PEER REVIEWED

Trace Mineral Concentrations in Dairy Cattle with Rupture of Abdominal Artery Aneurysms

Catherine G. Lamm[1], *DVM, DACVP;* **Karyn L. Bischoff**[2], *DVM, MS, DABVT;* **Hollis N. Erb**[2], *DVM, MS, PhD;* **Charles L. Guard**[2], *DVM, PhD;* **Joseph R. Hillebrandt**[2], *AAS, BS;* **Belinda Thompson**[2], *DVM;* **Bradley L. Njaa**[3], *DVM, MVSc, DACVP*

[1]*Oklahoma Animal Disease Diagnostic Laboratory, Oklahoma State University, Stillwater, OK 74078*

[2]*Department of Population Medicine and Diagnostic Sciences, Cornell University, Ithaca, NY 14853*

[3]*Department of Veterinary Clinical Sciences and Department of Pathobiology, Oklahoma State University, Stillwater, OK 74078*

Corresponding author: Dr. Catherine G. Lamm, Oklahoma State University, Oklahoma Animal Disease Diagnostic Laboratory, Stillwater, OK 74078

Abstract

Rupture of abdominal artery aneurysm (AAA) in cattle is sporadic and typically affects adult Holstein dairy cows. In this retrospective study, liver concentrations of copper (Cu), iron (Fe), manganese (Mn), sulfur (S), and zinc (Zn) were evaluated using inductively coupled plasma atomic-emission spectroscopy. Up to three liver sample types (fresh/frozen, fixed, or paraffin embedded) were collected from 22 affected adult Holstein dairy cows (36 samples) and nine similarly aged control Holstein cows with histologically normal vessels that died from other causes (25 samples). There was no significant difference in the liver concentrations of Cu, Mn, S, and Zn between groups for any tissue sample type (all $P \geq 0.022$; alpha=0.017). Liver Fe concentrations were lower in the affected animals for all tissue sample types ($P \leq 0.0067$). Liver Fe concentrations were measured in 11 additional cattle that were exsanguinated at slaughter to determine if exsanguination results in low liver Fe concentrations. There was no significant difference between liver Fe concentrations in cattle with ruptured AAAs versus cattle that were exsanguinated, but both were significantly different from cattle that died from other causes (P=0.0066). This study found no significant differences in liver Cu, Mn, S, and Zn in cattle that died from AAA rupture compared to cattle that died from other causes. Significantly lower liver Fe concentrations in cattle that died from AAA rupture were apparently due to exsanguination.

Key words: aneurysm, artery, bovine, iron

Résumé

La rupture de l'anévrisme de l'artère abdominale est sporadique chez les bovins et affecte le plus souvent les vaches laitières Holstein adultes. Dans cette étude rétrospective, la concentration hépatique en cuivre, en fer, en manganèse, en soufre et en zinc a été évaluée avec la spectroscopie de masse utilisant un plasma à couplage inductif. On a recueilli jusqu'à trois types d'échantillons de foie (frais/gelé, fixé ou enduit de paraffine) chez 22 vaches laitières Holstein adultes affectées (36 échantillons) et chez neuf vaches témoins Holstein du même âge avec des vaisseaux histologiquement normaux et qui étaient mortes d'autres causes (25 échantillons). Il n'y avait pas de différence significative entre les deux groupes au niveau de la concentration hépatique en cuivre, en manganèse, en soufre et en zinc pour chacun des trois types d'échantillons (tous les $P > 0.022$, seuil alpha=0.017). La concentration hépatique en fer était moins élevée chez les individus affectés pour chacun des trois types d'échantillons ($P \leq 0.0067$). La concentration hépatique en fer a été mesurée chez 11 autres bovins saignés à l'abattoir pour déterminer si la saignée entraîne une plus faible concentration hépatique en fer. Il n'y avait pas de différence significative au niveau de la concentration hépatique en fer entre les bovins avec des ruptures de l'anévrisme de l'artère abdominale et les bovins saignés; toutefois, ces deux groupes montraient des différences significatives par rapport aux bovins morts d'autres causes (P=0.0066). Cette étude n'a pas démontré de différence significative au niveau de la concentration hépatique en cuivre, en manganèse, en soufre et en zinc entre les bovins avec rupture de l'anévrisme de l'artère abdominale et ceux morts d'autres causes. Toutefois, la concentration hépatique en fer moins élevée chez les bovins morts suite à une rupture de cet anévrisme semble être causée par la saignée.

Introduction

Rupture of an abdominal artery aneurysm (AAA) is a sporadic disease of adult Holstein dairy cattle

which results in exsanguination.[1,10,12,15] This disease is characterized by rupture of a major abdominal artery (e.g. aorta, celiac artery, gastric artery, ruminal artery, or mesenteric artery) leading to hemoabdomen.[10] Typically only a single abdominal artery ruptures, though histologic changes are evident in other major abdominal arteries.[10] Histologically, there are marked changes within the vessel-wall architecture, primarily affecting the tunica media. There is multifocal loss of the tunica media, disruption of the extracellular matrix (ECM) components, smooth muscle hyperplasia, and mineralization.[10] Of the ECM components, elastin appears to be the most affected with an overall decrease in elastin content and fraying and disruption of the remaining elastin. These changes appear to disrupt the contractile unit within the vessel wall, driving the development of aneurysms through the progressive loss of vascular recoil and overall vessel-wall integrity.

