PEER REVIEWED

The Magnitude and Patterns of Ruminal Hydrogen Sulfide Production, Blood Thiamine Concentration, and Mean Pulmonary Arterial Pressure in Feedlot Steers Consuming Water of Different Sulfate Concentrations

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Abstract

Twenty-seven single-source, yearling crossbred steers (713 lb; 324 kg) were used to evaluate the effect of water sulfate concentration on the patterns and magnitude of ruminal gas cap hydrogen sulfide, blood thiamine concentration and mean pulmonary arterial blood pressure (mPAP). Target water sulfate concentrations were 125, 500 and 2000 mg sulfate/L of drinking water, while the observed water sulfate concentrations were 136, 583 and 2,360 mg/L. Ruminal gas cap hydrogen sulfide concentrations were measured three times per week for 10 weeks, then once a week for seven additional weeks. On days 29 and 113, whole-blood samples were collected and analyzed for thiamine concentration. Pulmonary arterial blood pressures were measured on day 100.

Ruminal gas cap hydrogen sulfide concentrations decreased to day 15, then increased and peaked by day 31, and finally decreased and remained relatively stable. The peak in hydrogen sulfide concentration was associated with an event of polioencephalomalacia in the treatment group consuming water with 2,360 mg/L sulfate, and the greatest frequency of polioencephalomalacia events in cattle in an adjacent commercial feedlot consuming water with a similar sulfate concentration. Ruminal hydrogen sulfide concentration increased with increasing water sulfate concentration ($P < 0.05$). A treatment effect on blood thiamine concentration was not detected ($P = 0.29$). Mean pulmonary arterial pressure increased linearly with increasing water sulfate concentration ($P < 0.05$). Ruminal gas cap hydrogen sulfide concentrations greater than 1000 ppm provide evidence of excessive sulfur consumption. Pathological concentrations of ruminal sulfides appear central to the pathogenesis of sulfur-associated polioencephalomalacia.

Résumé

Un total de 27 bouvillons de l'année (713 lbs; 324 kg), de race croisée et de même origine, ont été utilisés pour évaluer l'effet de la concentration de sulfate dans l'eau sur les changements temporels et l'amplitude de la production d'hydrogène sulfuré gazeux dans le rumen, de la concentration de thiamine dans le sang et de la pression artérielle pulmonaire moyenne (mPAP). Les concentrations visées de sulfates dans l'eau étaient de 125, 500 et 2000 mg de sulfate par litre d'eau potable. Les concentrations atteintes dans l'eau potable furent de 136, 583 et 2360 mg/L. La concentration gazeuse d'hydrogène sulfuré dans le rumen était mesurée trois fois par semaine pendant 10 semaines et par la suite une fois par semaine lors de sept autres semaines. Aux jours 29 et 113, des échantillons sanguins ont été prélevés et analysés pour déterminer la concentration de thiamine. La pression artérielle pulmonaire était mesurée au jour 100.

Les concentrations gazeuses d'hydrogène sulfuré ont diminué jusqu'au jour 15 pour ensuite augmenter et atteindre un maximum au jour 31 avant de diminuer à nouveau et rester relativement stable par la suite. Le maximum de la concentration d'hydrogène sulfuré était associé avec un épisode de polioencéphalomalacie dans

le groupe consommant de l'eau de potable avec une concentration de sulfates de 2360 mg/L et aussi avec des épisodes de polioencéphalomalacie survenus dans un parc d'engraissement commercial proche où l'eau potable avait une concentration en sulfates du même ordre. La concentration d'hydrogène sulfuré dans le rumen augmentait avec la concentration de sulfates dans l'eau ($p < 0.05$). Aucun effet du traitement n'a été détecté au niveau de la concentration de thiamine dans le sang ($p = 0.29$). La pression artérielle pulmonaire moyenne augmentait linéairement avec la concentration de sulfates dans l'eau ($p < 0.05$). L'observation de concentrations gazeuses d'hydrogène sulfuré dans le rumen plus grandes que 1000 ppm supporte l'hypothèse d'une consommation excessive de soufre. Des concentrations pathologiques d'hydrogène sulfuré dans le rumen semblent cruciales dans la pathogenèse de la polioencéphalomalacie associée au soufre.

