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Investigation of congestive heart failure in beef cattle in a feedyard at a moderate altitude in western Nebraska

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Abstract. Right-sided congestive heart failure (brisket disease) commonly occurs in cattle raised at elevations >2,500–3,500 m. We investigated clinical cases resembling brisket disease at a western Nebraska feedyard at a moderate altitude (1,369 m). Over a 15-mo period (2009–2010), we examined 17 cases (16 steers and 1 heifer), all purebred Angus. All animals had clinical right-sided heart failure: brisket and ventral abdominal edema, and severe chronic passive congestion of the liver. Gross examination confirmed right ventricular hypertrophy (left ventricle plus septum: right ventricle weight ratio mean: 1.33 vs. 2.8–4.0 reference interval). Microscopically, all 17 cases had interstitial fibrosis (mean score: 2.4 ± 0.8) and 6 had replacement fibrosis of the right ventricle, whereas 14 had interstitial fibrosis (mean score: 1.2 ± 0.2) and 0 had replacement fibrosis of the left ventricle. Lesions of arteriosclerosis were seen in 9 of 16 cases in 51 of 571 (8.9%) right ventricular coronary arteries, and in 10 of 16 cases in 52 of 366 (14.2%) left ventricular coronary arteries. The probability of coronary arteriosclerosis was greater in papillary ventricular muscle (OR = 11.3; $p < 0.0001$), left ventricle (OR = 4.8; $p < 0.0001$), and larger arteries (OR = 1.01; $p < 0.0001$). Pulmonary arteries and arterioles had lesions compatible with hypoxia-induced pulmonary hypertension. We hypothesize that moderate hypobaric conditions significantly contributed to disease in cattle genetically predisposed to hypoxia-induced pulmonary hypertension. Adiposity, coronary arteriosclerosis, and left ventricular fibrosis may have contributed to the condition; however, the cattle died prior to development of advanced obesity.

Key words: bovine; congestive heart failure.

Introduction

Brisket disease, also known as high-mountain disease or high-altitude disease, is a common condition in cattle raised at elevations >2,500–3,500 m.[9] The pathogenesis involves hypoxia-induced pulmonary arterial constriction and remodeling leading to pulmonary hypertension and eventual right heart failure (cor pulmonale).[6] A syndrome thought to represent brisket disease occurs in Holstein cattle at lower elevation (1,600 m).[18] A 2016 large-scale retrospective observational study found an increasing incidence of right-sided congestive heart failure (RHF) in feedlot cattle at elevations ≤1,282 m in the midwestern United States and Canada.[21] Although the design in that study did not control for confounding variables associated with altitude, it was noted that cattle in U.S. feedlots at the highest altitude (1,282 m) were 9 times more likely to die of RHF than cattle at the lowest altitude (596 m). The authors noted that very little was known about the risk factors for this syndrome, but cattle treated for respiratory disease were 2–3 times more likely to die as a result of RHF.

The lack of knowledge about risk factors for RHF in feedlot cattle at low and moderate altitudes, coupled with anecdotal reports of an apparent increase in deaths in western Nebraska feedlots as a result of a condition resembling brisket disease, led us to conduct a postmortem study that included histopathology on affected cattle in one feedyard. We describe herein the results of that investigation.

Materials and methods

Cases and organ/tissue requests

We conducted our study at a feedyard in western Nebraska that had a significant number of RHF cases and was located at an elevation of ~1,369 m. The case definition was cattle

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Table 1. Signalment and history of 17 right-heart failure cases from a Nebraska cattle feedyard.

Case	Sex	Age (d) at arrival in feedlot	Number of days in feedlot	Animal died (D) or slaughtered (S)	Age (d) at death or slaughter	Weight (kg) at death or slaughter
1	Steer	194	137	D	331	264
2	Steer	194	140	D	334	400
3	Steer	214	171	D	385	414
4	Steer	191	183	S	374	483
5	Steer	202	183	S	385	483
6	Steer	210	183	S	393	483
7	Steer	197	207	D	404	450
8	Heifer	239	173	D	412	432
9	Steer	192	119	D	311	259
10	Steer	232	170	D	402	505
11	Steer	205	181	D	386	441
12	Steer	182	180	D	362	341
13	Steer	190	189	D	379	491
14	Steer	212	196	D	408	355
15	Steer	211	203	D	414	427
16	Steer	229	221	D	450	350
17	Steer	186	226	D	412	464
$\bar{x} \pm$ SEM		205 ± 1	180 ± 2		385 ± 2	414 ± 4

SEM = standard error of the mean.

that exhibited: 1) clinical evidence of brisket edema and one or more of the antemortem signs of jugular pulse, dyspnea, or cyanosis; and 2) postmortem lesions of heart enlargement, pleural or pericardial effusion, or pulmonary edema. Identification of heart enlargement postmortem was by subjective interpretation of size and shape.

We included in our study 17 cattle (16 steers and 1 heifer) fitting the case definition over a 15-mo period, from January 19, 2009 to April 22, 2010; all were purebred Angus (Table 1). The steers originated from a herd located immediately adjacent to the feedlot, and the heifer originated from a herd in Cokeville, Wyoming, elevation 1,888 m. Of the 17 cattle, 14 died and 3 were slaughtered prematurely because of advanced clinical signs. All 14 cattle that died were autopsied; slaughter was done at a commercial abattoir. We weighed dead cattle on an 18-m (60-ft) truck scale that measured to the nearest 4.5 kg (10 lb), with accuracy certified by the State of Nebraska. The weight of each dead case animal was determined by subtracting the weight of an empty payloader with driver from the weight of the payloader with driver and animal. We weighed live case animals on a chute scale measuring to the nearest 0.45 kg (1 lb) immediately prior to loading on the truck for shipment to the abattoir. We confirmed the accuracy of the chute scale by periodically comparing measured weights with that obtained from the aforementioned truck scale. Weight measurements of live and dead cattle were converted to kg.

Autopsies and/or sample collection at slaughter were performed by 5 lay-workers (4 at the feedlot and 1 at the abattoir) who were highly experienced in recognizing the postmortem lesions described in the case definition and had been trained to do so by one of the coauthors (T Edwards). At autopsy or slaughter, samples of heart, lungs, liver, and kidney were collected and shipped overnight to the laboratory in Lincoln, Nebraska, in mailers containing icepacks and formalin jars.

Submission forms and other materials to facilitate proper collection of specimens and supportive information were provided to the feedyard personnel. The materials included a Styrofoam specimen box with an outer cardboard mailing container (ThermoSafe 656 EPS; Sonoco ThermoSafe, Arlington Heights, IL), and wide-mouth, screw-cap polycarbonate jars (Nalgene; Nalge Nunc International, Rochester, NY) containing 10% neutral-buffered formalin. The submission form provided instructions on specimen collection, and requested contact information, animal description [ear tag or other identification (ID)], lot ID, date of death, date of arrival at feedlot, sex, breed, body weight (BW) at entry into the feedlot, BW at death or harvest, and comments and observations from the autopsy.

The request for tissues included whole, intact, fresh hearts, and formalin-fixed slices of lung, liver, and kidney. Formalin-fixed slices of heart were accepted if the submitter elected not to send the intact heart. The request for tissues was a slice 3-mm thick to improve fixation and thereby minimize postmortem change. The request for tissues was lung, 8 slices total, 4 from each lung; liver, 4 slices total, 1 from each lobe; kidney, slices from 2 lobules; heart (as an alternative to receipt of the intact organ), ≥5 slices total, each through the entire wall, including 1 from each atrium and ventricle, and 1 from the interventricular septum. We also requested slices from any organs with definitive or suspect

gross anatomic lesions, including lung, liver, kidney, and heart.

