PEER REVIEWED

The Association Between Serum Mineral and Vitamin A and E Concentration and Respiratory Disease in Beef Calves Entering Backgrounding Lots

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Abstract

The objectives of this study were 1) to measure morbidity and mortality rates attributable to respiratory disease in beef calves originating from southwestern Utah, 2) to evaluate herd of origin vitamin/mineral serum concentration at backgrounding lot entry, and 3) to evaluate a potential association between serum concentrations of 29 minerals and vitamins A and E at backgrounding lot entry with respiratory disease in recently weaned beef calves. This was a prospective longitudinal study with matched case-control and cross-sectional studies nested within the main study design. Pen-specific morbidity rates attributable to respiratory disease ranged from 1.2% to 81.8%. Serum concentrations of vitamins A and E in calves sampled (n=224) from eight southern Utah ranches were commonly less than published low-normal values. In these data, there was no detectable association between serum vitamin A and E or mineral imbalances and respiratory disease. Herds that experience a high rate of bovine respiratory disease (BRD) should be evaluated for mineral and vitamin deficiencies and supplemented as needed. If not deficient, it should not be expected that supplementation would reduce the rate of BRD in the face of mismanagement.

Résumé

Les objectifs de cette étude étaient 1) de mesurer le taux de morbidité et de mortalité associé aux maladies respiratoires chez les veaux de boucherie de l'Utah du sud-ouest, 2) d'évaluer les concentrations en minéraux et en vitamines du troupeau d'origine lors de l'entrée au parc de semi-finition, et 3) d'évaluer l'existence d'une association potentielle entre la concentration des vitamines A et E et de 29 minéraux et le développement de maladies respiratoires chez les veaux de boucherie récemment sevrés. L'étude était du type longitudinale prospective et comportait une composante cas-témoin apparié et une composante transversale toutes deux imbriquées dans le design de l'étude principale. Le taux de morbidité par parc attribuable aux maladies respiratoires variait de 1.2% à 81.8%. Les concentrations sériques des vitamines A et E chez des veaux (n = 224) échantillonnés à partir de huit fermes du sud de l'Utah étaient souvent plus faibles que les valeurs normales basses rapportées dans la littérature. Dans cet ensemble de données, il n'y avait pas d'association discernable entre la concentration des vitamines A et E, ou l'état de déséquilibre minéral, et le développement de maladies respiratoires. Il faudrait examiner les carences en vitamines ou en minéraux dans les troupeaux où les maladies respiratoires bovines sont fréquentes et envisager l'apport supplémentaire si nécessaire. Si les carences ne sont pas perceptibles, on ne devrait pas s'attendre à ce l'apport supplémentaire réduise le taux des maladies respiratoires bovines lorsque le troupeau n'est pas bien géré.

Introduction

Deficiencies of certain vitamins and minerals can negatively affect the immune system in cattle and decrease their ability to respond to vaccination.[1,3,5,7,10,12,14-16] Partial immunosuppression due to a mineral or vitamin deficiency may also increase morbidity and mortality rates in deficient cattle. Minerals with an important role in bovine immunocompetence are copper, selenium, zinc, cobalt, iron and vitamins A and E.[1-3,14,15] In one recent study, 53% of beef cattle herds surveyed in the western US and 46% of the animals surveyed within

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those herds were deficient in copper.[4] Excessive concentrations of molybdenum, sulfur and iron in feed or water have been reported to interfere with the absorption or use of other critical trace elements.[3,15]

Livestock producers and veterinarians in southwestern Utah had previously reported that the incidence of bovine respiratory disease (BRD) in weaned calves entering backgrounding lots was higher than expected.[a] Calves appeared healthy and vigorous at weaning, but shortly after weaning and transport of a moderate distance to backgrounding lots, a high percentage of these calves were diagnosed with BRD. Some backgrounding lots reported that morbidity attributable to BRD was over 50%. Concern arose among livestock producers and veterinarians that a deficiency or excess of one or more vitamins and minerals was a contributing factor for higher than expected BRD morbidity rates in calves originating from ranches in southwestern Utah.

The objectives of this study were 1) to measure morbidity and mortality rates attributable to BRD in beef calves originating from southwestern Utah, 2) to evaluate herd of origin vitamin/mineral serum concentration at backgrounding lot entry, and 3) to evaluate a potential association between serum concentrations of 29 minerals and vitamins A and E at backgrounding lot entry with BRD in recently weaned beef calves.

Material and Methods

Experimental Design

This was a prospective longitudinal study with matched case-control and cross-sectional studies nested within the main study design.

