inc., Plain City, Ohio). Samples were centrifuged at 1000g for 10 minutes and the supernatant aspirated so as not to disturb the sperm pellet. The supernatant was then centrifuged at 3000g for 15 minutes and stored in cryovials at -70^{0}C. Samples were thawed, centrifuged, assayed for protein concentration, and divided into aliquots prior to refreezing. Protein concentration was determined by a standard BCA protein assay. All samples were subsequently diluted to a standard concentration of 50µg/10µl. The isoelectric points and molecular heterogeneity of BSP proteins were determined by measuring their electrophoretic mobility on 2-D PAGE (15% polyacrylamide). The final pH gradient extended from 3.82 to 8.24. After being electrophoresed the gels were stained for proteins with Coomassie blue, air-dried, then scanned by a megapixel camera. The large 2-D gels had to be trimmed to fit into the drying chamber. Thus, only protein spots less than 45 kDa were available for imaging.

Three distinct protein spot constellations (a,b,c) were identified by visual inspection. The image analysis software located 6 protein spots in constellation "a". These had a molecular weight (MWt) of 26 kDa and an isoelectric point (pI) ranging from 4.2 to 4.8. Constellation "b" also contained 6 protein spots (MWt of 27 kDa and a pI range of 6.6 to 8.0). The major protein spots were located in constellation "c". This constellation had the appearance of a right-angled triangle with its base towards the acidic end of the gel. Although 13 protein spots were located, only spots c2, c3, c5, c8, and c13 were present in all 12 samples. The constellation lay in a MWt range of 14.7 to 19.3, and between pI 5.3 and pI 7.4.

We have demonstrated that streaking can be eliminated by using 50µg protein for 2-D PAGE, and that this does not preclude the identification of the major protein spots. Killian's 2 low fertility proteins may lie in the "c" constellation, and one of the high fertility proteins may lie in the "b" constellation.[1]. Furthermore, we believe that the major proteins identified by Manjunath's group (BSP-A_1;BSP-A_2;BSP-A_3;BSP-30kDa) may correspond to protein spots c3,c5,c13 and constellation "a".[2,3]

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Type C Enterotoxemia in Young Calves

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Type C enterotoxemia is an extremely fatal disease caused by two toxins, alpha and beta, produced as a metabolic by-product of the bacteria *Clostridium perfringens* Type C. This problem, commonly known as enterotoxemia, purple gut, or clostridial enteritis, occurs in calves from one week of age to two months of age. Death loss in infected calves approaches 100% but, generally, no more than 10% of calves in a problem herd are affected.

Cl. perfringens Type C bacteria are found in the intestines of all cattle and in the soil; normally toxins produced by these bacteria are inactivated by existing defense mechanisms. Enterotoxemia occurs when a calf's ability to inactivate these toxins is overwhelmed by excessive growth of the *Cl. perfringens* Type C bacteria. High amounts of carbohydrates and proteins coupled with anaerobic conditions in the small intestine usually provide the environment necessary for rapid growth of the bacteria that triggers enterotoxemia.

Abrupt diet changes or any change in nursing pattern alters the amount of milk the calf consumes and interferes with bacteria balances in the gut. These events can trigger the explosive bacteria growth that precedes enterotoxemia. Calves nursing high-producing cows with especially full udders are also susceptible to enterotoxemia as the calf overeats and gets indigestion that causes intestinal stasis. This, then, prevents normal flushing of toxins necessary for a healthy bacteria balance.

Clinical Signs

When Type C enterotoxemia strikes, a dead calf is often the first sign of a problem. Enterotoxemia causes death usually within 24 hours. Other manifestations of enterotoxemia include colic, kicking at the abdomen, lying down, and occasionally rolling. Some animals have diarrhea. If a calf survives more than four hours, blood, usually dark in color, can be present in feces. As the disease progresses, depression is pronounced; convulsions, muscle rigidity, and arching of the back are other signs.

Diagnosis

Extremely high death losses make an accurate diagnosis of enterotoxemia critical to any cattle producer. Quality postmortem examinations and diagnostic workups are essential to diagnosis. Postmortem results usually show severe damage and darkening in the small intestine, often purple in appearance. Hemorrhagic enteritis with ulcerations are commonly observed on histopathological examination of the intestine. Lesions are most severe in the ileum. Blood frequently is present in the intestine; gas bubbles are sometimes present in the intestine wall. The condition is sometimes confused with hemolytic *E. coli* infections, intestinal torsions or volvulus, and intestinal congestion with blood accumulation occurring near death but attributable to other reasons, making laboratory confirmation important in obtaining an accurate diagnosis.

Treatment

Treatment of affected calves is rarely successful.

Treatments include commercial antitoxin products, hyperimmune serums of equine origin which provides passive immunity for approximately three weeks. Antibiotics are routinely administered and clostridial bacteria are generally susceptible to penicillin, but because of the severe intestinal damage produced by entertoxemia, response is variable. Supportive therapy such as fluids, electrolytes, vitamins, and other supportive therapy are also used.

Prevention

Prevention is the key to combating enterotoxemia.

Occurrence of infectious disease depends upon the immune status of the animal and the level of exposure to the bacteria.