Elastin is one of the major components of the large caliber arteries. Elastin is joined with fibrillin to form elastin fibers.[9] Elastin fibers are connected through an intricate network of microfibrils to the contractile fibers within the smooth muscle cell, forming a single contractile unit.[7] Elastin fiber loss plays a role in the development of artery rupture in several other species, including humans, turkeys, and swine.[6,8,14,16] In conditions affecting turkeys and swine, elastin fiber loss is driven by dietary copper (Cu) deficiency.[8,16] In rats, decreased dietary iron (Fe) results in decreased vascular wall elastin.[13] Studies using fibroblasts derived from humans have shown that Fe directly and specifically affects the genes which regulate the production of elastin.[5] Interactions between trace minerals and vascular ECM components are documented within a variety of species.[5,13,16]

There is an absence of literature relating to trace mineral concentrations in cattle with AAA. The purpose of this study was to determine whether Holstein dairy cows with rupture of an AAA had abnormal concentrations of trace minerals within the liver at the time of death.

Materials and Methods

Study population

The Cornell University Anatomic Pathology computer data base was searched for cases submitted through the surgical or necropsy services from January 1980 through June 2005. A 25-year search period was chosen because rupture of an AAA is a rare disease, ranging from zero to three affected cattle submitted for necropsy per year. Additional cases fitting the following criteria submitted separately by the Ambulatory Clinic at Cornell University through the Section of Anatomic Pathology were also included: a domestic bovid (*Bos taurus*) of any age with rupture of an AAA and subsequent hemoabdomen confirmed on postmortem examination. Sections of the abdominal aorta, celiac artery, and gastric artery from each of the affected animals were examined histologically. Liver was stored either frozen, formalin-fixed, or embedded for trace mineral analysis using inductively coupled plasma atomic-emission spectroscopy (ICP-AES).

Controls

Nine adult Holstein dairy cows submitted to Cornell University for necropsy or necropsied in the field by the Cornell Ambulatory Clinic between July 2004 and June 2005 were selected prospectively by convenience as controls. All control cattle died from disease unrelated to the cardiovascular system. Sections of the abdominal aorta, celiac artery, and gastric artery from each of the controls were examined histologically. Cattle with histologic abnormalities in the vessels were excluded as controls in the study. Selection of control cattle was limited to a one-year period because archived tissue from unaffected cattle did not have large vessels for histologic examination to confirm the lack of vascular changes. Liver was stored either frozen, formalin-fixed, or embedded for trace mineral analysis.

Slaughter animals

Fresh liver from an additional 11 cattle was collected from two slaughter facilities following routine slaughter and exsanguination. Cattle came from at least two different farms. Liver was frozen for trace mineral analysis; vessels from slaughter animals were not examined histologically.

Analytical toxicology

Up to three types of liver samples were available for each affected and control animal included in the study: frozen, fixed, and paraffin embedded tissues. Only frozen livers from slaughter cattle were analyzed. All samples from both affected and control cows were tested for Cu, Fe, manganese (Mn), sulfur (S), and zinc (Zn) concentrations using modified Association of Official Agricultural Chemists official method of analysis 985.01.[2] The wet weight of the samples was determined. Samples for Fe, Cu, Mn, and Zn were ashed at 932°F (500°C). Samples were then dissolved in nitric acid and hydrochloric acid. Samples for S analysis were placed in closed Teflon™ vessels using a wet digest with the same acids, and put in an oven for 48 hours at 176°F (80°C). Then ICP-AES was used on the processed samples to determine concentrations of Cu, Fe, Mn, S, and Zn.[a] The resulting mineral concentrations were calculated on a wet-weight basis.

Statistical analysis

Wilcoxon rank-sum tests were used to generate *P*-values for each mineral tested. Because paraffin

embedding artificially elevates mineral concentrations within liver samples, each tissue sample type was compared separately for statistical analysis.[3] Statistical analysis was performed using Statistix 9.[b] All *P*-values were interpreted 2-sided and with $P<0.017$ required for significance (because there were three tests for each mineral; 0.05/3 = 0.0166).

Results

Case, control, and slaughter samples

Between January 1980 and June 2005, 33 cows with AAA rupture from 29 different farms were submitted to the laboratory, and at least one tissue sample type from 22 cows was available for testing. One to three tissue sample types were available for each affected cow (36 total samples). A total of 25 samples were tested from the nine control cows (Tables 1 to 3). Frozen tissues from 11 cattle, representing at least two farms, were collected from two slaughterhouses.