Examination and processing of organs and tissues

Whole, intact fresh hearts (12 cases, and 3 controls) were examined using a standard protocol.[16,25] Case animal hearts were weighed on a pan scale (Avery Berkel 3359; Avery Weigh-Tronix, Fairmont, MN) that measured to the nearest 0.01 lb. We converted measured weights to kg. Control animal hearts were weighed on a pan scale (Yamato DP-6200; Yamato, Colorado Springs, CO) that measured to the nearest 0.02 kg. We confirmed the accuracy of each scale with calibration weights. We measured case and control animal heart valves with a 35- or 30-cm ruler, respectively, each having 1-mm divisions (1-mm accuracy). Suture material (2-0) (case animals) or twist-tie wire (control animals) was laid along the edge of each heart valve, and then onto the ruler to determine the distance. We measured the total heart weight (HW), right ventricular free wall weight, left ventricle (LV) plus septum (LV + S) weight, aortic valve (AV) circumference, pulmonic valve (PV) circumference, left atrioventricular valve (LAV) circumference, and right atrioventricular valve (RAV) circumference. The HW/BW (%), right ventricle (RV)/BW (%), RV/HW, (LV + S)/RV, (LV + S)/HW, (LV + S)/BW (%), LAV/RAV circumference, AV/PV circumference, AV/LAV circumference, PV/LAV circumference, PV/RAV circumference, and AV/RAV circumference ratios were calculated. Measured and calculated values were compared to a normal reference interval (RI) for adult cattle,[16,25] if available. Additionally, mean values from 3 of the 17 cases (4, 10, and 13) were compared with that of 3 healthy weight-matched control feedlot heifers from another study.[5]

Formalin-fixed slices of heart, lung, liver, and kidney were examined grossly, and slices of fresh intact heart were prepared as described above and fixed in 10% neutral-buffered formalin for 24 h. All formalin-fixed tissues were processed, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin (H&E)[32] for histologic examination. Additional selected tissue sections were stained by the Verhoeff–Van Gieson,[14] Masson trichrome,[14] or Von Kossa[17] methods to demonstrate elastin, collagen, or mineral, respectively.

Normal pulmonary arteries, arterioles, veins, and venules were identified on the basis of features described previously.[1,2,11] Normal proximal arteries contained 2–3 layers of smooth muscle in the tunica media, and were adjacent to terminal bronchi having complete tubular structures.[11] Normal intermediate arteries were adjacent to airways containing the origins of alveolar ducts, and had one layer of smooth muscle in the tunica media.[11] Normal distal "non-muscularized" arteries or arterioles were "found lying to one side or in the space separating adjacent alveolar ducts."[11] These vessels contained one cell layer of smooth muscle in the tunica media[11] and had diameters as small as 20 μm.[1] Hyperplasia was interpreted as an increase in the numbers of cell layers for the particular type of vessel in the intima, media, or adventitia. Pulmonary veins were identified by the presence of sphincters, and hypertrophy of sphincters was assessed on veins cut through the middle of the vessel, either in cross-section or longitudinally. Sphincter hypertrophy was identified by increased thickness of the sphincter muscle such that the lumen was occluded or nearly so. Veins containing hypertrophied sphincters were often noted to be congested.

In order to assess hypertrophy objectively, morphometric measurements of cross-sectional diameters of cardiac myocytes were determined on H&E-stained sections of the right and left ventricular wall from the 17 RHF cases and of the LV from 3 control heifers.[5] Formalin-fixed right ventricular tissue had not been collected from the control heifers.[5] On one section per tissue per animal, cross-sections of 10 myocytes with clearly demarcated boundaries in different fields were randomly selected for measurement; the ocular micrometer was oriented across the nucleus. Mean values derived from the 10 measurements on each tissue were used for statistical analyses. Statistical comparisons of mean values of cardiac myocyte diameters in right and left ventricle included all RHF cases (n = 17), whereas mean values of LV in RHF cases 4, 10, and 13 (n = 3) were compared with that of healthy weight-matched control feedlot heifers (n = 3) from another study.[5]

We assessed the prevalence and severity of arteriosclerosis in coronary arteries in sections of right and LV stained with H&E, Masson trichrome, and Verhoeff–Van Gieson. Coronary arteries measuring ≥50 μm in diameter in myocardium, endocardium, and epicardium were collectively included in the assessment of prevalence and scoring. A semiquantitative scoring scheme based on the pathogenesis of coronary arteriosclerosis in cattle[4] was developed and used to determine prevalence and score the severity of lesions: 1) Tunica intima is segmentally or diffusely hyperplastic with accompanying irregularity, fragmentation, and duplication of the internal elastic membrane, and collagen deposition. 2) Tunica intima is diffusely hyperplastic with diffuse internal elastic membrane fragmentation and duplication, and contains immigrated smooth muscle cells and collagen deposition with the last extending into the tunica media. 3) Intimal changes as in 2 above, with collagen deposition extending through the tunica media in association with loss of medial myocytes. 4) Changes as in 3 above, with further intimal proliferation and degenerative changes in the tunica media that have resulted in narrowing of the vascular lumen. 5) Changes as in 4 above, with occlusion of the vascular lumen.

The severity of perivascular, interstitial, and replacement fibrosis[3,10,15,26] in the right and left ventricular myocardium was assessed on sections stained with Masson trichrome using a semiquantitative scoring scheme: 0, no detectable lesions; 1–3 represented increasing amounts and distribution

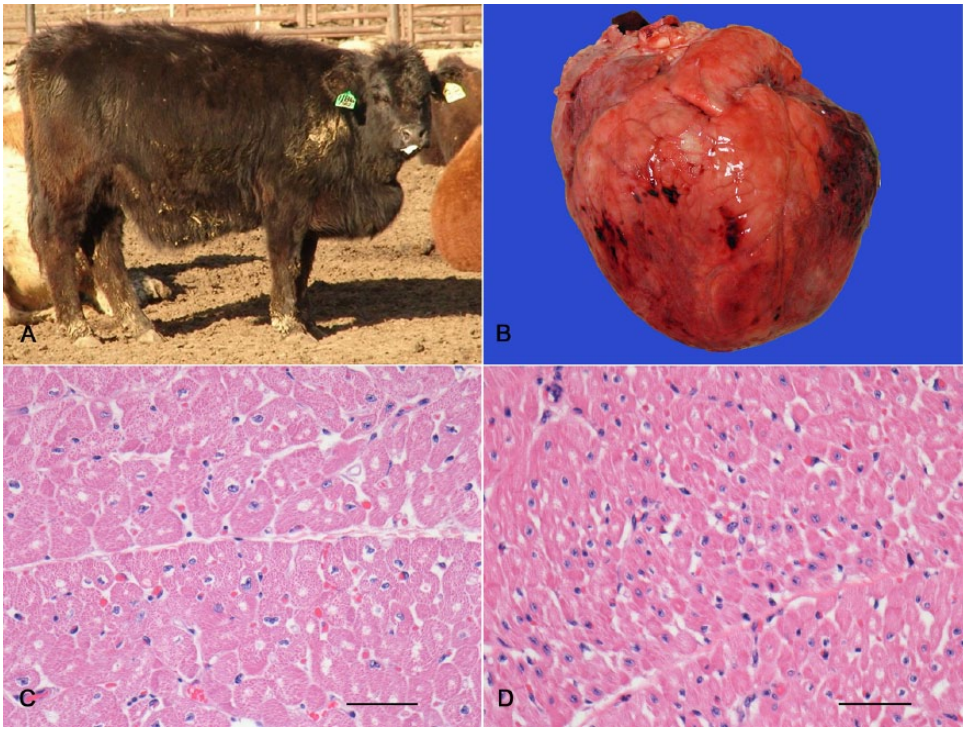

Figure 1. A. Purebred Angus steer at western Nebraska feedyard in which all cases in our study occurred, exhibiting clinical signs of severe brisket and ventral edema, fitting the case definition. This steer was not present when the study began, but represents the clinical appearance of the 17 cattle in our study. **B.** Severe right ventricular hypertrophy causing marked rounding of the heart (right side of photo) of case 11. Ecchymotic hemorrhages in right and left ventricular epicardium. **C.** Right ventricular cardiac myocytes have increased diameter and prominent aggregates of myofilaments in case 1. The mean cross-sectional diameter of cardiac myocytes in the right ventricle was 30.7 µm. H&E. Bar = 50 µm. **D.** Left ventricular cardiac myocytes are ~50% of the diameter of hypertrophied myocytes in right ventricle with normal-sized myofilaments. The mean cross-sectional diameter of cardiac myocytes in the left ventricle was 14.2 µm. H&E. Bar = 50 µm.

of collagen deposition in perivascular and interstitial locations of the myocardium. Replacement fibrosis was identified as foci of collagen deposition associated with confluent loss of cardiac myocytes (scars), with scores of 1–3 representing increasing sizes and numbers of foci.