Introduction

Polioencephalomalacia (PEM) is a sporadic, yet important neurological disease of ruminants.[39] The condition may arise as isolated events or outbreaks affecting a substantial proportion of the animals at-risk.[5] *Polio* refers to gray matter, *encephalo* refers to brain, and *malacia* means softening or necrosis. Therefore, PEM is descriptive terminology for a specific pattern of histological lesions.[19,20] Brain edema, subcortical hemorrhages and vasculopathy may be present in addition to cortical laminar necrosis in severely affected cases.[15,18,24]

Although PEM is descriptively specific, it is nonspecific with regard to etiology. Polioencephalomalacia has been attributed to thiamine deficiency,[34,35] lead toxicity[40] and water deprivation/salt toxicity.[33] Increasingly, PEM has been associated with consumption of excessive amounts of sulfur.[24] Reported sources of sulfur include elemental sulfur,[5] feed additives such as ammonium sulfate and gypsum,[36] feed stuffs such as grain co-products, molasses and cruciferous crops,[16,29] and water sulfates.[28]

It is unlikely that sulfur and sulfate directly cause PEM because they are of low cellular toxicity. However, microbial reduction of sulfur-containing compounds within the rumen liberates highly toxic sulfides.[22] Among other effects, sulfides result in ATP depletion through inhibition of cytochrome oxidase, an enzyme in the electron transport chain.[3,10] Tissues with high metabolic demand, such as the brain, are at greatest risk from sulfide-induced ATP depletion. Therefore, sulfur-associated PEM is more likely a consequence of sulfide toxicity than sulfur toxicity.[27]

This hypothesis is supported by observation documented by Gould and colleagues where the onset of clinical signs of PEM was associated with increased sulfide concentrations in ruminal fluid of calves fed a high-sulfur diet.[14] Moreover, PEM was induced in sheep subsequent to an orogastric challenge with preformed sulfide ions,[27] and ruminal gas cap hydrogen sulfide concentrations were also elevated during a naturally-occurring outbreak of PEM.[24] In an investigation of seasonal outbreaks of PEM in feedlot cattle consuming water containing approximately 2,500 mg/L sulfate,[28] the majority of the PEM events occurred during the hottest months of the year, from 15 to 35 days following arrival at the feedlot, and were associated with elevated ruminal fluid sulfide concentrations. The objective of the described research was to evaluate the effect of water sulfate concentration on the pattern of ruminal gas cap hydrogen sulfide concentration, blood thiamine concentration and mean pulmonary arterial blood pressure.

Material and Methods

Twenty-seven single source, crossbred yearling steers weighing 713 lb (324 kg) were used. Upon arrival at the feedlot, steers had *ad libitum* access to long-stem hay plus water with a sulfate concentration of approximately 500 mg/L. Steers were moved through an animal handling facility the next morning (day -1), individually identified, weighed, administered a modified live virus vaccine (bovine rhinotracheitis virus, bovine viral diarrhea virus, bovine respiratory syncytial virus, parainfluenza 3 virus),[a] a bacterin-toxoid against *Clostridium perfringens* types C and D,[b] a broad-spectrum anthelmintic,[c] and implanted with a growth promotant.[d] Steers were randomly and equally assigned to one of three treatments. On day 0, cattle were reweighed and moved to one of three pens that corresponded to their treatment assignment. Cattle were reimplanted[e] on day 56, and harvested on day 113.

Water sulfate concentration was the treatment factor of interest, and target concentrations were 125, 500 and 2000 mg/L. Targeted water sulfate concentration was achieved by blending well water (high-sulfate) with purified well water (low-sulfate).[26] The amount of well and purified water to be blended was based on daily measurements of their total dissolved solids. Historically, sulfate constituted 64% of well water total dissolved solids. Therefore, for the purpose of blending treatment water, water sulfate content was assumed to be 64% of total dissolved solids. Well water was purified using a commercially available reverse osmosis system.[f] Purified water was stored in an 8000-L tank, whereas well water was stored in a 6000-L tank. Well and purified water were then blended to achieve appropriate treatments. Two-hundred-fifty milliliter water samples (well, reverse osmosis and treatment water samples) were collected three times a week. Samples

were composited by week and analyzed for sulfate concentration.[g]

