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Bronchopneumonia with interstitial pneumonia in beef feedlot cattle: Characterization and laboratory investigation

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Abstract

Bronchopneumonia with interstitial pneumonia (BIP) has been considered a variant of acute interstitial pneumonia (AIP) rather than a distinct disease. This study compared 18 BIP, 24 bronchopneumonia (BP), and 13 AIP cases in feedlot beef cattle. Grossly, BIP cases typically had cranioventral lung lesions of similar morphology and extent as BP cases, but the caudodorsal lung appeared overinflated, bulged on section, and had interlobular edema and emphysema. Gross diagnosis of BIP had 83% sensitivity and 73% specificity relative to histopathology. Histologic lesions of BIP in cranioventral areas were of chronic BP, while caudodorsal lesions included alveolar and bronchiolar damage and inflammation, interstitial hypercellularity, and multifocal hemorrhages. In BIP cases, cranioventral lung lesions were more chronic than caudodorsal lesions. Histologic scores and microbiology data were comparable in cranioventral lung of BIP versus BP cases and caudodorsal lung of BIP versus AIP cases, with differences reflecting a more chronic disease involving less virulent bacteria in BIP versus BP. *Mycoplasma bovis* infection was similarly frequent among groups, and a viral cause of BIP was not identified. Lesion morphology and similar blood cytokine concentrations among groups argued against sepsis as a cause of lung injury. Surfactant dysfunction was identified in BIP and BP, and was only partially the result of protein exudation. These and other findings establish BIP as a distinct condition in which chronic cranioventral BP precedes acute caudodorsal interstitial lung disease, supporting a role of chronic inflammation in heightened sensitivity to 3-methylindole or another lung toxicant.

Keywords

adenovirus, atypical interstitial pneumonia, bovine hokovirus, bovine respiratory disease, calves, lung, metabolism, 3-methylindole, *Mycoplasma bovis*, surfactant, virology

Interstitial lung diseases in cattle may be caused by ingested or inhaled toxicants, viral infection, parasite larval migration, sepsis, or hypersensitivity. A form of interstitial lung disease resulting in alveolar and/or bronchiolar damage is historically recognized in cattle grazing lush pasture, where it is known as fog fever or acute bovine pulmonary emphysema and edema. L-tryptophan in forage is metabolized by rumen microbes to 3-methylindole that is absorbed into the blood and further metabolized in the lung, where cytotoxic intermediates of 3-methylindole cause injury to the alveolar and airway epithelium. A similar form of interstitial lung disease is also important and well recognized in feedlot cattle, where it is known as acute or atypical interstitial pneumonia.[1,3,5,8,15,22,23,25] While 3-methylindole has been implicated by several feedlot-based studies, it remains uncertain whether 3-methylindole is the cause of AIP in feedlot cattle.[1,10,16,24]

It has been recognized since at least the 1970s that some feedlot cattle dying of AIP also have bronchopneumonia (BP) in the cranioventral lung regions, and this has been termed feedlot interstitial pneumonia (a variant of feedlot acute interstitial pneumonia [AIP]), secondary interstitial pneumonia, or bronchointerstitial pneumonia.[3,22,23] Here, we propose "bronchopneumonia with interstitial pneumonia" (BIP) as a more morphologically correct term. BIP comprises 11% to 17% of total mortalities in western Canadian feedlots (Kent Fenton, unpublished data, 2017). The goal of this study was to characterize the pathological and microbiologic features of BIP. Particular emphasis was placed on comparing the cranioventral lung lesions in cattle dying of BIP versus BP, and caudodorsal

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interstitial lung lesions in cattle dying of BIP versus AIP. In addition, the study tested the hypotheses that the frequency of *Mycoplasma bovis* or viral infections, blood cytokine concentrations as indicators of sepsis, or pulmonary surfactant function would differ in BIP, AIP, and BP cases.

Materials and Methods

Case Collection

Cases were a convenience sample of naturally occurring mortalities on 6 large-scale commercial beef feedlots in Alberta and Saskatchewan, Canada, from January 2018 to January 2022. Based on gross and histopathological diagnoses, the study included 18 cases of BIP, 24 cases of BP, and 13 cases of AIP. From each case, lung samples were tested as follows: fresh tissue for routine bacterial culture and mycoplasmal culture (cranioventral and caudodorsal lung, separately), fresh tissue for polymerase chain reaction (PCR) testing for viruses (caudodorsal lung only), and formalin fixation for histopathology (cranioventral and caudodorsal lung, separately). Clotted blood was collected from the heart, and the serum was used for cytokine analysis. Bronchoalveolar lavage fluid was harvested from 13 cases for surfactant analysis (Supplemental Table S1).

