



RESEARCH ARTICLE

# Preliminary evidence that hydrostatic edema may contribute to the formation of diffuse alveolar damage in a Holstein calf model [version 1; referees: 2 approved]

Joseph M. Neary , Dee Church

Departments of Animal and Food Sciences, College of Agricultural Sciences and Natural Resources, Texas Tech University, Lubbock, TX, 79409, USA

**V1** First published: 26 Mar 2018, 7:374 (<https://doi.org/10.12688/f1000research.14153.1>)  
 Latest published: 26 Mar 2018, 7:374 (<https://doi.org/10.12688/f1000research.14153.1>)

**Abstract**

**Background:** Two notable findings of clinically healthy feedlot cattle suggest they may have pulmonary hydrostatic edema during the finishing phase of production: increased pulmonary arterial wedge pressures and pulmonary venous hypertrophy. The goal of this study was to determine if increased pulmonary arterial wedge pressure (PAWP) in a Holstein calf could lead to diffuse alveolar damage consistent with the early, exudative phase of acute interstitial pneumonia of feedlot cattle.

**Methods:** Six male Holstein dairy calves were given daily subcutaneous injections of the nonspecific  $\beta$ -adrenergic agonist isoprenaline (10 mg/kg/d), to induce left ventricular diastolic dysfunction, or sterile water for 14 days. On Day 14, pulmonary arterial pressures and wedge pressures were measured, echocardiography performed, and the ratio of mitral valve flow velocity (E) to septal lengthening velocity ( $e'$ ) calculated. Calves were euthanized on Day 15 and lung lesions semi-quantitatively scored.

**Results:** Mean PAWP was  $12 \pm 1$  mm Hg in calves that received isoprenaline and  $7 \pm 1$  mm Hg in controls ( $P = 0.01$ ). Calves that received isoprenaline tended to have greater relative wall thickness than control calves ( $P = 0.15$ ) and greater E/ $e'$  ratios ( $P = 0.16$ ), suggestive of concentric hypertrophy and diastolic dysfunction, respectively. Calves that received isoprenaline also tended to have a left ventricle and interventricular septum that was  $29 \pm 10$  g heavier than control calves ( $P = 0.10$ ) when controlling for body mass. Hyaline membranes, the hallmark feature of diffuse alveolar damage, were evident in lung sections from all calves that received isoprenaline but none of the controls.



**Conclusions:** Consistent with prior pathological and physiological studies of feedlot cattle, this study provides preliminary evidence that cattle presenting with clinical signs and pathology consistent with early stage acute interstitial pneumonia could be attributable to hydrostatic edema associated with left ventricular failure.

**Keywords**

acute respiratory distress syndrome, pneumonia, congestive heart failure, hypertension, left ventricle

**Open Peer Review**

Referee Status:  

	Invited Referees	
	1	2
<b>version 1</b> published 26 Mar 2018	 report	 report
1 <b>Amelia Woolums</b>  , Mississippi State University, USA		
2 <b>Min Li</b> , University of Colorado Denver, USA		

**Discuss this article**

Comments (0)

**Corresponding author:** Joseph M. Neary ([joe.neary@ttu.edu](mailto:joe.neary@ttu.edu))

**Author roles:** **Neary JM:** Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Software, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Church D:** Data Curation, Investigation, Methodology, Project Administration, Writing – Original Draft Preparation

**Competing interests:** No competing interests were disclosed.

**Grant information:** The author(s) declared that no grants were involved in supporting this work.

**Copyright:** © 2018 Neary JM and Church D. This is an open access article distributed under the terms of the [Creative Commons Attribution Licence](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

**How to cite this article:** Neary JM and Church D. **Preliminary evidence that hydrostatic edema may contribute to the formation of diffuse alveolar damage in a Holstein calf model [version 1; referees: 2 approved]** *F1000Research* 2018, 7:374 (<https://doi.org/10.12688/f1000research.14153.1>)

**First published:** 26 Mar 2018, 7:374 (<https://doi.org/10.12688/f1000research.14153.1>)

## Introduction

Feedlot cattle are susceptible to two diseases of the cardiopulmonary system as they approach slaughter weight: congestive heart failure<sup>1</sup> and acute interstitial pneumonia (AIP)<sup>2</sup>. In human medicine, and for the purpose of our study, acute interstitial pneumonia was defined as an acute respiratory distress syndrome (ARDS) of unknown etiology that is not primarily attributable to hydrostatic edema due to congestive left ventricular failure, pulmonary venous hypertension, or fluid overload<sup>3</sup>. In veterinary medicine, a consensus definition of ARDS has been proposed<sup>4</sup>. Diagnosing feedlot cattle using the proposed criteria, however, is not feasible largely because the diagnostic tools for ruling out hydrostatic edema are not available; consequently, it has been said that definitive diagnosis of AIP in cattle requires histopathologic evaluation of lung tissue obtained postmortem<sup>5</sup>. The histological counterpart of ARDS is typically diffuse alveolar damage (DAD), defined by the presence of hyaline membranes<sup>6</sup>.