Steers were fed typical feedlot diets composed primarily of steam flaked corn, and their formulations are presented elsewhere.[26] Diets were delivered to the steers twice daily at a constant time of the day. Feed samples were routinely taken and analyzed for dry matter and sulfur content.[h]

Ruminal gas cap hydrogen sulfide concentrations were measured in all animals three times a week for 10 weeks, then once a week for seven further samplings. Samples were collected at the same time of day for the duration of the study where possible. The procedure for measuring hydrogen sulfide concentration was performed as described.[12] In brief, the left paralumbar fossa was clipped, disinfected with a 2% chlorhexidine surgical scrub and rinsed with a 4% chlorhexidine solution. An 89-mm, 18-gauge spinal needle was inserted through the body wall and into the gas cap of the rumen. A modified precision gas sampler[i] was attached to the hub of the spinal needle and 100 mL of gas was drawn through a hydrogen sulfide detector tube.[j] Measurements were divided by 0.87 to adjust for the dead space (13 mL) created by modifications to the precision gas sampler.

On days 29 and 113 of the study, whole-blood samples were collected via jugular venipuncture into heparinized blood collection tubes[k] and stored at -20°C (-4.0°F) until further processing. Blood samples were thawed, and analyzed for total blood thiamine concentration using a thiamine dependent *Lactobacillus* bioassay.[31] Blood thiamine concentrations were compared to published reference values.[17]

During the final month of the trial, mean pulmonary arterial blood pressures (mPAP) were measured using a direct invasive technique as described.[21,38] A saline-filled catheter was passed normograde down the right jugular vein, through the right heart chambers and into the pulmonary artery. The catheter was attached to a transducer and pressures were electronically measured.[l] Proper location of the catheter was determined by visual appraisal of the characteristic patterns of blood pressure.

At harvest, brains were removed and fixed in 10% neutral buffered formalin. Samples of pulmonary tissue, 10 x 5 x 5 cm, were collected from the base of the cranial, middle and caudal lobes of the right lung. Lung samples were fixed in 10% neutral buffered formalin. Fixed brain and lung samples were sectioned at 10-mm intervals and grossly examined. Representative samples were embedded in paraffin, sectioned at 5 μm, routinely stained with hematoxylin and eosin, and examined using light microscopy techniques.

Data relating to the occurrence of clinical manifestations of PEM in cattle at the adjacent commercial feedlot were collected over a five-month period as described.[28] This time period coincided with the duration of the described research. The one-time capacity of the commercial feedlot was approximately 38,000 animals. Cattle in that feedlot consumed similar diets, and the same well water, as cattle housed in the research facility. The previously reported[28] graphic of the frequency of PEM events was modified to facilitate visual comparison of PEM risk period and ruminal gas cap hydrogen sulfide concentration measured in those cattle consuming water with the greatest sulfate concentration.

Statistical analyses

Ruminal gas cap hydrogen sulfide data were divided into four time periods based on a preliminary visual appraisal of the patterns. The time periods were: Period 1, days 1 through 15; Period 2, days 17 through 31; Period 3, days 33 through 69; and Period 4, days 71 through 113. Gas cap sampling was performed three times weekly during Periods 1, 2 and 3, and once weekly during Period 4. Ruminal gas cap hydrogen sulfide concentrations were transformed to a natural log scale to normalize variance. Water sulfate concentration and trial day were considered continuous variables in all analyses. Full second order models were used and terms removed if their P value was greater than 0.10 while maintaining hierarchy within terms. First order autoregressive covariance matrices were used to model the correlation within experimental units over time for the analyses of hydrogen sulfide.[23] Least square means were compared using the LSD technique.