Gross Diagnosis and Gross Image Analysis

For all cases, a targeted postmortem examination was performed by a feedlot veterinarian or trained veterinary technician, including a standardized set of postmortem photographs. The photos were reviewed, and a gross diagnosis was allocated by a single experienced feedlot veterinarian (RKF). A diagnosis of BP was indicated by purple to dark red, consolidated lung in the cranioventral regions (with or without other lesions such as abscesses or caseous foci) that was sharply demarcated from the unaffected caudodorsal lung. In AIP cases, lesions were generalized throughout the lung (often more prominent in caudodorsal lung) with no line of demarcation; the entire lung appeared overinflated, interlobular septa were expanded by edema and/or emphysema, individual lobules often varied from pale pink to dark purple (lobular or "checkerboard pattern"), and lung tissue bulged on cut surface. In BIP cases, both lesion types were present with the BP-affected cranioventral lesion being sharply demarcated from the caudodorsal lesion of interstitial pneumonia.

Subsequently, cases were assigned to a final diagnosis group based on histopathology. Sensitivity, specificity, positive predictive value, and negative predictive value of gross diagnoses of BIP were calculated.

For each case, a single in situ photograph of the intact right lung was analyzed. The proportion of lung grossly affected by BP (based on the surface area visible in the photograph) was measured using image analysis (ImageJ; National Institute of Health & Laboratory for Optical and Computational Instrumentation, University of Wisconsin) and compared between BIP and BP cases. Cases were excluded if poor animal positioning or photo quality impaired evaluation of the entire lateral surface of the right lung.

Histopathology

Tissue samples were fixed in formalin, processed routinely, and stained with hematoxylin and eosin. Histologic scores and diagnoses were made by consensus (LAJH or LS, and JLC) without knowledge of the gross diagnosis or microbiology data. All lung sections were scored for the presence or absence of specified histologic lesions (Supplemental Tables S4 and S5). The duration of cranioventral and caudodorsal lung lesions was classified as acute (absence of fibrosis), subacute (presence of immature granulation tissue), or chronic (presence of mature fibrosis that was densely eosinophilic with fewer and smaller fibroblast nuclei).

Histologic criteria for diagnosis of bronchopneumonia were neutrophils and macrophages filling the lumen of alveoli and bronchioles. Histologic criteria for alveolar and bronchiolar damage (a form of interstitial/bronchointerstitial lung disease) were alveoli lined by hyaline membranes or type II pneumocytes and loss of bronchiolar epithelium with attenuation of remaining epithelial cells, respectively. Cases were diagnosed as BIP if bronchopneumonia was a predominant histologic lesion in sections of cranioventral lung and alveolar and bronchiolar damage were prominent in sections of caudodorsal lung. However, the lesion types were not required to be anatomically segregated: The cranioventral lung sections of some BIP cases had alveolar and bronchiolar damage in addition to the bronchopneumonia required for the diagnosis of BIP, and the caudodorsal lung sections of some BIP cases had bronchopneumonia in addition to the alveolar and bronchiolar damage required for the diagnosis of BIP.

Microbiology

For each case, 3 pooled samples each of fresh cranioventral and caudodorsal lung were submitted for routine bacterial culture and for specialized mycoplasmal culture to the Animal Health Laboratory, University of Guelph (Supplemental Methods). For routine cultures, all bacterial growth was identified using matrix-assisted laser-desorption ionization time-of-flight mass spectrometry. For mycoplasmal culture, isolates were identified by indirect fluorescent antibody tests. For each case, 3 pooled samples of fresh caudodorsal lung were routinely tested using real-time PCR for bovine alphaherpesvirus 1 (BHV-1), bovine respiratory syncytial virus (BRSV), bovine parainfluenzavirus 3 (BPIV3), bovine coronavirus (BCV), and bovine viral diarrhea virus (BVDV) types 1 and 2 (Supplemental Methods). At a later time, samples from 12 BIP, 20 BP, and 10 AIP cases were tested by PCR for bovine hokovirus (*Ungulate tetraparvovirus* 1), based on results of high-throughput sequencing on 3 cases per group (data not shown; Supplemental Methods), and bovine adenovirus serotypes 1 to 8 (Supplemental Methods).

Cytokine ELISA

Interleukin (IL)-1β, IL-6, and IL-8 were measured in heart blood serum from 5 cases each of BIP, BP, and AIP to evaluate sepsis or cytokine storm as a possible cause of alveolar damage in BIP. Where possible, cases were matched in each mortality group by the length of time on the feedlot prior to death. Commercial ELISA kits for bovine IL-1β (Invitrogen), bovine IL-6 (Invitrogen), and bovine IL-8 (Mabtech, Stockholm) with internal positive and negative controls were used following manufacturer protocols.