Statistical analysis

Descriptive statistics were conducted, and differences in measured heart parameters between cases and controls were tested using the Student *t*-test for equal or unequal variances as tested by the *F*-test for equality of variances (Excel 2016; Microsoft, Redmond, WA). The comparison of artery diameter between cases and controls was tested using linear regression with heart as a random effect (PROC MIXED, SAS v.9.4; SAS Institute, Cary, NC). Multivariable logistic regression with a random effect of heart was used to test for factors associated with lesions of coronary arteriosclerosis (PROC GLIMMIX, SAS v.9.4; SAS Institute). The difference in coronary fibrosis severity scores between cases and controls was tested using the Wilcoxon rank sum test (PROC NPAR1WAY, SAS v.9.4; SAS Institute). Factors associated with coronary fibrosis severity in cases were tested using the Wilcoxon signed rank test with *t* approximation (PROC UNIVARIATE, SAS v.9.4; SAS Institute). In all analyses, statistical significance was $\alpha = 0.05$.

Results

Signalment and history

Specimens originated from 17 cattle at 1 feedlot that fit the clinical (Fig. 1A) and postmortem case definition (Table 1). The mean age on arrival at the feedlot was 205 d, and the mean time in the feedlot prior to death or slaughter was 180 d. The mean age and weight at death or slaughter was 385 d and 414 kg, respectively.

Gross pathology

We received whole, fresh hearts from 12 animals and formalin-fixed tissue slices from the hearts of 5 animals. One of the fresh hearts had portions of the left atrium and aorta removed; hence, it was excluded from gross measurements and calculations. All 11 intact hearts appeared enlarged and globose, suggesting right ventricular hypertrophy (RVH; Fig. 1B).

Table 2. Heart measurements in right-heart failure cases from a Nebraska cattle feedyard.

Case	Heart weight (kg)	Heart weight/ body weight (%)	(Left ventricle + septum)/ right ventricle	Left atrioventricular valve/ right atrioventricular valve	Aortic valve/ pulmonic valve
4	2.84	0.59	1.00	0.83	1.00
8	2.39	0.55	1.38	0.54	0.92
9	1.62	0.62	1.42	0.56	0.81
10	3.00	0.59	1.41	0.63	1.00
11	3.11	0.71	1.09	0.29	0.84
12	2.77	0.81	2.15	0.86	0.83
13	2.82	0.57	1.29	0.60	0.79
14	2.59	0.73	1.07	0.53	0.72
15	2.95	0.69	1.23	0.44	0.97
16	2.45	0.70	1.00	0.77	1.03
17	2.34	0.50	1.64	0.61	1.02
$\bar{x}$ ± SEM	2.63 ± 0.13	0.64 ± 0.03	1.33 ± 0.10	0.60 ± 0.05	0.90 ± 0.03

SEM = standard error of the mean.

Table 3. Comparison of heart measurements ($\bar{x}$ ± SEM) in right-heart failure cases from a Nebraska cattle feedyard with healthy control feedlot heifers from another study.

Parameter	Cases (n = 11)	Cases* (n = 3)	Controls* (n = 3)	p value
HW (kg)	2.63 ± 0.13	2.89 ± 0.06	2.55 ± 0.13	0.083
BW (kg)	413 ± 23	493 ± 6	517 ± 17	0.269
HW/BW (%)	0.64 ± 0.03	0.59 ± 0.01	0.50 ± 0.03	0.064
RV (kg)	0.91 ± 0.07	1.06 ± 0.10	0.42 ± 0.03	0.003
RV/BW (%)	0.22 ± 0.01	0.22 ± 0.02	0.08 ± 0.01	0.004
RV/HW	0.45 ± 0.02	0.45 ± 0.01	0.16 ± 0.01	<0.0001
LV + S (kg)	1.18 ± 0.06	1.29 ± 0.06	1.50 ± 0.06	0.079
(LV + S)/BW (%)	0.29 ± 0.01	0.26 ± 0.01	0.29 ± 0.02	0.281
(LV + S)/HW	0.45 ± 0.02	0.45 ± 0.01	0.59 ± 0.02	0.004
(LV + S)/RV	1.33 ± 0.10	1.23 ± 0.12	3.64 ± 0.28	0.001
AV circumference (cm)	14.5 ± 0.7	15.5 ± 0.5	12.3 ± 0.6	0.014
PV circumference (cm)	16.1 ± 0.6	16.8 ± 0.8	12.2 ± 0.9	0.016
LAV circumference (cm)	17.8 ± 0.7	17.9 ± 1.2	16.1 ± 1.7	0.510
RAV circumference (cm)	31.5 ± 3.0	26.4 ± 2.8	16.2 ± 0.9	0.026
AV/PV	0.90 ± 0.03	0.93 ± 0.07	1.02 ± 0.03	0.322
AV/LAV	0.83 ± 0.04	0.88 ± 0.06	0.78 ± 0.06	0.276
AV/RAV	0.49 ± 0.04	0.60 ± 0.06	0.76 ± 0.03	0.069
PV/LAV	0.92 ± 0.05	0.96 ± 0.14	0.76 ± 0.03	0.240
PV/RAV	0.54 ± 0.04	0.65 ± 0.08	0.75 ± 0.03	0.286
LAV/RAV	0.60 ± 0.05	0.69 ± 0.07	0.99 ± 0.07	0.039

SEM = standard error of the mean. See text for explanation of abbreviations. Control data originating from Buntyn et al.[5]
* Two-tailed t-test comparing means from 3 approximately weight-matched heifers (1–3) with that of cases 4, 10, and 13.

Measurements of the whole hearts from the 11 case animals confirmed heart enlargement as a result of RVH (Tables 2, 3). The mean HW/BW, as a measure of heart enlargement, was 0.64% in the cases (n = 11); in the weight-matched subset, it was 0.59% (n = 3), compared to 0.50% in healthy control feedlot heifers (n = 3)[5] (p = 0.06; Table 3). Mean HW/BW was higher than the RI for adult cattle (0.48%).[16,25] The mean RV free wall weight, RV/BW, and RV/HW values were >2 times that of the controls (p = 0.003 and $p < 0.0001$, respectively), confirming RVH. Further, the mean (LV + S)/RV of the cases was less than one-half that of the controls[5] (p = 0.001), also consistent with RVH. The RI of (LV + S)/RV adult cattle is 2.8–4.0; a value <2.8 is indicative of RVH.[16,25]

Measurements on the hearts were suggestive of dilation of the RAV ostium (Table 3). The mean RAV was significantly greater in the cases compared to the controls[5] (p = 0.03). The mean LAV/RAV of the cases was significantly lower (p = 0.04)