Results

Mean ± SEM water sulfate concentrations were greater than the targeted concentrations, and were 136.1 ± 6.3, 582.6 ± 16.9 and 2360.4 ± 68.2 mg/L.[25] The diet contained 0.16% sulfur on a dry matter basis. Ruminal hydrogen sulfide concentrations decreased during Period 1. This was followed by a noticeable peak in H_2S concentrations that lasted through day 31 (Figure 1). Subsequent to the peak, all groups experienced episodic fluctuations in hydrogen sulfide concentration for the remainder of the trial. For Period 1, hydrogen sulfide increased quadratically with increasing water sulfate concentration ($P = 0.09$) and decreased quadratically over time ($P < 0.01$). For Period 2, hydrogen sulfide increased quadratically with increasing water sulfate concentrations ($P < 0.01$). Day had a quadratic effect on hydrogen sulfide concentration ($P < 0.01$). During Periods 3 and 4, ruminal gas cap hydrogen sulfide concentrations increased quadratically with increasing water sulfate concentration ($P < 0.01$) and decreased over time ($P < 0.01$).

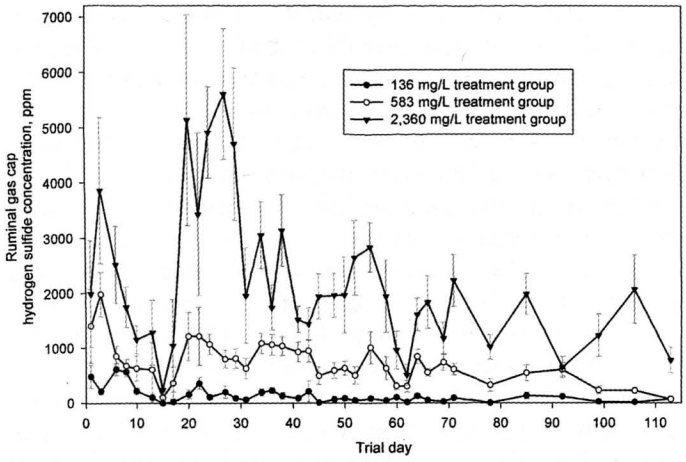

Figure 1. Ruminal gas cap hydrogen sulfide concentrations (ppm) by day of study.

On day 29, six steers had blood thiamine concentrations below the normal reference range of 75 to 185 nmol/L (Table 1).[17] These steers were consuming water with a sulfate concentration of 136 (n = 1), 583 (n = 3), and 2,360 (n = 2) mg/L. However, none were considered deficient (less than 50 nmol/L). No steers had blood thiamine concentrations below the normal reference range on day 113. There was no evidence for a treatment effect on total blood thiamine concentration (P = 0.29). Blood thiamine concentration increased significantly from day 29 (93.9 nmol/L) to day 113 (113.1 nmol/L). Averaged across sample days, blood thiamine least square means were 105.72, 97.94 and 106.77 nmol/L for cattle receiving 136, 583, and 2360 mg sulfate / L, respectively. Results are presented in Table 1.

Mean pulmonary arterial pressures increased linearly with increasing water sulfate concentration (P < 0.01). The mPAP of cattle in the 2,360 mg/L treatment group was greater than those of the other treatment groups (P < 0.05; Table 1).

A steer from the 2,360 mg/L treatment group displayed clinical signs of PEM on day 17. The steer demonstrated blindness, ataxia, and adopted a sawhorse stance with its head resting on the cement feedbunk. The animal was seemingly unaware of feedlot personnel. The steer was treated with 30 mg of dexamethasone and 2 g thiamine intramuscularly and intravenously, respectively. With the exception of the steer's vision, improvements were observed for all clinical signs over a period of a week, at which time the animal appeared to be drinking and eating sufficiently to re-enter the trial on day 24. Data from that steer collected from days 17 through 24 were omitted from analysis. The steer's hydrogen sulfide concentration on the sampling day prior to the onset of illness was 13,448 ppm, which was the second greatest concentration recorded among all study animals. Following the completion of the research, gross and histological examination of brain tissue from the steer revealed gross and microscopic lesions consistent with a prior PEM event. No abnormalities were detected either grossly or histologically in brain tissue of other animals. Although cattle from the 2,360 mg/L treatment group often displayed short-lived episodes of shallow breathing following eructation, particularly during Period 2, no pathological alterations were discovered during gross and histological examinations of pulmonary tissue.

The majority (98%) of clinical PEM events in the adjacent commercial feedlot occurred within 59 days following arrival at the feedlot, with 78% occurring from days 15 through 31. The peak in ruminal hydrogen sulfide concentration in steers consuming the high-sulfate water coincided with the apparent period of risk for PEM development (Figure 2).