If an animal dies in the acute, exudate phase of AIP, then hyaline membranes and hemorrhage may be the only lesions observed<sup>5</sup>. This is problematic because these lesions are not pathognomonic for ARDS and may be attributable to hydrostatic edema. Furthermore, there are two notable findings of clinically healthy feedlot cattle that suggest they may be at risk of congestive left heart failure, and therefore pulmonary hydrostatic edema, during the finishing phase: increased pulmonary arterial wedge pressures (PAWP) and pulmonary venous hypertrophy<sup>7</sup>. This leads us to speculate whether cattle presenting with clinical signs and pathology consistent with early stage AIP could, at least in some instances, be attributable to hydrostatic edema associated with left ventricular failure. The goal of this study was to determine if increased PAWP due to left ventricular dysfunction in a Holstein calf model could lead to pulmonary lesions consistent with the early, exudative phase of AIP in feedlot cattle.

## Methods

### Overview

Six, day-old male, intact clinically healthy Holstein dairy calves were collected from a farm in West Texas and individually housed under normoxic conditions (altitude: 975 m). At 7-days of age (Day 1 of the study), calves (n = 3) were given daily subcutaneous injections of the nonspecific  $\beta$ -adrenergic agonist isoprenaline (10 mg/kg/d) (henceforth, ISO calves) or an equivalent volume of sterile water (controls, n = 3) for 14 days. On Day 14, pulmonary arterial pressures and wedge pressures were measured and echocardiography performed. Calves were euthanized on Day 15. The heart and lungs were weighed, and lung sections histologically evaluated.

### Ethical considerations

Institutional Animal Care and Use Committee approval was granted prior to initiation of the study (Protocol 17013-02). Efforts were made to ameliorate animal suffering by following recommendations for the housing and care of animals provided by the National Research Council<sup>8</sup>.

### Study site, housing, and feed

Day-old male, intact, male Holstein calves were obtained from one commercial dairy in West Texas. Six calves were included

in this preliminary study as, to our knowledge, no similar studies had been performed in this species. Calves were fed 4 to 5 L of colostrum within 12 hours of birth. They were given 3 L of non-medicated milk replacer (22% crude protein, 15% crude fat) twice per day throughout the study (Purina® Herd Maker®, Gray Summit, MO, USA) and had ad libitum access to water. From 1 week of age (Day 1) calves had *ad libitum* access to a calf starter (Purina®, Ampli-Calf® Starter 22, Gray Summit, MO, USA) with 22% crude protein (dry matter basis).

All calves were individually housed in pens with dimensions 1.8 m by 2.3 m (Agri-Plastics, Grassie, ON, Canada). Four calves were housed on a raised slatted floor inside temperature-controlled chambers (temperature  $17 \pm 3^\circ\text{C}$ ). Calves were housed according to body mass so that the heaviest calves at the start of the study (one control and one experimental calf) were housed in one chamber (Calves 4 and 5) and the lightest calves (Calves 1 and 2) in another chamber. One control (Calf 3) and one experimental calf (Calf 6), were housed in shaded outdoor pens with straw bedding on a sloped concrete floor. The pens were moved to new locations every 3 days and the inside of the pens cleaned with disinfectant (Virkon S, DuPont, Wilmington, DE, USA). Soiled straw was removed once daily, and all straw was replaced every 3 days.

### Isoprenaline/Isoproterenol

To our knowledge, isoprenaline (DL-Isoproterenol hydrochloride, 98%, Alfa Aesar, Ward Hill, MA, USA) has not been used in large animal models of heart failure with preserved ejection fraction (HFpEF). The dosage used in this study (10 mg/kg sid) was double the dosage used in a study of equivalent duration in a mouse model<sup>9</sup>. The isoprenaline was dissolved in sterile water (50 mg/mL) at room temperature prior to subcutaneous injection in the neck. Alternating sides of the neck were used. Controls were injected subcutaneously in the neck with an equivalent volume of sterile water (0.2 mL/kg). Treatments were given between 8:30 am and 9:30 am after all calves had been fed milk replacer. Injections were given whilst calves were manually restrained within their pen.

### Pulmonary arterial pressure measurement

Pulmonary arterial pressure (PAP) testing was performed on Day 14. Calves were restrained in a calf chute and a halter used to hold the calf's head to one side so that the right jugular groove was exposed. The neck was clipped and cleaned with chlorhexidine solution. A 7-French peel-away introducer (IS-07AS, Vascor Medical Corporation, Tarpon Springs, FL, USA) was placed in the jugular vein prior to inserted of a 110 cm, 7 French, polyurethane, modified J-tip wedge pressure catheter (172-110P, Vascor Medical Corporation, Tarpon Springs, FL, USA). A pressure transducer (TranStar DPT, Smiths Medical ASD, Inc., Dublin, OH, USA) was interposed between the catheter to the data acquisition system (IX-TA-220, iWorx Systems, Inc., Dover, NH, USA). The pressure waveforms were recorded and analyzed offline (LabScribe3, iWorx Systems, Inc., Dover, NH, USA). Pressures were analyzed at the end of expiration. The position of the catheter tip was determined by monitoring the change in the pressure waveform as the catheter tip was advanced through the right atrium, right ventricle, and finally into the pulmonary artery.