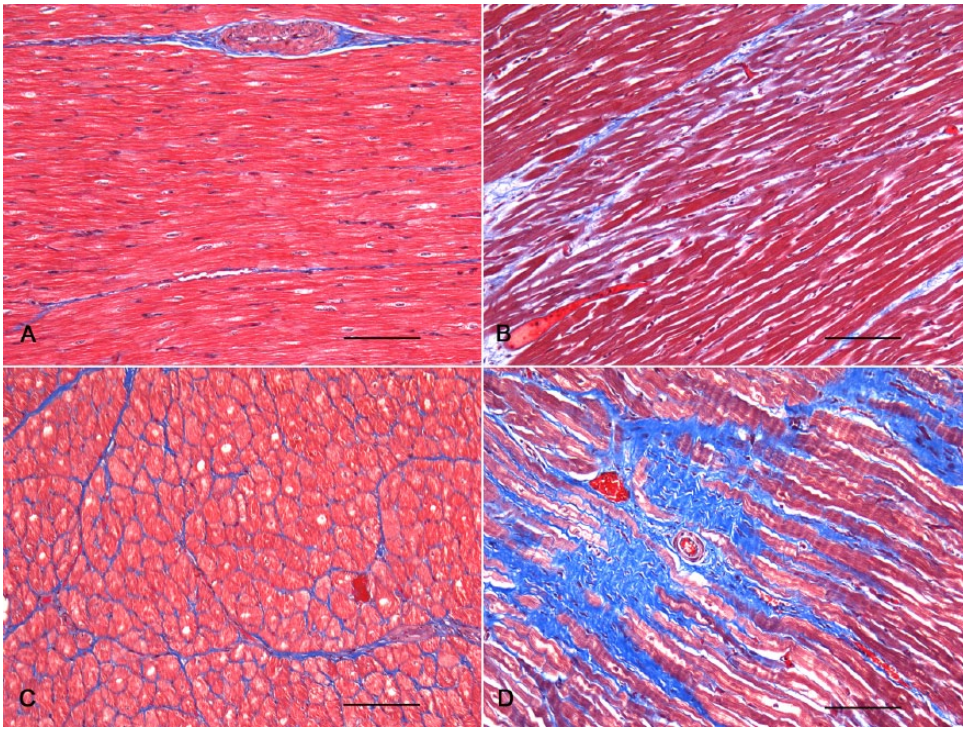

Figure 2. Interstitial myocardial fibrosis. Masson trichrome. **A.** Normal myocardium (score 0) in the left ventricle of case 2. Bar = 100 µm. **B.** Mild fibrosis (score 1) in the left ventricle of case 4. Bar = 100 µm. **C.** Moderate fibrosis (score 2) in the right ventricle of case 2. Bar = 100 µm. **D.** Severe fibrosis (score 3) in the right ventricle of case 9. Bar = 100 µm.

than the controls.[5] Normal LAV/RAV reference values for cattle are not available; however, based on data from dogs,[16] a LAV/RAV ratio of ≤0.60 is suggestive of dilation of the RAV ostium if the LAV measurement is normal. The mean LAV of the cases was not significantly different from the controls. The mean (LV + S)/HW value of the cases was significantly lower than the controls[5] ($p = 0.004$).

Grossly, liver specimens from all 17 cattle had severe, diffuse, chronic passive congestion ("nutmeg" appearance), compatible with chronic RHF. Case 9 had miliary hepatic abscesses. At autopsy, cases 1 and 2 had pulmonary consolidation suggestive of bronchopneumonia, but were found histologically only to have atelectasis, congestion, and hemorrhage. Grossly, case 12 had enlarged kidneys with focal hemorrhages; the intact fresh heart had LAV endocarditis, and the fixed renal specimen had acute infarcts. *Histophilus somni* was isolated from the inflamed heart valve.

Histopathology

Microscopically, all 17 cases had right atrial and ventricular cardiac myocyte hypertrophy. Hypertrophic cardiac myocytes had increased cellular diameter and increased prominence of myofilaments (Fig. 1C). The mean diameter of cardiac myocytes in the RV (mean ± SD; 26.5 ± 0.2 µm) was significantly greater than in the LV (14.8 ± 0.1 µm; $n = 17$; $p < 0.0001$; Fig. 1D). All 17 cases had perivascular and interstitial fibrosis in the RV myocardium (Fig. 2), whereas replacement fibrosis (Fig. 3A, 3B) was detected in the RV myocardium in 6 of 17 animals (Table 4). Among the cases, mean interstitial and replacement fibrosis scores were significantly greater in the RV compared to the LV ($p = 0.0005$ and $p = 0.03$, respectively; Table 4), whereas perivascular fibrosis was not significantly different ($p = 0.09$; Table 4). All 3 weight-matched control animals had mild perivascular fibrosis in the LV, the scores of which were not significantly different from the cases ($p = 0.18$), but none had evidence of interstitial or replacement fibrosis (Table 4). Foci of coagulative necrosis and mineralization of myocytes were scattered randomly in the RV myocardium in some case animals.

The heifer (case 8), had a 6 × 10 mm myocardial infarct with granulation tissue and fibrosis in the interventricular septum as a result of non-septic thrombosis of a >1 mm diameter branch of a coronary artery (Fig. 3C). The RV contained multiple large coalescing foci of chronic myocardial necrosis and fibrosis, and interstitial fibrosis in the right atrium (Fig. 3D). This heifer also had thrombi in >1 mm diameter pulmonary artery branches in the lungs.

Lesions of coronary arteries consistent with arteriosclerosis (any or all of intimal hyperplasia, fragmentation and replication of the internal elastic membrane, medial degeneration, luminal narrowing, and periadventitial fibrosis; Fig. 4) were seen in the RV in 9 of 16 cases in 51 of 571 (8.9%) arteries, with severity scores of 0–4 for individual arteries (Table 5).

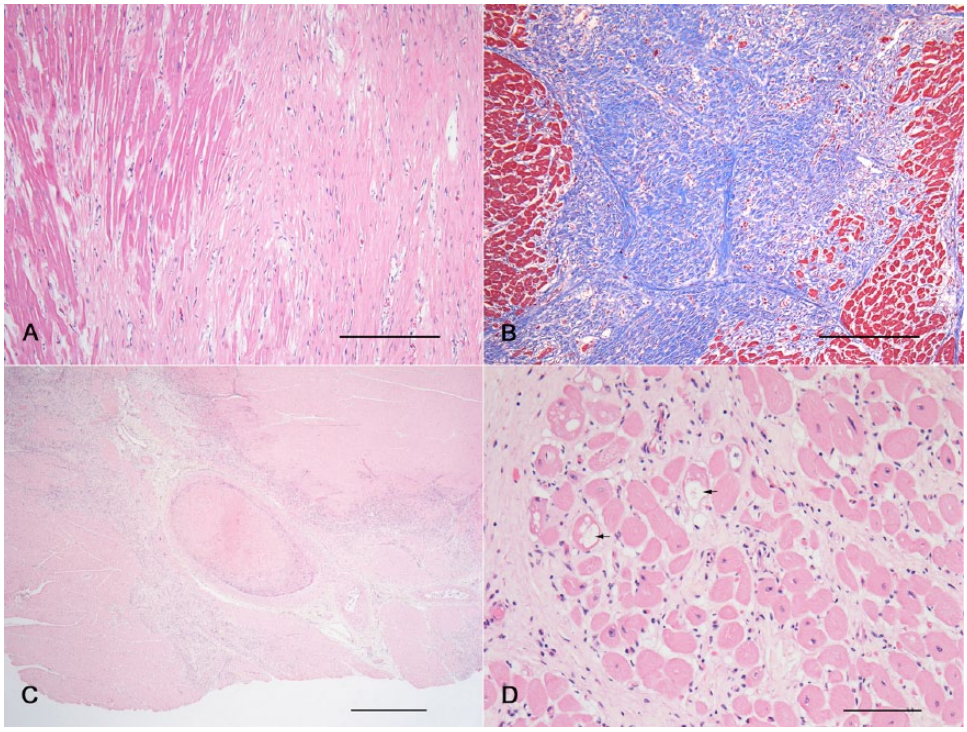

Figure 3. A. Severe replacement fibrosis (score 3) in right ventricular myocardium encompassing right half of field in case 13. H&E. Bar = 250 µm. **B.** Severe replacement fibrosis (score 3) in right ventricular myocardium centrally encompassing ~75% of the field in case 3. Masson trichrome. Bar = 250 µm. **C.** Non-septic occlusive thrombosis of an artery with infarction of interventricular septal myocardium in case 8. Necrotic myocardium replaced by granulation and scar tissue. H&E. Bar = 1,000 µm. **D.** Cardiac myocyte hypertrophy with vacuolar degeneration (arrows) and interstitial fibrosis in the right atrium in case 8. H&E. Bar = 100 µm.

Postmortem autolysis prevented adequate assessment of coronary artery changes in case 10.