Table 1. Blood thiamine concentration for animals* sampled on day 29 and day 113, and least mean pulmonary arterial blood pressure least squares means and SEM.

	Treatment group			
Trial day	125 (n = 9)	500 (n = 9)	2000 (n = 9)	SEM
	Blood thiamine concentration (nmol/mL)			
Day 29	103.67^{a}	80.56^{by}	97.56^{z}	4.66
Day 113	107.78^{xz}	115.33^{xz}	116.00^{x}	2.37
	Mean pulmonary arterial blood pressure (mmHg)			
Day 100	29.56^{a} ± 1.45	33.67^{a} ± 1.45	38.13^{b} ± 1.54*	

*Estimate is based on measurements in 8 animals.
**The SEM for blood thiamine concentration across sample days is 3.92 nmol/mL.
^{a,b,c} Within each row, estimates with different superscripts (a, b or c) differ at P < 0.05.
^{x,y,z} For blood thiamine concentration, estimates with differing superscripts (x, y, or z) differ over time as P < 0.05.

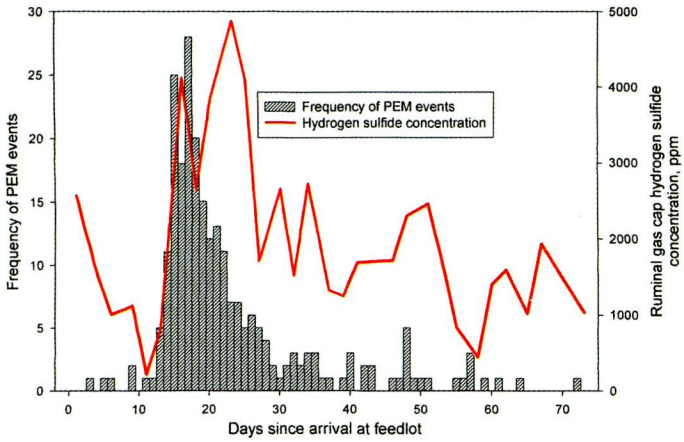

Figure 2. Hydrogen sulfide concentration for cattle receiving water with a mean sulfate concentration of 2,360 mg/L and frequency of polioencephalomalacia (PEM) events by days at the feedlot. Data for frequency of PEM events are modified from McAllister *et al*, *J Am Vet Med Assoc* 211:1275-1279, 1997.

Discussion

The targeted water sulfate concentrations to provide a low-, medium-, and high-level challenge. The high-level challenge is consistent with the water sulfate concentration observed in the adjacent commercial feedlot[28] and has been associated with PEM outbreaks by other authors.[30] The medium- and low-level concentration are commonly reported nationwide.[13]

A time-dependent pattern in ruminal hydrogen sulfide production was observed in this study. Concentration in the gas cap initially decreased, and was followed by a surge in hydrogen sulfide concentration. This peak coincided with introduction to the finishing ration, a case of PEM in the treatment group receiving the greatest water sulfate concentration, and temporally with the majority of PEM events in cattle consuming water containing approximately 2,500 mg/L sulfate.

Reasons for the decrease in ruminal gas cap hydrogen sulfide concentration prior to day 15 of the present study are uncertain. During this time period, the steers progressed sequentially through the step-up diets and received the finishing diet for the first time on day 13. The alterations in the rumen microbial environment associated with changing diets may wholly or partly have resulted in the decrease in ruminal sulfide concentrations. The surge in ruminal gas cap hydrogen sulfide concentrations demonstrated from days 17 through 31 may have been due to multiple factors. Because this surge was associated with the introduction of the finishing diet, a lower ruminal pH or other factors may have favored a disproportionately large increase in the numbers of dissimilatory sulfate reducers compared to assimilatory sulfate reducers. Sulfur assimilation results in microbial synthesis of sulfur-containing amino acids (cysteine and methionine). On the other hand, sulfur dissimilation leads to liberation of free sulfide ions while generating ATP for the microbe.[22] Ion partitioning drives the majority of the liberated sulfhydryl ion to hydrogen sulfide.[4] Hydrogen sulfide then moves freely into the ruminal gas cap. Therefore, a diet that lowers rumen pH, such as a feedlot-type diet, would result in greater ruminal gas cap hydrogen sulfide concentration than a roughage-based diet. Other factors that might have led to increased ruminal sulfide concentrations include induction of microbial enzymes involved in sulfur reduction or selection for a sub-group of potent dissimilatory sulfate reducers.[7] It is possible that the increased ruminal sulfide concentrations may have occurred secondary to the synthesis of a large pool of organic sulfur compounds within the rumen, followed by a rapid desulfuration and liberation of sulfides.