Managers of three cattle backgrounding operations located in southern Utah and eastern Nevada agreed to cooperate with our study objectives. All three operations received cattle from southwestern Utah ranches. During the fall of 2000, blood samples were collected from 787 steer beef calves, originating from eight ranches in southwestern Utah. All calves were from rangeland grazing operations with rather dry forage conditions, and were recently weaned and shipped from the ranch of origin directly to the backgrounding lot.

There was considerable variation in the vaccine protocols used in the various herds prior to backgrounding lot entry. Vaccines and antimicrobial drugs administered to calves at backgrounding lot entry were selected by individual backgrounding lot operators and varied between lots. However, all calves received a multivalent viral respiratory vaccine (either inactivated or attenuated) upon entry into the backgrounding lot as a part of initial processing.

Calves were individually identified with an ear tag and weighed during routine processing at entry into a backgrounding lot. Blood was collected from the jugular vein into royal blue, trace mineral blood tubes[b] and soon after placed in a light-protected container (cooler), on ice. Sera was separated by centrifugation within 2-3 hours of collection, kept refrigerated on ice and protected from light until returned to Utah State University (maximum of 6 hours) and then frozen at -4°F (-20°C) until it was thawed for analysis. Serum samples were evaluated for the following: aluminum, antimony, arsenic, barium, beryllium, boron, cadmium, calcium, chromium, cobalt, copper, iron, lead, lithium, magnesium, manganese, molybdenum, nickel, phosphorus, potassium, selenium, silicon, silver, sodium, strontium, thallium, tin, vanadium, vitamin A, vitamin E and zinc.

Individual calf illness events were recorded by backgrounding lot personnel during the next 60 days. A case of BRD was defined as any calf exhibiting clinical signs of depression, gauntness, or anorexia, and did not have clinical signs referable to another body system. Calves with clinical cases of BRD were identified and treated with an antimicrobial drug by experienced feedlot personnel. Eighty-four calves diagnosed with BRD during the first 33 days in the backgrounding lot were identified and matched 1:1 with calves not diagnosed with BRD. Calf matched pairs originated from the same ranch (lot) and had similar weight and breed characteristics, thus controlling for the variation in preconditioning, weaning and backgrounding lot processing protocols in the case/control study design.

Serum samples from at least 20 calves within each ranch of origin were randomly selected and analyzed to evaluate herd vitamin and mineral serum concentrations. Mean values for each herd were compared to published normal values for minerals and vitamins A and E.[8]

Elemental Analysis

Elemental analysis was performed at the Utah State Veterinary Diagnostic Laboratory.[c] An ICP/MS (Inductively Coupled Plasma/Mass Spectroscopy) analytical technique was utilized for elemental quantification. Serum samples from 224 enrolled or randomly selected animals were thawed at room temperature and analyzed for 29 minerals.

Elemental analyses were performed with a PerkinElmer Sciex Elan 6000 ICP/MS[d] equipped with an AS 91 autosampler. Samples were digested 1:1 in trace mineral-grade nitric acid at 90°C for 1 hour. One milliliter (ml) of the digest was added to 9 ml of 18.3 mOhm water to provide a 5% final acid content. All external standards were also prepared in a 5% final nitric acid matrix. Five point standard curves were utilized for all mineral analyses, except that of selenium. Selenium was analyzed by the standard addition method with three point standard curves. Standard curves and quality control samples were analyzed every 6 samples.

Vitamin A and E Analyses

The analyses for the vitamin A and E content were performed at the Wyoming State Veterinary Diagnostic Laboratory.[e] Serum samples for vitamin A and E analyses were thawed at room temperature. One-half to 1 ml of serum was added to a 10 ml glass tube with a teflon-lined cap. A sample was also spiked with 10 parts per million (ppm) vitamin A and 3 ppm vitamin E to calculate recovery. Three milliliters of 2% ascorbic acid in ethanol and 0.5 ml of saturated KC1 were added to each sample. The mixture was then extracted twice with 4 ml of petroleum ether. The two volumes of petroleum ether were dried without heat under nitrogen gas flow. The dried extract was reconstituted with 0.5 ml HPLC grade methanol. High performance liquid chromatography (HPLC) analysis was achieved with a Perkin Elmer 250 Binary LC Pump attached to a Perkin Elmer LC-235 Diode Array Detector.[f] Analyte separation used a Perkin Elmer Brownlee, Pecosphere 3 C18 Monofunctional 3 cm column, 10 µl injections, 1 mL/min flow rate of 3% distilled water in HPLC-grade methanol. Vitamin E was measured at 290 nm and vitamin A at 325 nm, with quantification using external standards.