Although fibrosis was less severe in the left than RV of the case animals, coronary arteriosclerosis was more severe. The mean diameter of cardiac myocytes in the LV of weight-matched case (14.4 ± 1.8 µm; n = 3) and control (15.3 ± 0.5 µm; n = 3) animals was not significantly different (p = 0.67). Interstitial fibrosis in the LV was seen in most (14 of 17) cases (Fig. 2D), but, as noted above, the mean score was significantly lower than in the RV (Table 4). Replacement fibrosis was not seen in the LV in case animals. Lesions of arteriosclerosis were seen in coronary arteries in the LV (Fig. 4) in 10 of 16 cases in 52 of 366 (14.2%) arteries, with scores of 0–4 in individual arteries (Table 5). Postmortem autolysis prevented adequate assessment of coronary artery changes in case 10. The probability of coronary arteriosclerosis in cases was greater in the left than in the RV (OR = 4.8, $p < 0.0001$; Table 5). The probability of coronary arteriosclerosis in the LV of controls was not significantly different from that of the case animals, although the proportions were 3.3% and 14.2%, respectively (Table 5). We interpret this to be a lack of statistical power because only 30 coronary arteries in the 3 control animals were present in the LV tissue sections, 1 of which contained lesions.

All 17 cattle had lung lesions compatible with pulmonary hypertension, and 8 animals (cases 1, 2, 4–8, 12, and 17) had no histologic evidence of other pulmonary disease. In the lungs of all cattle, the tunica media of small arteries and arterioles were hypertrophic with narrowed or occluded lumens. Pulmonary arteries and arterioles had any or all of the following: intimal hyperplasia (Fig. 5), hypertrophy of tunica media smooth muscle (Fig. 6), and proliferation of the adventitia and vasa vasorum (Fig. 7A, 7B). Distal small blood vessels (Fig. 7C) had undergone muscularization (Fig. 7D). In 14 of 17 cattle (excluding cases 10, 12, and 13), pulmonary veins and venules had hypertrophy of the sphincters and tunica media (Fig. 8A, 8B). Some cattle had mineralization of the elastic layers of the alveolar septa as seen in Von Kossa–stained sections (Fig. 8C, 8D). Most cattle had gross petechial and microscopic hemorrhages involving pulmonary arterioles, and frequently lobular atelectasis. Acute and chronic (organized) thrombi were seen in pulmonary arteries of 4 of 17 cattle (cases 1, 8, 11, and 16).

Nine cattle had histologic evidence of other pulmonary disease. Cases 3, 9–11, and 14–16 had mild acute or chronic bronchointerstitial pneumonia suggestive of an active or previous viral infection. Cases 10 and 11 had alveolar epithelial syncytial cells suggesting bovine respiratory syncytial virus (BRSV; species *Bovine orthopneumovirus*) infection. Cases 3 and 9 had bronchiolitis obliterans with fibrotic polyps occluding or partially occluding bronchiolar lumens, and case 9 had caseous microabscesses indicative of secondary

Table 4. Cardiac fibrosis scores in right-heart failure cases from a Nebraska cattle feedyard.

	Right ventricle			Left ventricle		
Animal	PV	I	R	PV	I	R
Control						
1	NA	NA	NA	1	0	0
2	NA	NA	NA	1	0	0
3	NA	NA	NA	1	0	0
$\bar{x} \pm$ SEM				1.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
Case						
1	2	2	0	1	1	0
2	2	2	0	0	0	0
3	2	3	3	2	2	0
4	2	2	0	3	1	0
5	2	2	1	2	1	0
6	2	2	0	2	1	0
7	1	2	0	2	2	0
8	2	3	3	0	2	0
9	2	3	1	2	1	0
11	2	3	0	2	1	0
12	2	3	2	1	1	0
13	2	3	2	1	1	0
14	2	2	0	2	2	0
15	2	2	0	2	2	0
16	3	3	0	1	0	0
17	3	1	0	2	1	0
$\bar{x} \pm$ SEM	2.1 ± 0.1^{x}	2.4 ± 0.8^{x}	0.8 ± 0.4^{x}	1.6 ± 0.3^{ax}	1.2 ± 0.2^{by}	0.0 ± 0.0^{ay}

I = interstitial fibrosis; NA = not available; PV = perivascular fibrosis; R = replacement fibrosis; SEM = standard error of mean. Means with unlike superscripts are significantly different ($p \leq 0.05$). Superscripts ($^{a, b}$) denote comparisons of common parameter (PV, I, R) means for left ventricle of controls versus cases. Superscripts ($^{x, y}$) denote comparisons of common parameter (PV, I, R) means of right versus left ventricle within cases.

bacterial infection. Cases 16 and 13 had mild or moderate acute purulent bronchopneumonia, suggestive of bacterial infection. Case 2 had mild chronic proliferative pleuritis. Case 12, from which *H. somni* was isolated, had severe chronic suppurative vegetative endocarditis of the LAV, and myocardial abscesses with intralesional bacterial colonies. This animal had no detectable lung involvement from the *H. somni* infection, but the kidneys contained multiple acute infarcts.

The livers of all 17 cattle had severe diffuse chronic passive congestion with central-to-central bridging fibrosis and fatty change of hepatocytes bordering regions of hepatocyte loss and congestion. These lesions were compatible with right-sided heart failure. Fourteen of 17 cattle had minimal-to-mild chronic interstitial lymphocytic inflammation or fibrosis in the kidneys.

Discussion

Our results support the hypothesis that a proportion of feedlot cattle at moderate altitudes (<1,524 m) develop cor pulmonale as a result of hypoxia-induced pulmonary hypertension. The clinical and pathologic manifestations of cattle in our investigation are very similar to those reported in a syndrome of progressive RVH in Holstein heifers at an elevation of 1,600 m.[18] Some practical gross pathology parameters for diagnosis of RVH in mature cattle that were useful in our study included: RV/BW >0.48, RV/HW >21%, and (LV + S)/RV ratio <2.8.[16,25]

In addition to generalized hypoxia caused by hypobaric conditions, cor pulmonale can result from localized hypoxia as a result of chronic obstructive pulmonary disease (COPD).[7] In cattle, chronic bronchopneumonia and bronchointerstitial pneumonia, principally caused by bacterial and viral infections, respectively, are causes of COPD. We noted bronchiolitis obliterans, as a form of COPD, in 2 of 17 cattle. Other cattle with bronchointerstitial pneumonia (2 of 17) had alveolar epithelial syncytial cells, a lesion seen with BRSV or bovine parainfluenza virus 3 (BPIV-3; species *Bovine respirovirus 3*) infection.[7] Bronchiolitis obliterans is a common sequel to bronchiolar epithelial necrosis and inflammation in BRSV or BPIV-3 infection, but especially the former.[7] Affected lung lobules were atelectatic, which, too, is a common accompaniment to the aforementioned viral-induced bronchiolar lesions. Hence, although virologic tests were not conducted, we presume 6 of 17 cattle were in various stages of infection with BRSV or BPIV-3, and this infection exacerbated the pulmonary hypoxia.