Whatever factors were responsible for the patterns of hydrogen sulfide concentration observed, it is apparent that ruminal microbes require a period of time from sulfur exposure until maximum generation of sulfide occurs. This phenomenon has been supported by others using both *in vitro*[1] and *in vivo* techniques.[9] Following the surge in ruminal sulfide concentration demonstrated by animals receiving 2,360 mg/L sulfate, sulfide concentrations demonstrated episodic fluctuations and tended to decrease over time. This may indicate an adaptation of the microbial population to a more stable consortium of assimilatory and dissimilatory sulfate reducers. Other investigators documented that cattle that had been in a feedlot for three weeks had greater ruminal fluid sulfide concentration (175 mM) than those that had been in the feedlot for two months (83 mM).[28]

Figure 2 included data collected from the commercial feedlot representing cattle of both sexes (steers and heifers) that arrived at the feedlot throughout the hottest months of the year (bars). These animals also varied greatly in origin and arrival weights, whereas the nine steers housed in the research facility were similar in type and arrived on the same day (line). Because of these and other differences between the data collected from the commercial and research facilities, some care should be used in inferring a causal association from Figure 2. Given interpretive care, the data presented herein support the hypothesis that elevated ruminal sulfide generation is central to the pathogenesis of sulfur-associated PEM.

Some investigators proposed that elevated ruminal sulfide results in ruminal destruction of thiamine,[11] or an increased requirement for thiamine.[32] Given these hypothetical arguments, excessive sulfur intake might induce a secondary or functional thiamine deficiency

that leads to PEM. Other experimental observations do not support decreased *de novo* thiamine synthesis in ruminants consuming high-sulfur diets.[1,2] Further, alterations in thiamine status is rarely evident in experimentally induced[37] or naturally occurring cases of sulfur-associated PEM.[24] In the present investigation, blood samples were collected toward the end of the surge in ruminal sulfide concentrations and at the completion of the trial. A significant increase in thiamine concentrations over time was observed. These data might be interpreted to support the theory that elevated ruminal sulfides negatively affect thiamine status. However, averaged over time there was no evidence of a treatment effect ($P = 0.29$). Although blood thiamine concentrations were on average lower on day 29, no values were considered deficient. The differences observed in thiamine concentration over time were likely biologically negligible.

The steer that developed PEM in the present study responded favorably to thiamine and corticosteroid administration. It is the authors' experience that animals suffering PEM, regardless of the cause, typically respond favorably to such treatment regimens. It is unclear why thiamine is therapeutic in PEM-affected animals without a detectable thiamine deficiency. Either animals have an increased requirement of thiamine, or thiamine is beneficial to the metabolically-impaired brain.

It is uncertain why mPAP increased with increasing water sulfate concentration. Other investigators have reported that a feature of sulfide poisoning is pulmonary edema,[5,6] and respiratory distress is a common finding in cases of sulfur-associated disease.[6,27] Furthermore, steers in the 2,360 mg/L group demonstrated short periods of shallow breathing immediately following eructation, particularly during Period 2. Because cattle inhale a substantial proportion of eructated gases,[8] inhaled hydrogen sulfide may have resulted in changes in pulmonary vascular tone or pulmonary injury. However, there was no evidence of pulmonary injury during routine light microscopic evaluation of lung samples. Elevated ruminal sulfide concentration may affect pulmonary arterial pressures through other mechanisms, such as a direct effect of absorbed sulfide upon central baroreceptors or another regulator of blood pressure. Although the exact mechanism is not known, it might be possible that high water sulfate concentrations increase the risk of high mountain disease in cattle.