Statistical Analysis

Data were entered into a spreadsheet[g] and descriptive statistics were performed. Respiratory disease morbidity rates were calculated by taking the total number of cattle diagnosed with respiratory disease at least once during the study period, divided by the total number of cattle in the pen or study. Mortality rates were defined as the number of dead cattle within the pen or total study, divided by the number of cattle placed in the pen or entered in the whole study. Mean serum concentrations of six clinically important vitamins and minerals were calculated using random samples from each herd of origin and summarized in tabular form.

Individual mineral/vitamin serum concentrations for all calves classified as either cases or controls were examined graphically and compared to published normal serum concentration ranges for each mineral/vitamin. Serum vitamin/mineral concentration for each case and control animal was classified as either: 1) within normal range; 2) low abnormal range; or 3) high abnormal range. However, most of the 31 vitamins/minerals examined in this study had only two classifications, within normal range and low abnormal range. Case-control pairs were examined in the statistical inferential analysis as a single observation by subtracting the value of the control from the case (case minus control) and performing the analysis on the differences between the case-control pairs. Differences between the cases/controls for each mineral/vitamin were examined in tabular form to determine the number of concordant (same classification) and discordant (different classification) pairs. Mineral/vitamins that had ≤5 discordant pairs or that had approximately equal numbers of discordant pairs were excluded from any further consideration in the analysis.

Logistic regression modeling (Proc Logistic[h]) was performed on the difference between the case/control pairs for all serum minerals/vitamins with enough discordant pairs to be biologically or clinically relevant. Regression models were run with no y-intercept and the outcome variable for all case/control pairs was set to one.[6,9]

Mineral/vitamin variables, which qualified for further analysis, were individually evaluated for an association with the likelihood of being diagnosed as a case using a Wald Chi-square *P*-value of < 0.25 as a cutoff. All variables which met this univariate criteria were included in a multi-variable model and evaluated using a manual backwards selection model building technique with a critical alpha of 0.05 for inclusion into the final model.

Results

All initial treatments of calves for BRD occurred during the first 33 days in the backgrounding lots, with some calves being re-treated. Descriptive information and morbidity and mortality rates for the herds of origin and the backgrounding lots are summarized in Table 1. There was great variation in the BRD morbidity rates observed in calves from the various ranches of origin, ranging from 1.2% to 81.8%. Overall group mortality rates ranged from 0% to 5.5%. This included calves that died from any cause during the study period because postmortem examinations could not be performed by investigators due to constraints of travel distance.

The regression model used to evaluate the relationship between vitamin/mineral serum concentration at backgrounding lot entry and BRD modeled the likelihood that a calf with low vitamin/mineral serum concentrations would be diagnosed as a case of BRD. Out of 31 variables initially screened, four qualified for inclusion in a multi-variable model. The four variables were sodium, selenium, vitamin E and manganese. None of the variables evaluated in the final multi-variable model were statistically associated with a diagnosis of BRD. However, calves which entered the backgrounding lot with low serum selenium concentrations (<0.08 ppm) tended to be less likely to be classified as a case of BRD (OR = 0.143, 95% C.I. = 0.02-1.16). All two-way interactions between the four variables in the multi-regression model were evaluated but were not statistically significant.

Classification of calves as deficient for selected clinically important vitamins and minerals, by herd of

Table 1. Overall and group specific descriptive statistics, crude mean daily weight gain, and morbidity and mortality rates for beef calves originating from ranches in southwestern Utah.

Herd No.	Lot	N[a]	Entry wt.[b] (lb±sd)	DOF[c]	Final wt.[d*] (lb±sd)	MDG[e*] (lb±sd)	% BRD[f] (n)	% dead[g] (n)
1	A	104	479±66	73	629±81	2.05±0.79	71.2 (74)	0 (0)
2	B	94	480±58	65	697±85	3.35±0.88	29.8 (28)	0 (0)
3	B	106	500±49	65	714±62	3.28±0.51	5.7 (6)	0 (0)
4*	A	110	401±60	59	499±72	1.55±0.84	81.8 (90)	5.5 (6)
5	A	100	522±47	49	636±64	2.33±0.71	13.0 (13)	1.0 (1)
6	C	86	465±69	75	684±81	2.92±0.48	1.2 (1)	0 (0)
7	C	103	490±54	68	681±60	2.82±0.47	1.9 (2)	0.9 (1)
8*	A	84	492±62	59	601±73	1.75±0.69	27.4 (23)	1.2 (1)
Totals		787	481±65	64	645±97	2.52±0.93	30.1 (237)	1.1 (9)