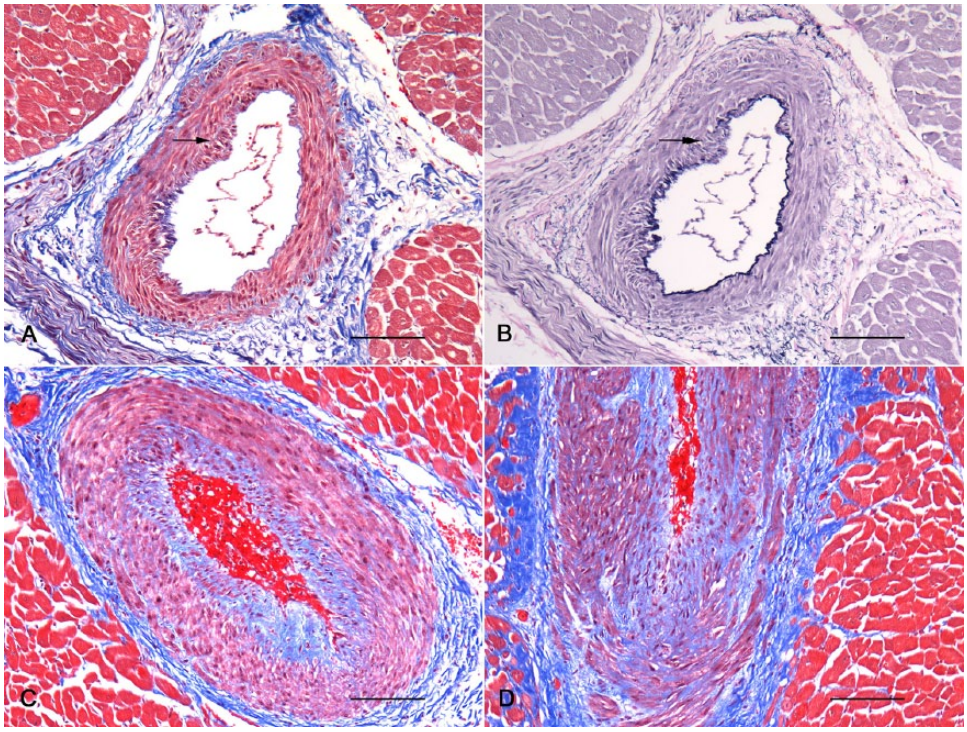

Figure 4. Intramural coronary arteries. Artery with multifocal intimal hyperplasia and fragmentation of internal elastic membrane (arrow) in papillary muscle of right ventricle of case 5 (score 1). **A.** Masson trichrome. Bar = 100 µm. **B.** Verhoeff–Van Gieson. Bar = 100 µm. **C.** Artery with diffuse intimal hyperplasia, smooth muscle migration into intima, and diffuse collagen deposition in tunica media in left ventricle of case 4 (score 2). Masson trichrome. Bar = 100 µm. **D.** Artery with diffuse intimal hyperplasia, smooth muscle migration into intima, diffuse collagen deposition penetrating tunica media, and periadventitial fibrosis in left ventricle of case 4 (score 3). Masson trichrome. Bar = 100 µm.

The finding of pulmonary vascular lesions compatible with hypoxia-induced pulmonary hypertension in the absence of pneumonia in 9 of 17 cattle strongly suggests that hypoxia caused by something other than pneumonia was the principal cause of RHF in the majority of the cattle examined. Interestingly, upon review of departmental records (Hibbs C. Bovine pulmonary hypertension. University of Nebraska–Lincoln Veterinary Extension Newsletter 1975;4:82), and through personal communication with an emeritus extension veterinarian (White RG, pers. comm., Nov. 2009), we found that the same ranch had seen deaths with lesions at autopsy suggestive of brisket disease since at least the mid-1970s.

Although different beef and dairy breeds, including crossbred cattle, are affected,[18,21] studies since at least the mid-1970s have provided evidence for genetic or epigenetic predisposition to hypoxia-induced pulmonary hypertension in cattle.[23,27,28,30,31] Pulmonary arterial pressure is moderately heritable in spring-born Angus cattle acclimatized and tested at high altitude.[28] Selection of cattle for rapid growth at low altitudes is expected to increase pulmonary arterial pressure scores and susceptibility to brisket disease at high altitudes.[28] One study found a high association of an endothelial PAS domain–containing protein 1 (*EPAS1*) double variant gene in the oxygen degradation domain of EPAS1 in Angus cattle with high-altitude pulmonary hypertension.[22] However, another study tested 8 *EPAS1* missense variants for association with clinical pulmonary hypertension and RHF in western plains feedlot cattle and found no association with disease (Heaton MP. In search of a genetic cause for heart failure in beef cattle [abstract]. University of Nebraska–Lincoln, School of Veterinary Medicine and Biomedical Sciences Seminar Series; Aug 2018; Lincoln NE). In the latter study, it was noted that future searches for a genetic cause may benefit from a genome-wide approach.

Brisket disease has been defined as a condition that occurs in cattle at an altitude of >1,524 m (5,000 ft).[9] At sea level (0 m; 0 ft), the barometric pressure is 101 kPa, yielding an expected partial pressure of oxygen in the pulmonary alveoli (P_{AO2}) of 13.3 kPa.[24] At an altitude of 1,524 m, the barometric pressure is 85 kPa and the expected P_{AO2} would be 10.0 kPa, a decrease of 24.8% compared to sea level. The altitude of the ranch in our study was 1,369 m (4,500 ft) having a barometric pressure of 86 kPa, and yielding an expected P_{AO2} of 10.4 kPa or a 21.8% P_{AO2} decrease from sea level. Hence, the altitude of the feedlot, although lower than that included in the current definition of brisket disease, would cause a moderate level of hypobaric hypoxia, and could have contributed

Table 5. Prevalence and severity of arteriosclerosis in coronary arteries in right-heart failure cases from a Nebraska cattle feedyard.

	No. of arteries with lesions/ no. of arteries examined (%)		Mean score of arteries with lesions		Mean diameter (μm) of arteries with lesions	
Animal	Right ventricle	Left ventricle	Right ventricle	Left ventricle	Right ventricle	Left ventricle
Control						
1	NA	1/13	NA	3.0	NA	500
2	NA	0/9	NA	NA	NA	NA
3	NA	0/8	NA	NA	NA	NA
Total	NA	1/30 (3.3)[a]	NA	3.0[a]	NA	500[a]
Case						
1	0/18	0/40	NA	NA	NA	NA
2	0/51	0/21	NA	NA	NA	NA
3	1/25	2/25	3.0	1.0	1,250	443
4	1/53	4/29	3.0	2.5	240	303
5	13/64	11/31	1.3	1.8	293	182
6	8/34	2/19	2.4	2.0	224	330
7	0/20	0/17	NA	NA	NA	NA
8	15/66	5/18	2.8	2.0	146	70
9	0/29	0/24	NA	NA	NA	NA
10	NA	NA	NA	NA	NA	NA
11	0/18	6/37	NA	1.8	NA	453
12	0/28	0/24	NA	NA	NA	NA
13	2/64	0/12	2.0	NA	263	NA
14	2/24	5/16	1.5	1.6	290	161
15	6/40	5/16	2.0	3.0	107	276
16	0/16	5/18	NA	2.8	NA	223
17	3/21	7/19	1.7	1.9	223	192
Total	51/571 (8.9)[x]	52/366 (14.2)[ay]				
$\bar{x} \pm$ SEM			2.1 ± 0.1^{x}	2.1 ± 0.1^{bx}	209 ± 21^{x}	240 ± 31^{ax}

NA = not available or not applicable; SEM = standard error of mean. Means with unlike superscripts are significantly different ($p \leq 0.05$). Superscripts ([a, b]) denote comparisons of means of common parameters for left ventricle of controls versus cases. Superscripts ([x, y]) denote comparisons of means of common parameters of right versus left ventricle within cases. Parameters compared were: number of arteries with lesions/number of arteries examined (%); mean score of arteries with lesions; and mean diameter (μm) of arteries with lesions.

significantly to pulmonary hypertension, vascular remodeling, and right-sided heart failure.

In general, cattle are more susceptible to hypoxia-induced pulmonary hypertension than are other species.[20] At least in part, cattle are prone to hypoxemia because they have small lungs relative to their oxygen requirements.[8] A 2016 large-scale study involving midwestern high plains feedyards in the United States and Canada found that cattle of different breeds developed RHF at moderate altitudes. The adjusted risk for RHF increased from 2 to 4 per 10,000 cattle from 2000 to 2012, suggesting that the problem is increasing and involves factors other than genetic predisposition and altitude.[21] These authors noted that hypoventilation and respiratory disease, in addition to hypobaric conditions, are causes of pulmonary alveolar hypoxia. They concluded that hypobaric hypoxia could not explain the increase in RHF death in all cases given that the same Canadian feedlots were studied over the entire period. However, they also noted that hypobaric hypoxia may be a factor in other cases, because in U.S. feedlots, cattle at the highest altitude (1,282 m) were 9 times more likely to die of RHF than cattle at the lowest altitude. In that same study, localized hypoxia resulting from respiratory disease was a risk factor, given that cattle treated for respiratory disease were 2–3 times more likely to die of RHF than cattle not treated for respiratory disease.[21]

Although high altitude is a well-established factor, previous studies[15,20,21] indicate that obesity and potentially coronary artery disease and left-heart dysfunction are likely major contributing factors to pulmonary hypertension and right-sided heart failure, and may have played a role in disease in the cattle in our study. Cattle are now fed to heavier finishing weights, with abdominal fat accumulation causing hypoxia through hypoventilation.[21] Pulmonary arterial pressure in feedlot steers increases with age (from cow–calf to feedlot–finishing phases, a maximum age of 18 mo) and adiposity.[20] "Significant LV fibrosis, abundant cardiac adipose depots, coronary artery injury, and pulmonary venous remodeling" was found in fattened beef cattle.[15] This series of lesions resembles human obesity-related pulmonary hypertension–left heart disease. These changes likely preceded