Conclusion and Implications

Excessive consumption of dietary sulfur, which includes water sulfate, appears to be an important cause of PEM in feedlot cattle. After a period of adaption to high-sulfur conditions, cattle experience a surge in ruminal sulfide generation that is temporally associated with the peak occurrence of PEM. Water sulfate greater than 500 mg/L may result in excessive sulfur intake during periods of high ambient temperature when the ration contains a recommended sulfur content. Efforts to reduce water sulfate during summer and prevent rumen acidosis should reduce the burden of PEM in situations where water has been implicated as an important source of dietary sulfate.

Footnotes

[a] Bovishield 4, SmithKline Beecham Animal Health, West Chester, PA
[b] Ultrabac CD, SmithKline Beecham Animal Health, West Chester, PA
[c] Ivomec-Plus, Merck AgVet Division, Merck and Co. Inc., Rahway, NJ
[d] Synovex-S, Fort Dodge Animal Health, Overland Park, KS
[e] Revalor-S, Hoechst-Roussel Agri-Vet Co., Somerville, NJ
[f] World Water Systems, Inc., Colorado Springs, CO
[g] Peterson Laboratories Inc. (now known as SLK Laboratories), Hutchinson, KS
[h] Grant County Feeders, Ulysses, KS
[i] Gastec precision gas sampler, Sensidyne, Clearwater, FL
[j] Gastec hydrogen sulfide analyzer tube, Sensidyne, Clearwater, FL
[k] Vacutainer, Becton Dickinson and Company, Franklin Lakes, NJ
[l] MDE Escort E100, Escort Monitors, Medical Data Electronics, Arleta, CA

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PYRAMID® 5
with MetaStim®

Serious Protection

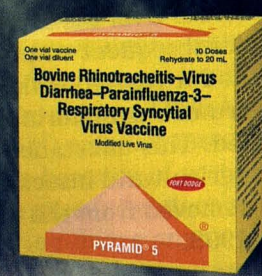
Type I & II BVD Protection

Two specifically selected strains offering broad protection and increased immune response.

Proven Safety

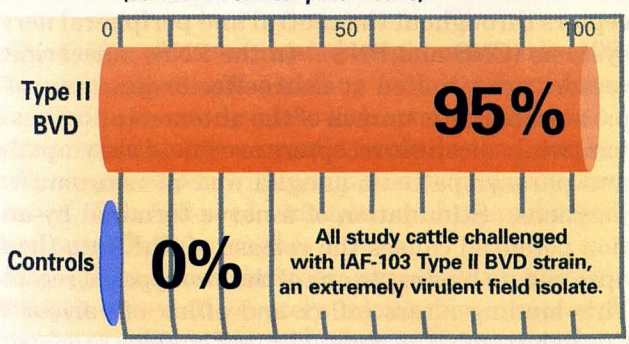
Licensed by the USDA for Sub-Q administration and proven safe when used according to label directions.

Stimulating Results Against BVD



Type II Respiratory Challenge Results[1]

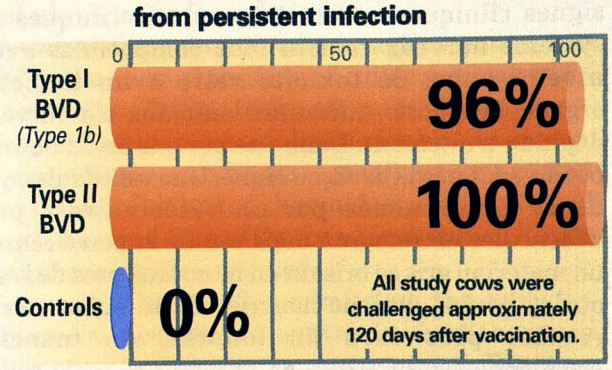
Protection from Disease
(sum of all clinical parameters)



***95%** of PYRAMID 5 vaccinated calves showed no clinical Type II BVD signs following virulent challenge.*

BVD Fetal Challenge Results[1]

% fetuses protected from persistent infection



Up to 100% fetal protection against Type I and Type II BVD challenges.

1. Data on file.

FORT DODGE®

PYRAMID® 5
with MetaStim®