**Echocardiography**

Echocardiography was performed on Day 14. Calves remained standing throughout the procedure. Fractional shortening and ejection fraction (cubed or Teichholz method<sup>10</sup>) were obtained from right parasternal long-axis views of the left ventricle obtained between intercostal spaces 3 to 6. Relative wall thickness was calculated as two-times the left ventricular free wall diastolic thickness divided by the left ventricular internal diastolic diameter. Early diastolic (e') septal lengthening velocities and early mitral flow (E) velocities were obtained from a left parasternal apical four-chamber view. The E/e' ratio is a measure of left ventricular filling pressure and, consequently, a diagnostic measure of diastolic dysfunction<sup>11</sup>.

**Postmortem examination and histology**

Calves were euthanized with intravenous pentobarbital sodium (85 mg/kg) on Day 15 of the study and exsanguinated. Lung lobes were individually weighed. The atria were separated from the ventricles at the atrioventricular junction. The right ventricular free wall (RV) was separated from the left ventricle and septum (LVS). The RV and LVS were individually weighed.

The left diaphragmatic lobe was perfused with formalin (10%, neutral buffered) at 15 to 20 cm H<sub>2</sub>O for approximately 5 minutes. After 5 days of formalin fixation, lung sections were collected midway along the dorsal aspect of the lobe for histology. Paraffinized sections (4 μm) were stained with hematoxylin and eosin and Masson's trichrome. Lung sections were semi-quantitatively scored based on severity (0 = no lesion; 1 = mild; 2 = moderate; 3 = severe). The investigator was blinded to the calves' identities and treatment group. The pulmonary parenchymal lesions scored included interstitial edema, intra-alveolar edema, hemorrhage and hemosiderin, inflammatory infiltrates, type II hyperplasia, and interstitial fibrosis. Pulmonary arterioles (< 500 μm) and veins were scored for medial hypertrophy and adventitial fibrosis. Veins were distinguishable from arteries as they have spiral bundles of muscle that give them a beaded appearance.

**Statistical analyses**

Statistical analyses were performed using commercial software (Stata version 12.1, College Station, TX). Calf 1 was excluded from statistical analyses because a sub-endocardial lesion was

detected postmortem that likely affected his cardiac function and, consequently, pulmonary vascular pathophysiology. Between group differences were evaluated using Student's *t*-test with equal variances. Student's *t*-test is a suitable statistical method for small sample sizes ( $n \leq 5$ ) even if group sizes are unequal as long as the effect size is expected to be large.<sup>12</sup> Linear regression was used to determine if there was a significant difference between groups in the mass of the right ventricle, left ventricle and interventricular septum, and lung, while controlling for body mass.

**Results**

**Cardiopulmonary pressures**

Calves that received ISO had a greater mean PAWP but a lower mean PAP than controls. Mean PAWP was 12 ± 1 mm Hg in the ISO group and 7 ± 1 mm Hg in the control group ( $P = 0.01$ ). Mean PAP was 23 ± 2 mm Hg in the ISO group and 43.5 ± 8 mm Hg in the control group ( $P = 0.05$ ). The control group had a greater heart rate (104 ± 9 bpm) than the ISO group (74 ± 4 bpm;  $P = 0.03$ ). Results are presented in **Table 1**.

**Echocardiography**

There was no statistical difference in ejection fraction or fractional shortening between the ISO and control groups indicating that left ventricular systolic function was preserved. Ejection fraction was 66 ± 6% in the control calves and 54 ± 6% in the ISO group ( $P = 0.29$ ). Fractional shortening was 36 ± 4% in the control calves and 29 ± 4% in the ISO group ( $P = 0.27$ ). ISO calves tended to have greater relative wall thickness than control calves ( $P = 0.15$ ) and greater E/e' ratios ( $P = 0.16$ ) which is suggestive of concentric hypertrophy and diastolic dysfunction, respectively. Results are presented in **Table 2**.