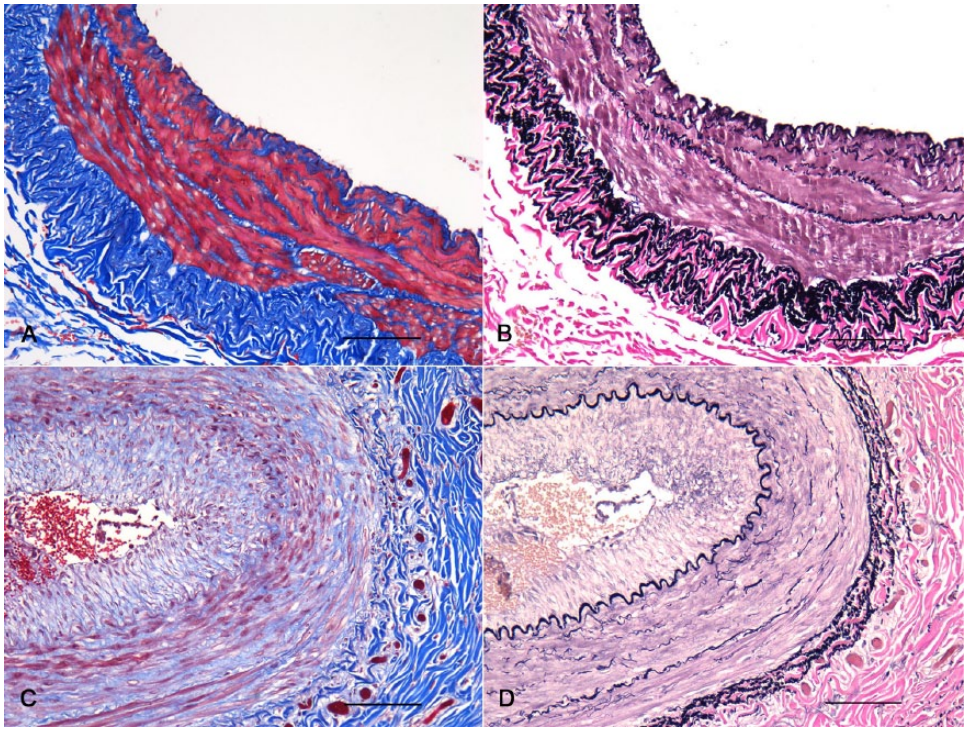

Figure 5. Elastic pulmonary arteries. Normal elastic artery in lung of control heifer 1. **A.** Masson trichrome. Bar = 100 µm. **B.** Verhoeff–Van Gieson. Bar = 100 µm. **C.** Elastic artery in lung of case 3 remodeled by severe diffuse hyperplasia and fibrocytic infiltration of tunica intima (fibroelastosis), collagen deposition in tunica media, and hyperplasia of adventitia. Masson trichrome. Bar = 100 µm. **D.** Verhoeff–Van Gieson. Bar = 100 µm.

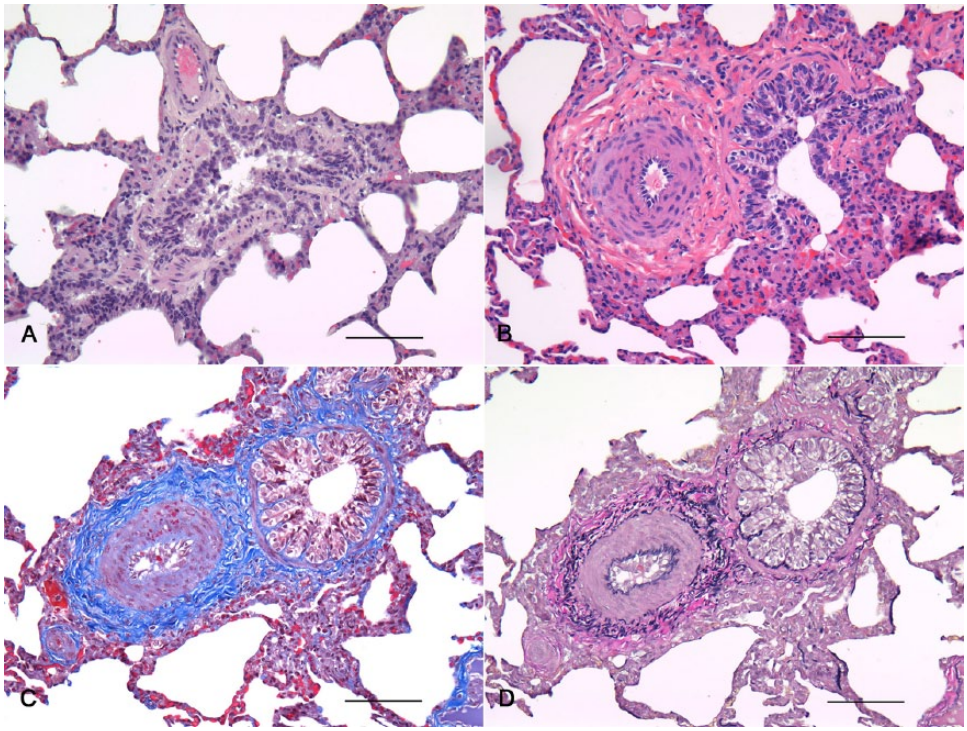

Figure 6. A. Proximal pulmonary arterioles. **A.** Normal arteriole in lung of control heifer 1. Bar = 100 µm. **B.** Arteriole in lung with tunica media thickened by hypertrophy of myocytes in case 2. Tunica adventitia has increased deposition of collagen and duplication of external elastic membrane. H&E. Bar = 100 µm. **C.** Masson trichrome. Bar = 100 µm. **D.** Verhoeff–Van Gieson. Bar = 100 µm.

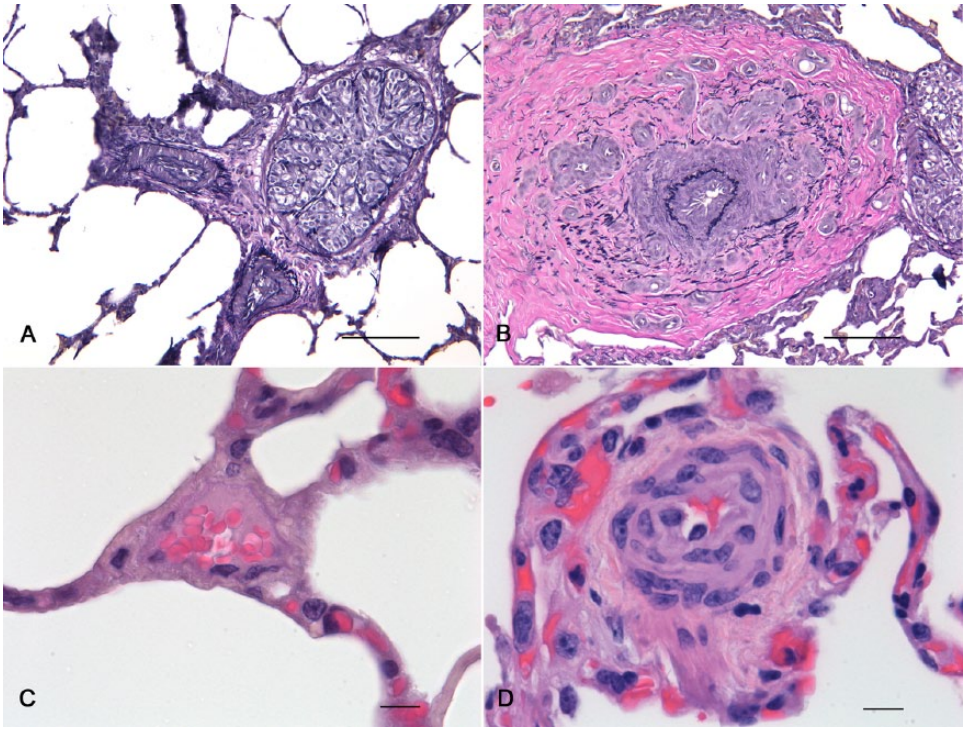

Figure 7. A. Proximal pulmonary arterioles in lung of control heifer 2 displaying intact internal and external elastic membranes. Verhoeff–Van Gieson. Bar = 100 µm. **B.** Proximal pulmonary arteriole in lung with severe diffuse proliferation of adventitia and vasa vasorum in case 2. Verhoeff–Van Gieson. Bar = 100 µm. **C.** Non-muscularized distal blood vessel in an alveolar septum of lung of control heifer 1. H&E. Bar = 10 µm. **D.** Muscularized distal arteriole in alveolar septum of lung of case 1. H&E. Bar = 10 µm.