Newborn calves receive immunity against *Cl. perfringens* related diseases from the cow's colostrum. Because colostrum is critical in controlling enterotoxemia in newborn calves both natural immunity and immunity from vaccination are important in a cow herd. For this reason veterinarians have tried many different vaccination programs aimed at entertoxemia prevention.

A recent study supports the conclusion drawn by many practicing veterinarians that there is need to vaccinate at least first calf heifers prepartum. In this study, precalving vaccinations in first calf beef heifers resulted in different *Cl. perfringen* Type C antibody levels post-colostrally at birth. Vaccination of cows prepartum against *Cl. perfringens* beta toxin increased pathogen-specific IgG levels in the dam's colostrum and in the offspring's serum. Vaccination programs need to be individually designed to meet varying herd conditions and disease incidence. Veterinary involvement is essential to designing a preventive program, assisting in the monitoring of the program, then making adjustments to the program as needed.

Field reports from several practicing veterinarians indicate a reduced incidence of the abomasitis, abomasal tympanitis, and abomasal ulcer complex in herds where the cows and/or calves have been vaccinated for *Cl. perfringens* C and D.

Other than vaccination, enterotoxemia prevention is generally confined to decreasing exposure to the bacteria and to controlling conditions that might lend themselves to promotion of the disease. Use of clean calving areas or pastures and pairing out of newborn calves and dams to other pastures are practices that limit exposure to *Cl. perfringens* and other infections. Calves obtain the bacteria orally so practices promoting cleanliness are important. Contamination of teats is an example of a route of exposure.

Although difficult to accomplish in all cases, management allowing calves to nurse regularly, and preventing overeating, is helpful. Regular feeding of cows to meet their requirements may also prevent excessive milk production. Calves with increased growth rate from heavy milking cows may be more susceptible to enterotoxemia, according to frequent owner observations.

Conclusion

It is important to remember that the bacteria, *Cl. perfringens* Type C, are normally found in the gut of cattle as well as in the environment. Occurrence of the disease depends upon conditions in the small intestine which promote rapid growth of the bacteria and production of toxins. Prevention programs need to be designed with these factors in mind and can best be accomplished through producer-veterinary practitioner prevention programs.

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Clinical Observations By Practitioners

Dr. Diana L. Scollard, a practicing veterinarian in Absarokee, MT, before 1994 advocated vaccinating heifers in the fall with C & D, boostering two weeks before calving, giving baby calves C & D toxoid at birth, then boostering calves at branding for best results against enterotoxemia. Because this program provided limited results, in 1994 Dr. Scollard vaccinated all heifers in the spring and fall with Scourguard 3KC then gave the calves ALPHA-7 and Nasalgen at birth.

At branding time and for purposes of comparison, 15 calves were given antitoxin and a booster shot of ALPHA-7. All remaining calves received no additional vaccinations. There were no enterotoxemia death losses experienced in these cattle in 1994.

Concern about using an oil adjuvanted 7-way clostridial vaccine are alleviated by Dr. Scollard's experiences. Dr. Scollard reports, "At branding approximately 20 calves of 280 or 7% were showing an injection site reaction, something quite comparable to what we got with the previous year's program and way better than what we would get using a double 7-way program where 25% injection site reaction would be fairly normal."

Dr. Steven L. Graf, All Creatures Vet Clinic of Almena, KS, relates similar experiences. According to Dr. Graf, he received the *"most encouraging results of anything we've tried in the last five years to combat enterotoxemia"* when he switched to one dose 7-way ALPHA-7. In the past Dr. Graf used 7-way clostridials or C and D toxoids in one or two dose regimens, sometimes three, in pregnant cows, combined with a 7-way vaccination to newborn calves and two repeat injections at four to six weeks of age and at branding. This program was coupled with antitoxin treatments of sick calves and/or whole herd antitoxin treatment at four to six weeks, with less than optimum results. As Dr. Graf says, *"It's very frustrating to post four or five of a producer's best doing calves with enterotoxemia after he's been on such a program."* With the new program Dr. Graf does see some post injection swelling following vaccination with the oil adjuvanted ALPHA-7 but as Dr. Graf states, "Although no more [injection site swelling] than with traditional 5 cc vaccines we have used."

Dr. Michael Slattery in the fall of 1993, included ALPHA-7 in a 200 head Angus cow herd. In the spring of that year, this herd experienced a loss of 23 calves to enterotoxemia. In the fall of that year, the cows and first calf heifers received one dose of ALPHA-7 in addition to a killed IBR, BVD, PI_3 and Scourguard 3K 60 to 90 days before calving. Baby calves were vaccinated with ALPHA-7 at birth. In 1994 no calves were lost to enterotoxemia and Dr. Slattery believes this program accounts for the significant reduction in death loss.

Dr. Bob Sager, Belgrade, MT reports losses of 24-30 head of calves out of 3,500 to 4,000 to acute enterotoxemia even after two doses of a popular 7-way product. In 1994 Dr. Sager says, *"We used almost exclusively ALPHA-7 with post vaccination enterotoxemia reduced over 90% in the same problem herds."*

FUTURE MEETINGS

American Association of Bovine Practitioners

1997	Montreal	September	18-21
1998	Spokane	September	24-27
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2000	Rapid City	September	21-24
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