[a]Total number of calves in the group from the herd of origin.
[b]Mean weight (lb±sd) of calves in the group at processing upon backgrounding lot arrival.
[c]Days on feed in the backgrounding lot when weighed at the end of study period.
[d]Mean weight (lb±sd) of surviving calves in the group when weighed at the end of study period.
[e]Crude mean daily weight gain (lb±sd) of surviving calves in the group when weighed at the end of study period.
[f]Bovine respiratory disease morbidity rate of group for the first 30 days on feed in backgrounding lot. n = number in group diagnosed and treated for respiratory disease by producer.
[g]Overall mortality rate in group due to any cause when evaluated at the end of the study period.
*The background operator sold 12 calves from group four and 4 calves from group eight prior to measuring final weights on calves. These observations were dropped from the data set in the calculation of final mean calf weights and crude mean daily weight gains in these groups.

origin, are summarized in Tables 2 and 3. The proportion of calves with vitamin E and vitamin A serum concentrations less than published low-normal values ranged from 15.0-75.0% and 40.0-90.0%, respectively, and was not correlated with the proportion of respiratory disease diagnosed in the herd.

Discussion

Respiratory disease morbidity rates in these groups of southern Utah calves varied greatly, but 2 of 3 backgrounding lots reported BRD morbidity rates that were within expected parameters for lightweight, high-risk calves entering a backgrounding unit. Higher than expected BRD morbidity rates experienced by backgrounding lot A appeared to be an effect specific to that backgrounding lot and unrelated to the origin of incoming calves.

The observed trend that calves with low serum selenium concentrations were less likely to be diagnosed as a case of BRD was probably a spurious observation due to the low numbers of discordant pairs (n = 8) for serum selenium concentration in these data. Most case-control pairs in these data had the same serum selenium concentration classification (n = 76). Selenium is commonly regarded as an important element for immune function in cattle, which also suggests that our observation in these data conflicts with what is currently known about the biological importance of selenium in the bovine immune system. This observation should therefore be regarded with caution until this relationship, if it exists, can be verified or disproved in a larger, prospective study.

This investigation did not detect an association between BRD and any of the minerals and vitamins evaluated. This may be due to several reasons. First, an association between BRD and the vitamins/minerals may not exist in this population of cattle. However, if a relationship does exist, the current investigation may not have detected that relationship due to the low power of this study and the potential effect of non-differential misclassification of cases due to the use of producer defined cases of BRD in this study. Unfortunately, this could not be avoided because of the distance between the investigators and the backgrounding lots and the failure of participating backgrounding lot treatment crews to record data that could have been used for a more rigid definition of BRD. Also, some minerals under investigation (i.e., beryllium, thallium) could not be examined for a relationship to BRD because serum concentrations of these minerals were below detectable levels (as expected).

This study was a unique evaluation for a potential relationship between BRD and vitamin/mineral deficiencies of individual beef calves at backgrounding lot entry for a large number of minerals and vitamins. The authors recognize the value of liver biopsy tissue for evaluation of body stores of some minerals, such as copper.

Table 2. Mean serum concentration (ppm±sd) of selected vitamins and minerals in beef calves originating from ranches in southwestern Utah at entry into a backgrounding lot.

Herd No.	N[a]	Vitamin E	Vitamin A	Copper	Mn[b]	Selenium	Zinc
1	36	3.96±2.68	0.27±.14	0.84±.14	.018±.014	0.13±.03	1.26±.13
2	34	3.03±1.38	0.27±.12	0.78±.13	.015±.011	0.09±.01	1.19±.13
3	20	4.35±1.30	0.29±.13	0.30±.13	.014±.008	0.35±.06	1.12±.22
4	39	2.70±1.32	0.27±.12	0.81±.16	.011±.004	0.14±.04	1.22±.20
5	20	3.40±1.90	0.18±.10	0.91±.21	.008±.005	0.06±.02	0.99±.23
6	20	3.66±1.42	0.33±.15	0.94±.13	.014±.007	0.09±.02	1.08±.12
7	20	2.30±1.69	0.31±.23	0.99±.30	.022±.010	0.10±.03	0.95±.25
8	35	4.22±3.33	0.28±.15	1.01±.16	.032±.022	0.20±.05	0.96±.23
Mean[c]		3.44±2.17	0.27±.14	0.83±.25	.017±.014	0.14±.08	1.12±.22
Normal Range[d]		3-10	>0.3	0.6-1.5	.006-.07	0.08-0.3	0.8-1.4

[a]Total number of calves sampled from each herd.
[b]Manganese
[c]Mean serum vitamin or mineral concentration (ppm±sd) for combined sample groups.
[d]Published normal value for serum vitamin or mineral concentration (ppm) in beef calves.[8]

Table 3. Proportion of calves originating from ranches in southwestern Utah with serum concentrations of selected vitamins and minerals that were less than published low-normal values.