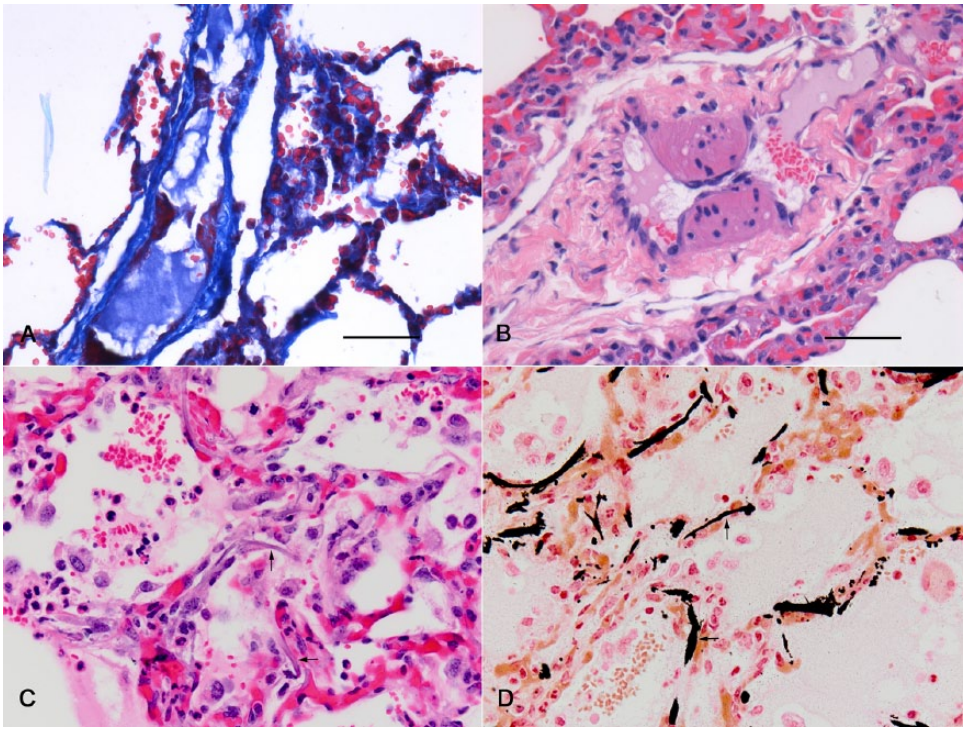

Figure 8. A. Pulmonary vein with normal-sized sphincters in lung of control heifer 1. Masson trichrome. Bar = 50 µm. **B.** Pulmonary vein with hypertrophied sphincter in lung of case 1. H&E. Bar = 50 µm. **C.** Mineralization of alveolar septa (basophilic spicules, arrows) in lung of case 1. H&E. **D.** Mineralization of alveolar septa (black spicules, arrows) in lung of case 1. Von Kossa.

"muscularization, medial hypertrophy, adventitial fibrosis, and vasa vasorum hyperplasia of the pulmonary" arteries and, in turn, was "associated with the sequela of right ventricular remodeling."[15]

Although coronary artery remodeling is thought to play a role in the pathogenesis of obesity-related congestive heart failure in feedyard cattle,[15] interestingly, intramural coronary arteriosclerosis is now recognized to commonly occur subclinically in slaughtered veal calves and beef cattle.[4] Notably, 1 of 3 controls in our study had coronary arteriosclerosis. Our findings of greater probability of coronary arteriosclerosis in the LV and papillary muscle are also consistent with that previous study.[4] Our study did not lead to conclusions about the significance of coronary arteriosclerosis in feedyard cattle congestive heart failure, but suggests that further research on its potential role is warranted.

Fibrosis of the myocardium occurs in 3 main patterns: perivascular, interstitial, and replacement.[3,10,26] Perivascular and interstitial fibrosis are also described as reactive, with increased deposition of collagen and other extracellular matrix proteins initially around blood vessels (perivascular). In the interstitial pattern, fibrosis is more diffusely distributed along the interstitium and then extends between and around individual cardiac myocytes.[3,10,26] Replacement fibrosis results from death of cardiac muscle cells (e.g., necrosis, secondary to ischemia or other causes). The 3 patterns are considered to represent increasing levels of severity. In our study, we developed a semiquantitative assessment of the levels of severity of each pattern to allow us to compare weight-matched controls and cases, and in the case of the latter, the right and left ventricle. We conclude that interstitial and replacement fibrosis, but not perivascular, was more severe in our cases than in the controls. Similarly, within the cases, RV was more severely fibrotic than LV (both interstitial and replacement), although perivascular fibrosis was not significantly different. The consistent presence of interstitial fibrosis in the LV, albeit less severe than the RV, is similar to the findings in feedyard cattle obesity-related congestive heart failure syndrome.[15] A major difference is that, in obesity-related congestive heart failure, the RV and LV had similar mean severity scores for all 3 patterns of fibrosis.[15]

Although the cattle in our study were in the process of finishing, they died at an earlier age and weighed ~100 kg less than those in the fattened beef feedlot study.[15] Based on the difference in weight, we estimate that the cases in our study had ~2% lower body fat content than the fat cattle in that study.[15,19] Histologically, the amount of adipose tissue deposition in the hearts was consistent with this estimate, in that it appeared to be less than that shown in figures in the feedyard cattle obesity-related congestive heart failure study.[15] We hypothesize that cattle in our study had less severe LV fibrosis because they died before reaching similar weights and levels of adiposity. Further, based on these differences from the obesity-related congestive heart failure study,[15] we hypothesize that some cattle are predisposed, possibly genetically, to this syndrome.

Another interesting finding in our study was non-septic pulmonary thrombosis in cases 1, 8, 11, which resulted in myocardial infarction in case 8. Pulmonary thrombosis was presumably the result of altered hemodynamics and vascular integrity, especially related to intimal hyperplasia in pulmonary arteries. Pulmonary thrombosis in association with aneurysms was reported in feedlot cattle (25 of 1,988; 1.3%) in Colorado,[13] but considered to be a different entity than cases of brisket disease described in the same study by the same authors in a different publication.[12] Our study suggests that pulmonary thrombosis is a sequela to brisket disease in some cattle.

Pulmonary vascular lesions in cattle in our study support the conclusion that hypoxia-induced pulmonary hypertension was the cause of RHF, and the moderate altitude to which these cattle were exposed played a significant role in the pathogenesis, which further suggests that these cattle were genetically predisposed to pulmonary hypertension in response to hypoxia. Muscularization, which is the conversion of normally non-muscular or partially muscular distal pulmonary precapillary vessels into muscularized vessels,[29] was consistently seen in the lungs of case animals in our study. Muscularization is "the most characteristic, yet least understood, change in the pulmonary vasculature that occurs in response to chronic hypoxic exposure."[29] As noted above, muscularization was also seen in the report of feedyard cattle with obesity-related congestive heart failure.[15] Cases in our study consistently had profound intimal hyperplasia in pulmonary elastic arteries, a lesion also seen in brisket disease.[2,18] This change is rarely seen in obesity-related congestive heart failure[15] (Krafsur GM, pers. comm., Oct. 5, 2018). Despite this difference, other lesions, such as mild LV fibrosis (perivascular and interstitial) were consistently seen in cases in our study, and may represent early features of the pathogenesis reported in the obesity-related congestive heart failure syndrome.

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Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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