**Pathology**

There was no difference in body mass at the end of the study ( $P = 0.51$ ). The mean body mass was 46.6 kg ± 3.3 kg for the control calves and 42.8 ± 3.4 kg for the ISO group. Calf 1 had endocardial fibrosis extending from the atrio-ventricular boundary to the dorsal aspect of the papillary muscle and approximately 0.5 cm into the myocardium (Supplementary file). Calf 5 had a 1 cm abscess in the right diaphragmatic lobe and Calf 6 had acute fibrinous pneumonia affecting the ventral 20% of the right cranio-ventral lung lobe (Supplementary file). The lungs of Calf 2

**Table 1. Cardiopulmonary pressures and pulmonary arterial wedge pressures (PAWP) obtained from male intact Holstein calves after 14 days of receiving the nonspecific β-adrenergic agonist (β-AA) isoprenaline (10 mg/kg/d) and control calves that received an equivalent volume of sterile water (0.2 mL/kg).**

Group	Calf	Right ventricle	Pulmonary artery	Mean PAWP	Heart rate
Control	1*	51/-12 (19)	30/11 (20)	5	149
	3	65/5 (35)	68/40 (51)	6	95
	5	68/-6 (30)	48/15 (36)	7	112
β-AA	2	42/1 (12)	39/6 (20)	10	71
	4	43/3 (8)	46/3 (22)	13	70
	6	42/7 (16)	43/19 (27)	12	81

\*Left ventricular endocardial lesion identified post-mortem

appeared hyper-inflated (Figure 1) and Calf 6 had a mottled icteric liver with rounded margins.

The ISO group tended to have a left ventricle and interventricular septum that was  $29 \pm 10$  g heavier than control calves ( $P = 0.10$ ) when controlling for body mass. There was no difference between groups in the mass of the lung ( $P = 0.81$ ) or right ventricle ( $P = 0.36$ ) when controlling for body mass. Results are presented in Table 3.

In general, tissue areas with lesions consistent with DAD were multifocal and often located adjacent to a lobule with a normal healthy appearance. Occasionally, a gradual change from healthy to DAD was observed within the same lobule. All ISO calves had mild or moderate interstitial and intra-alveolar

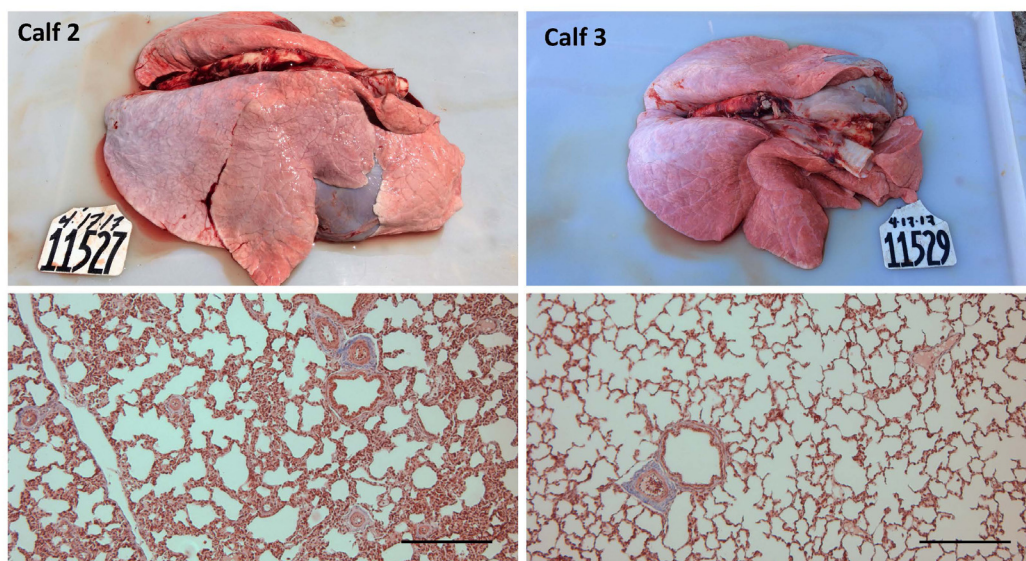
edema, mild or moderate hemorrhage, and type II hyperplasia. Two control calves (Calves 3 and 5) showed mild interstitial edema in some areas. Interstitial inflammatory infiltrates were evident in two calves, particularly Calf 1. No calves showed evidence of interstitial fibrosis. Calves 4 and 6 showed moderate epithelisation of alveoli. Calf 6 also had severe emphysema and honeycomb parenchymal remodeling (Figure 1). Results are presented in Table 4.

Mild or moderate medial hypertrophy and adventitial fibrosis of pulmonary arterioles was evident in all calves. ISO calves had more substantial medial hypertrophy of the pulmonary veins than controls. Pulmonary veins were not evident in Calf 6. Adventitial fibrosis was not observed except in Calf 4. Results are presented in Table 5.

**Table 2. Echocardiographic measures of systolic (ejection fraction and fractional shortening) and diastolic (E/e' ratio) function and left ventricular wall thickness relative to internal diastolic diameter obtained from male intact Holstein calves after 14 days of receiving the nonspecific  $\beta$ -adrenergic agonist ( $\beta$ -AA) isoprenaline (10 mg/kg/d) and control calves that received an equivalent volume of sterile water (0.2 mL/kg).**

Group	Calf	Ejection fraction,%	Fractional shortening,%	Relative wall thickness	E/e'
Control	1*	60	32	0.55	13.2
	3	61	30	0.34	8.1
	5	60	32	0.37	8.3
$\beta$ -AA	2	43	22	0.38	11.2
	4	63	34	0.52	10.0
	6	57	30	0.49	8.7

\* Left ventricular endocardial lesion identified post-mortem



**Figure 1. Lungs and pulmonary histology of male intact Holstein calves after 14 days of receiving the nonspecific  $\beta$ -adrenergic agonist isoprenaline (10 mg/kg/d sid) (Calf 2) or equivalent volume of sterile water (0.2 mL/kg) (Calf 3). Masson's trichrome. Scale bar = 0.25 mm.**

**Table 3.** Heart, lung, and body masses of male intact Holstein calves after 14 days of receiving the nonspecific  $\beta$ -adrenergic agonist ( $\beta$ -AA) isoprenaline (10 mg/kg/d) and control calves that received equivalent volume of sterile water (0.2 mL/kg).