Herd No.	N[a]	Vitamin E[b] (%)	Vitamin A[c] (%)	Copper[d](%)	Mn[e](%)	Selenium[f](%)	Zinc[g] (%)	BRD[h](%)
1	36	47.2	61.1	2.8	11.1	2.8	0.0	71.2
2	34	58.8	67.7	14.7	11.8	11.8	0.0	29.8
3	20	15.0	65.0	95.0	0.0	0.0	10.0	5.7
4	39	64.1	66.7	10.3	10.3	2.6	0.0	81.8
5	20	45.0	90.0	5.0	30.0	80.0	25.0	13.0
6	20	35.0	40.0	0.0	10.0	25.0	0.0	1.2
7	20	75.0	70.0	0.0	5.0	10.0	20.0	1.9
8	35	48.6	54.3	0.0	0.0	0.0	22.9	27.4
Total	224	50.5	63.8	13.4	9.4	13.0	8.5	30.1

[a]Total number of calves sampled from each herd.
[b]Proportion of calves with serum vitamin E concentration < 3 ppm.
[c]Proportion of calves with serum vitamin A concentration < 0.3 ppm.
[d]Proportion of calves with serum copper concentration < 0.6 ppm.
[e]Proportion of calves with serum manganese concentration < 0.006 ppm.
[f]Proportion of calves with serum selenium concentration < 0.08 ppm.
[g]Proportion of calves with serum zinc concentration < 0.8 ppm.
[h]Proportion of calves in herd diagnosed with respiratory disease.

However, we also concur with the conclusion that "low serum copper concentration suggests that liver copper stores have been exhausted and the animal is in a deficient state".[11,13] We wanted to identify those calves that were deficient at entry to the backgrounding lot. If their liver storage was low, but still adequate to maintain serum concentrations, then it could also be adequate to maintain normal immune function. However, if the serum concentration was low then it could be having an effect on immune function, regardless of the concentration present in the liver. Some calves may have had adequate concentrations of copper present in the serum but bound in some form to render it unusable to the calf. That aspect was not evaluated. However, the evaluation of liver status would not have answered that question either.

Our primary objective was not to predict a herd deficiency. Rather, we wanted to determine the serum concentration of individual calves at a time of beginning and continuing stress and relate that to later development of disease. However, it was also important to determine herd serum concentration by the same

methods used to evaluate the serum concentration of individual animals.

Conclusions

Some herds were deficient or marginally deficient in serum concentration of specific trace elements (copper, manganese, selenium, zinc) and vitamin A or E. All of these have been shown to be important for immunity and disease resistance. Potential deficiencies can be evaluated on a herd basis. Some of the herds involved in this study were deficient in specific minerals or vitamins, and the supplementation and further monitoring of these herds should be implemented.

In these data there was no detectable association between serum vitamin A and E or mineral concentration and BRD. In our opinion, producers and veterinarians should focus BRD prevention efforts on management procedures and vaccination schedules near weaning.

It appears it may be worthwhile for cow/calf producers to evaluate their vitamin/mineral supplementation program by blood serum analyses or other appropriate methods for the nutrient of concern to identify likely deficiencies. If deficiencies are found, then it is probably worthwhile to implement a program of supplementation to overcome the specific deficiencies and monitor the mineral or vitamin supplementation program with follow-up analyses. However, our data could not confirm or refute that trace element or vitamin supplements will greatly change the illness rate for recently weaned calves. If this rate is excessive, other management steps to reduce stress and exposure at weaning should be implemented.

Footnotes

[a] Multiple, personal communications with producers and veterinarians of the area, 1999.
[b] Royal blue top vaccutainer tubes (Silicon coated, 7ml), Becton, Dickinson and Co., Franklin Lakes, NJ
[c] Utah State Veterinary Diagnostic Laboratory, 5700 Old Main Hill, Logan, UT 84322-5700
[d] PE Sciex Elan 6000 ICP/MB, PerkinElmer Inc., Shelton, CT
[e] Wyoming State Veterinary Diagnostic Laboratory, 1174 Snowy Range Rd., Laramie, WY 82070
[f] PE LC-235 Diode Array Detector w/ PE 250 Binary LC pump, PerkinElmer Inc., Shelton, CT
[g] Excel, Microsoft Corp., Redmond, WA
[h] Proc Logistic, SAS Institute Inc., Cary, NC

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