Analytical toxicology

Cattle with AAA rupture had lower liver Fe concentrations than control animals for all tissue sample types tested (Tables 1 to 3). No association was found between Cu, Mn, S, and Zn liver concentrations and the development of rupture of an AAA ($P>0.017$; Tables 1 to 3). The difference between affected and control cow liver Fe concentrations was statistically significant for all tissue sample types ($P\leq0.0067$). Exsanguinated slaughter cattle had frozen liver Fe concentrations ranging between 43 mg/kg and 111 mg/kg (wet weight). No significant difference was found in liver Fe concentrations between cattle with ruptured AAAs and cattle exsanguinated at slaughter, but both were significantly different compared to control cattle based on frozen tissue only ($P=0.0066$).

Discussion

Based on research in other animals, including turkeys and swine, it has long been speculated that Cu

Table 1. Liver trace mineral concentrations from **fresh or frozen** samples from 22 adult Holstein dairy cows with rupture of an abdominal artery aneurysm and nine unaffected Holstein cows.

Mineral	Affected?	No. tested	Liver concentration (mg/kg)			*P*-value
			Min	Med	Max	
Copper	Yes	9	30	176	331	0.21
	No	7	39	108	228	
Iron	Yes	4	55	57	62	0.0061
	No	7	80	95	149	
Manganese	Yes	4	2	3	3	0.0394
	No	7	0	1	3	
Sulfur	Yes	1	-	1800	-	0.50
	No	3	1300	1300	1500	
Zinc	Yes	4	31	42	64	0.83
	No	7	22	51	133	

Table 2. Liver trace mineral concentrations from **formalin-fixed** samples from 22 adult Holstein dairy cows with rupture of an abdominal artery aneurysm and nine unaffected Holstein cows.

Mineral	Affected?	No. tested	Liver concentration (mg/kg)			*P*-value
			Min	Med	Max	
Copper	Yes	7	14	114	228	0.37
	No	9	49	135	527	
Iron	Yes	7	54	66	106	0.0005
	No	9	101	132	217	
Manganese	Yes	7	1	2	6	0.58
	No	9	0	2	6	
Sulfur	Yes	3	2000	2200	2400	0.23
	No	4	1600	1900	2200	
Zinc	Yes	7	20	38	74	0.022
	No	9	34	60	185	

Table 3. Liver trace mineral concentrations from **paraffin embedded** samples from 22 adult Holstein dairy cows with rupture of an abdominal artery aneurysm and nine unaffected Holstein cows.

Mineral	Affected?	No. tested	Liver concentration (mg/kg)			*P*-value
			Min	Med	Max	
Copper	Yes	20	7	202	488	0.83
	No	9	61	177	323	
Iron	Yes	20	43	104	216	0.0067
	No	9	95	166	226	
Manganese	Yes	20	1	2	5	0.94
	No	9	1	2	4	
Sulfur	Yes	15	1300	2800	4100	0.89
	No	4	2200	2400	3100	
Zinc	Yes	20	16	54	130	0.0591
	No	9	45	74	219	

deficiency plays a role in the development of aneurysm and rupture of abdominal arteries in cattle.[8,16] This study failed to demonstrate any association between the rupture of an AAA and liver Cu concentrations at the time of death. In fact, 31/36 of samples from affected cows had liver Cu concentrations considered adequate or elevated. As with Cu, there was no association between abnormal concentrations of Mn, S, and Zn and the presence of a ruptured AAA in cattle. One limitation of this study was that, due to the sporadic nature of the disease, the liver samples from affected animals could only be harvested postmortem. It is possible that affected cattle had historically low trace mineral concentrations which were corrected before the time of death.

The reference range for liver Fe concentrations varies widely depending on the source. The standard reference range used by many veterinary diagnostic laboratories is 45 to 300 mg/kg (wet weight) for beef and dairy cattle.[11] A liver Fe reference range specific for dairy cows is 100-250 mg/kg (wet weight), increasing with age.[4] Cattle with AAA rupture had low liver Fe concentrations, less than 80 mg/kg (wet weight), in fresh or frozen liver samples at the time of death (4/4), as did most of the slaughter cattle (10/11). Only 4/7 control cattle had liver Fe concentrations <80 mg/kg (wet weight). No significant difference was found between liver Fe concentrations in cattle with ruptured AAAs and cattle exsanguinated at slaughter. These results suggest that exsanguination, either through rupture of a vessel or at slaughter, can significantly lower liver Fe concentrations.

Conclusions

Based on the data presented in this study, Cu, Mn, S, or Zn concentrations in the liver at the time of death and the rupture of AAA in cattle were not significantly different from control cattle dying of disease unrelated to the cardiovascular system. Liver Fe concentrations less than 80 mg/kg are associated with exsanguination and can be seen in cattle with ruptured AAA as well as in slaughtered cattle. Additional studies evaluating trace mineral concentrations over time are required to definitively rule out Cu, Mn, S, Zn, or Fe mineral deficiency as a cause of AAA in cattle.

Acknowledgements

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Endnotes

[a]Spectroflame ICP, Spectro Analytical Instruments, Inc., Malborough, MA

[b]Statistix 9 (2008) Analytical Software, Tallahassee, FL

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