Group	Calf	Body mass, kg	Left ventricle and septum, g	Right ventricle, g	Lung, g
Control	1*	32.5	161	69	466
	3	43.3	191	82	650
	5	49.8	208	83	797
$\beta$ -AA	2	42.8	207	86	597
	4	48.7	245	90	812
	6	37	174	74	556

\* Left ventricular endocardial lesion identified post-mortem.

**Table 4.** Pulmonary parenchymal lesions consistent with diffuse alveolar damage in male intact Holstein calves after 14 days of receiving the nonspecific  $\beta$ -adrenergic agonist ( $\beta$ -AA) isoprenaline (10 mg/kg/d) and control calves that received equivalent volume of sterile water (0.2 mL/kg).

Group	Calf	Interstitial edema	Hyaline membrane	Hemorrhage	Inflammatory infiltrates	Type II hyperplasia	Fibrosis
Control	1*	0	0	1	2	1	0
	3	1	0	0	0	0	0
	5	1	0	0	0	0	0
$\beta$ -AA	2	2	2	2	1	2	0
	4	2	2	2	0	2	0
	6	2	1	1	0	1	0

\*Left ventricular endocardial lesion identified post-mortem

**Table 5.** Semi-quantitative assessment of medial hypertrophy and adventitial fibrosis in pulmonary arteries (< 500  $\mu$ m) and veins from male intact Holstein calves after 14 days of receiving the nonspecific  $\beta$ -adrenergic agonist ( $\beta$ -AA) isoprenaline (10 mg/kg/d) and control calves that received an equivalent volume of sterile water (0.2 mL/kg).

Group	Calf	Pulmonary arteriole		Pulmonary vein	
		Medial hypertrophy	Adventitial fibrosis	Medial hypertrophy	Adventitial fibrosis
Control	1*	1	2	0	0
	3	1	2	1	0
	5	2	1	1	0
$\beta$ -AA	2	1	2	2	0
	4	1	2	2	2
	6	1	1	-	-

## Discussion

The findings of this study indicate that left ventricular dysfunction could feasibly contribute to the development of diffuse alveolar damage, the pathologic correlate of acute interstitial pneumonia, an ARDS of feedlot cattle of unknown etiology. Two weeks of daily injections with isoprenaline led to the development of heart failure with preserved ejection fraction (HFpEF); left ventricular systolic function was preserved but diastolic function was reduced. Given that subclinical HFpEF in a Holstein model has many of the pathologic features and physiologic measurements consistent with ARDS<sup>13</sup>, this preliminary study indicates that the diagnosis of AIP in a feedlot animal can only be considered as tentative if left ventricular dysfunction has not been ruled out as a primary cause.

The PAWP of the ISO calves were significantly greater than controls but substantially less than the PAWP of 26 mm Hg previously reported in feedlot cattle at the end of the feeding period at the altitude of 1,220 m<sup>7</sup>. Pulmonary arterial wedge pressures are typically less than 15 mm Hg in non-feedlot cattle and other mammals<sup>14</sup>; therefore, the DAD seen in the ISO calves in this study is likely attributable to the acute doubling of PAWP over a 2-week period. Feedlot cattle may be able to tolerate much greater PAWP if pressures were to rise insidiously throughout the feeding period. There are a variety of adaptations, in addition to pulmonary arterial and venous remodeling<sup>15</sup>, that may occur in response to rising pulmonary vascular pressures: first, the lymphatic system is recruitable – it is able to increase the rate of lung-water clearance by over 10-fold<sup>16,17</sup>; and second, alveolar fibrosis may occur to reduce alveolar-capillary membrane permeability to water<sup>18</sup>. The time period over which pulmonary vascular pressure rise is, therefore, likely to be a key determinant of the pulmonary adaptability.

It is also feasible that isoprenaline induced DAD through forward hemodynamic effects. Increased cardiac contractility and pulse pressure may have had deleterious downstream effects on the pulmonary vasculature<sup>19,20</sup> paving the way for increased fluid flux into the pulmonary interstitium. Whether attributable to backward or forward hemodynamic effects, an increase in pulmonary capillary pressure will lead to mechanical injury of the alveolar-capillary membrane leading to increased capillary permeability and impaired gas exchange<sup>21</sup>. Although acute damage can be reversed, long term blood-gas barrier disruption leads to lung fibrosis, inflammation, impaired alveolar fluid clearance, and muscularization of pulmonary vessels<sup>21,22</sup>.

Interestingly, the two calves housed in shaded outdoor pens on straw bedding had the greatest pulmonary arterial pressures within their respective treatment groups. In a prior study, calves

housed on straw that developed bloody scours had histologic evidence of DAD and greater pulmonary arterial pressures than calves housed on slatted flooring that did not develop scours<sup>23</sup>. It was speculated that intestinal disease may contribute to the development of pulmonary disease in cattle<sup>23</sup>. Calves housed on straw bedding in our study may have been in the incubation phase of intestinal disease.

Even though there were only 6 calves in this study, the goal of the study, to determine if left ventricular dysfunction could lead to lesions consistent with the early, exudative phase of AIP, was met. For ethical reasons, decompensated heart failure was not induced; therefore, it is not possible to say whether overt left ventricular failure clinically manifests as an ARDS-like event. Human medical reports suggest, however, that it can present as an ARDS which is why the diagnosis of ARDS requires that acute dyspnea attributable to hydrostatic edema must first be ruled out<sup>3</sup>. Large-scale prospective cohort studies of feedlot cattle are necessary to determine if pulmonary arterial pressures and PAWP are positively associated with risk of respiratory diseases, such as AIP. We speculate that elevated pulmonary vascular hydrostatic pressures may act synergistically with mediators of acute alveolar damage, such as pathogens or airborne irritants, to promote the development and progression of respiratory disease.

## Data availability

All raw data underlying this study is available from Harvard Dataverse under a CC0 Public Domain Dedication

Images of the heart and lungs are available at <http://dx.doi.org/10.7910/DVN/HD1GEI><sup>24</sup>

Pulmonary histology is available at <http://dx.doi.org/10.7910/DVN/UR9MAC><sup>25</sup>

Hepatic histology images are available at <http://dx.doi.org/10.7910/DVN/ZBIVAG><sup>26</sup>

Pulmonary vascular pressure recordings are available at <http://dx.doi.org/10.7910/DVN/SKBMWZ><sup>27</sup>

## Competing interests

No competing interests were disclosed.

## Grant information

The author(s) declared that no grants were involved in supporting this work.

## References

1. Neary JM, Booker CW, Wildman BK, *et al.*: **Right-Sided Congestive Heart Failure in North American Feedlot Cattle.** *J Vet Intern Med.* 2016; **30**(1): 326–334.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
2. Loneragan GH, Gould DH, Mason GL, *et al.*: **Involvement of microbial respiratory pathogens in acute interstitial pneumonia in feedlot cattle.** *Am J Vet Res.* 2001; **62**(10): 1519–1524.  
[PubMed Abstract](#) | [Publisher Full Text](#)
3. ARDS Definition Task Force, Ranieri VM, Rubenfeld GD, *et al.*: **Acute respiratory distress syndrome: the Berlin Definition.** *JAMA.* 2012; **307**(23): 2526–2533.  
[PubMed Abstract](#) | [Publisher Full Text](#)
4. Wilkins PA, Otto CM, Baumgardner JE, *et al.*: **Acute lung injury and acute respiratory distress syndromes in veterinary medicine: consensus definitions: The Dorothy Russell Havemeyer Working Group on ALI and ARDS in Veterinary Medicine.** *J Vet Emerg Crit Care.* 2007; **17**(4): 333–339.  
[Publisher Full Text](#)
5. Woolums AR: **Feedlot Acute Interstitial Pneumonia.** *Vet Clin North Am Food Anim Pract.* 2015; **31**(3): 381–9, vi.  
[PubMed Abstract](#) | [Publisher Full Text](#)
6. Sweeney RM, McAuley DF: **Acute respiratory distress syndrome.** *Lancet.* 2016; **388**(10058): 2416–2430.  
[PubMed Abstract](#) | [Publisher Full Text](#)
7. Neary JM: **Epidemiological, physiological and genetic risk factors associated with congestive heart failure and mean pulmonary arterial pressure in cattle.** *Doctoral dissertation, Department of Clinical Sciences, Colorado State University, 2014.*  
[Reference Source](#)
8. Institute for Laboratory Animal Research: **Guide for the Care and Use of Laboratory Animals.** 8th Ed. 2011.  
[PubMed Abstract](#)
9. Ma X, Song Y, Chen C, *et al.*: **Distinct actions of intermittent and sustained  $\beta$ -adrenoceptor stimulation on cardiac remodeling.** *Sci China Life Sci.* 2011; **54**(6): 493–501.  
[PubMed Abstract](#) | [Publisher Full Text](#)
10. Teichholz LE, Kreulen T, Herman MV, *et al.*: **Problems in echocardiographic volume determinations: echocardiographic-angiographic correlations in the presence of absence of asynergy.** *Am J Cardiol.* 1976; **37**(1): 7–11.  
[PubMed Abstract](#) | [Publisher Full Text](#)
11. Paulus WJ, Tschöpe C, Sanderson JE, *et al.*: **How to diagnose diastolic heart failure: a consensus statement on the diagnosis of heart failure with normal left ventricular ejection fraction by the Heart Failure and Echocardiography Associations of the European Society of Cardiology.** *Eur Heart J.* 2007; **28**(20): 2539–2550.  
[PubMed Abstract](#) | [Publisher Full Text](#)
12. de Winter JC: **Using the Student's *t*-test with extremely small sample sizes.** *Pr Assessment, Res Evaluation.* 2013; **18**(10): 1–12.  
[Reference Source](#)
13. Matute-Bello G, Downey G, Moore BB, *et al.*: **An official American Thoracic Society workshop report: features and measurements of experimental acute lung injury in animals.** *Am J Respir Cell Mol Biol.* 2011; **44**(5): 725–738.  
[PubMed Abstract](#) | [Publisher Full Text](#)
14. Will DH, Alexander AF, Reeves JT, *et al.*: **High altitude-induced pulmonary hypertension in normal cattle.** *Circ Res.* 1962; **10**(2): 172–177.  
[PubMed Abstract](#) | [Publisher Full Text](#)
15. Reid LM: **The pulmonary circulation: remodeling in growth and disease. The 1978 J. Burns Amberson lecture.** *Am Rev Respir Dis.* 1979; **119**(4): 531–546.  
[PubMed Abstract](#)
16. Uhley HN, Leeds SE, Sampson JJ, *et al.*: **Role of pulmonary lymphatics in chronic pulmonary edema.** *Circ Res.* 1962; **11**(6): 966–70, Accessed June 23, 2017.  
[PubMed Abstract](#) | [Publisher Full Text](#)
17. Datar SA, Johnson EG, Oishi PE, *et al.*: **Altered lymphatics in an ovine model of congenital heart disease with increased pulmonary blood flow.** *Am J Physiol Lung Cell Mol Physiol.* 2012; **302**(6): L530–40.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
18. Townsley MI, Fu Z, Mathieu-Costello O, *et al.*: **Pulmonary microvascular permeability. Responses to high vascular pressure after induction of pacing-induced heart failure in dogs.** *Circ Res.* 1995; **77**(2): 317–325, Accessed June 23, 2017.  
[PubMed Abstract](#) | [Publisher Full Text](#)
19. Li M, Scott DE, Shandas R, *et al.*: **High pulsatility flow induces adhesion molecule and cytokine mRNA expression in distal pulmonary artery endothelial cells.** *Ann Biomed Eng.* 2009; **37**(6): 1082–1092.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
20. Li M, Stenmark KR, Shandas R, *et al.*: **Effects of pathological flow on pulmonary artery endothelial production of vasoactive mediators and growth factors.** *J Vasc Res.* 2009; **46**(6): 561–571.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
21. Guazzi M: **Alveolar-capillary membrane dysfunction in heart failure: evidence of a pathophysiologic role.** *Chest.* 2003; **124**(3): 1090–1102, Accessed August 17, 2017.  
[PubMed Abstract](#) | [Publisher Full Text](#)
22. Chen Y, Guo H, Xu D, *et al.*: **Left ventricular failure produces profound lung remodeling and pulmonary hypertension in mice: heart failure causes severe lung disease.** *Hypertension.* 2012; **59**(6): 1170–1178.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
23. Gonyeau K, Subbiah S, Klein D, *et al.*: **Development of pulmonary arterial hypertension and diffuse alveolar damage in 2-month old Holstein dairy calves following an acute episode of bloody scours [version 1; referees: 1 approved, 1 approved with reservations].** *F1000Res.* 2017; **6**: 1826.  
[Publisher Full Text](#)
24. Neary J: **Heart and lungs of Holstein calves.** Harvard Dataverse, V1. 2018.  
[Data Source](#)
25. Neary J: **Pulmonary histology of Holstein calves.** Harvard Dataverse, V1. 2018.  
[Data Source](#)
26. Neary J: **Hepatic histology of Holstein calves.** Harvard Dataverse, V1. 2018.  
[Data Source](#)
27. Neary J: **Cardiopulmonary pressures in Holstein calves.** Harvard Dataverse, V1. 2018.  
[Data Source](#)



# Open Peer Review

Current Referee Status:  

Version 1

Referee Report 28 November 2018

<https://doi.org/10.5256/f1000research.15397.r37946>



**Min Li**

Developmental Lung Biology, Cardiovascular Pulmonary Research Laboratories, University of Colorado Denver, Denver, CO, USA

In this small pilot study authors tried to determine if increased pulmonary arterial wedge pressure (PAWP) in a Holstein calf could lead to diffuse alveolar damage (DAD), which provide histologic evidence to diagnose acute interstitial pneumonia (AIP). The results showed that left ventricular dysfunction can associate with, or be one of the cause that a feedlot steer has the sign of AIP (hyaline membranes and type II pneumocyte hyperplasia).

"If applicable, is the statistical analysis and its interpretation appropriate?"

Due to the small number of animals used in the study, a qualified statistician is suggested to verify the statistical analyses.

"Are the conclusions drawn adequately supported by the results?"

More histology images (figure 1) with clear labels of different type of lesions and pathology findings will help to support the findings shown in Table 4 and 5.

Specific comments:

## 1. Methods

Postmortem exam and histology

Whether a board certified veterinary pathologist identified and scored the histologic lesions?

## Results

More histology images (Figure 1) with clear labels of different type of lesions and pathology findings will help to support the findings shown in Table 4 and 5.

2. In the introduction or discussion part, I would like authors to refine the novelty of this study. Increased PAWP due to left ventricular dysfunction can lead to hydrostatic edema, diffuse alveolar damage, all of

which are pathologically correlate with acute interstitial pneumonia. This concept is acknowledged in the cattle field and the results from this study confirmed this concept and suggest that in feedlot, ARDS can not be diagnosed without first rule out LV dysfunction.

**Is the work clearly and accurately presented and does it cite the current literature?**

Yes

**Is the study design appropriate and is the work technically sound?**

Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**

Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**

I cannot comment. A qualified statistician is required.

**Are all the source data underlying the results available to ensure full reproducibility?**

Yes

**Are the conclusions drawn adequately supported by the results?**

Partly

**Competing Interests:** No competing interests were disclosed.

**Referee Expertise:** Cattle model of hypoxia induced pulmonary hypertension, including pro-inflammatory phenotype and metabolic reprogramming of pulmonary vascular cells

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

Referee Report 23 October 2018

<https://doi.org/10.5256/f1000research.15397.r37016>



**Amelia Woolums** 

Department of Pathobiology and Population Medicine, Mississippi State University, Starkville, MS, USA

"If applicable, is the statistical analysis and its interpretation appropriate?"

For a small pilot study, the approach to statistical analysis is acceptable. However, in some cases the statistical analysis may have provided results that are misleading. For example: in the Results section, the authors state that there was no difference in body mass between calves in the two groups. How was this tested? If by the Student's t-test: per the Methods section, the t-test is only appropriate for small samples of unequal size if the effect is expected to be large. But the difference in body mass between the groups was not large, so it's not convincing that the results of this analysis are accurate. Body mass of the calves is not a particularly important outcome of this study, but if differences are going to be reported, a different statistical test should be used.

Similarly, while the ejection fraction between the two groups was not significantly different, the distribution

of values for this outcome in the calves in the two groups looks like EF would likely have been significantly lower in treated calves if more animals had been included. That is, the study likely lacked power to identify a true difference in ejection fraction. Thus this model may not actually induce left heart failure with preserved ejection fraction, as the authors contend.

"Are the conclusions drawn adequately supported by the results?"

Generally yes, with the caveat that the conclusions are based on a very small number of animals. The data depicted in Table 4 support the authors' contention that experimentally-induced left heart dysfunction was associated with lung lesions typical of early acute interstitial pneumonia: hyaline membranes and type II pneumocyte hyperplasia.

Specific comments:

Methods

Postmortem exam and histology

What were the qualifications of the investigator who identified and scored the histologic lesions? Was that person a board certified veterinary pathologist?

Results

Do the investigators have any idea why the ISO treated calves had lower heart rates than the control calves?

Discussion

The writers note: "...this preliminary study indicates that the diagnosis of AIP in a feedlot animal can only be considered as tentative if left ventricular dysfunction has not been ruled out..."

Strictly speaking, AIP (acute interstitial pneumonia) is a pathologic diagnosis, which can result from a variety of possible primary insults. This study does suggest that acute left heart dysfunction can be associated with the lung lesion consistent with early AIP (hyaline membranes and type II pneumocyte hyperplasia). A histologic diagnosis of AIP can be made based on evidence of diffuse alveolar damage; this work suggests that any cause of acute left heart failure could be one of several possible causes of AIP.

This is in contrast to the clinical definition of ARDS which, as the authors note in the introduction, currently requires rule out of respiratory failure solely due to heart failure. However, it is usually not possible in the feedlot to make the measurements necessary to confirm ARDS and rule out acute left heart failure, based on current definitions. This research does support the concept that a feedlot steer or heifer with acute severe dyspnea may be suffering from a lung lesion resulting solely from acute congestive left heart failure, as well as a lung lesion resulting from infection, pneumotoxicity, or other possible causes of AIP.

**Is the work clearly and accurately presented and does it cite the current literature?**

Yes

**Is the study design appropriate and is the work technically sound?**

Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**

Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**

Partly

**Are all the source data underlying the results available to ensure full reproducibility?**

Yes

**Are the conclusions drawn adequately supported by the results?**

Partly

**Competing Interests:** No competing interests were disclosed.

**Referee Expertise:** Lung disease of cattle, including lung infection, and immunity to lung disease caused by viruses or bacteria

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

---

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact [research@f1000.com](mailto:research@f1000.com)

**F1000Research**