Surfactant Analysis

Surfactant pool sizes and function were analyzed to evaluate surfactant dysfunction as a possible cause of alveolar and bronchiolar damage in BIP. Bronchoalveolar lavage fluid was collected from 2 areas of caudodorsal lung at postmortem from 4 BIP, 8 BP, and 1 AIP cases. Bronchoalveolar lavage fluid was also collected from lungs of 6 clinically and pathologically normal cattle at slaughter, including a "fresh" sample taken within 2 hours of slaughter, and an "autolyzed" sample taken from a different location after 24 hours of refrigeration to simulate the delay in isolating surfactant from feedlot cases. Detailed procedures are described in Supplemental Methods. Bronchoalveolar lavage fluid was fractionated by centrifugation, and phospholipid and total protein concentrations were measured.[19] The functional ability of the large aggregate surfactant subfraction to reduce surface tension was evaluated using a constrained sessile drop surfactometer, including adsorption and dynamic cycling through repeated periods of compression and expansion.[20] To determine the impact of inflammatory exudate protein on functional abnormalities, unextracted surfactant preparations were compared with organic extracts of large aggregate samples (4 BIP, 4 BP, 4 normal-fresh, and 3 normal-autolyzed); the organic extracts had been previously shown to contain surfactant lipids and hydrophobic surfactant proteins B and C.[2] The former and latter samples are referred to as "unextracted surfactant" and "extracted surfactant," respectively.

Statistical Analysis

Lesion duration and the frequency of histologic lesions or microbial pathogens were compared between BIP vs BP and BIP vs AIP using Pearson χ^2 tests. P values $< .05$ were considered significant. Continuous variables, including extent of gross lung lesions, cytokine concentrations, and surfactant measurements, were tested for normality using Shapiro-Wilk tests and for equality of variance using Levene tests. For non-normal continuous data, groups were compared by Mann-Whitney U tests. For continuous data with unequal variances, a Welch t test was used. For normally distributed data with equal variances, differences were analyzed by Student t test. For surfactant data, groups were compared with 2-way analysis of variance (ANOVA). Statistical analyses were performed in R software implemented via an open-source statistics interface (JASP, University of Amsterdam).

Table 1. Accuracy of gross diagnosis compared to diagnosis by histopathology.

	Histologic diagnosis			
	BIP	BP	AIP	Total
Gross diagnosis				
BIP	15	6	4	25
BP	2	18	0	20
AIP	1	0	9	10
Total	18	24	13	55

The data show the number of cases.
Abbreviations: BIP, bronchopneumonia with interstitial pneumonia; BP, bronchopneumonia; AIP, acute interstitial pneumonia.

Results

Gross Pathology

Typical gross lesions of BIP, BP, and AIP cases are shown in Fig. 1. Of 55 feedlot deaths included in the study, 25 were initially diagnosed as BIP based on gross examination. Based on subsequent histologic examination of lung, 10 cases initially diagnosed as BIP were reclassified to either BP (n = 6) or AIP (n = 4), and 3 of the 30 cases initially diagnosed as BP (n = 2) or AIP (n = 1) were reclassified to BIP (Table 1). Thus, using histopathology as the gold standard, gross diagnosis of BIP had a sensitivity of 83% and a specificity of 73%. As BIP was estimated to represent 11.2% of all deaths on western Canadian feedlots,[11] gross diagnosis of BIP had a positive predictive value of 28%, a negative predictive value of 97%, and an accuracy of 74%.

The percentage of lung affected by bronchopneumonia was estimated by image analysis of an in situ photo of the right lung. In the 18 histologically diagnosed BIP cases, cranioventral bronchopneumonia affected a mean of 53% (95% confidence interval = 45%–61%) of the right lung. In the 21 BP cases with photos of sufficient quality, cranioventral bronchopneumonia affected a mean of 45% (95% confidence interval = 26%–53%) of the right lung. The percentage of lung with lesions of BP was not significantly different between the 2 groups ($P = .21$).

Histopathology

Histologic sections of cranioventral and caudodorsal lung were evaluated systematically (Figs. 2–4, Supplemental Tables S4 and S5). In the cranioventral lung from BIP cases, alveoli and bronchiolar lumens contained neutrophils and macrophages in 18/18 cases (100%) and fibrin in 16/18 cases (88%). Attenuation of bronchiolar epithelial cells (bronchiolar necrosis) was present in 18/18 cases (100%), and alveolar type II pneumocyte proliferation was present in 12/18 cases (67%). There were

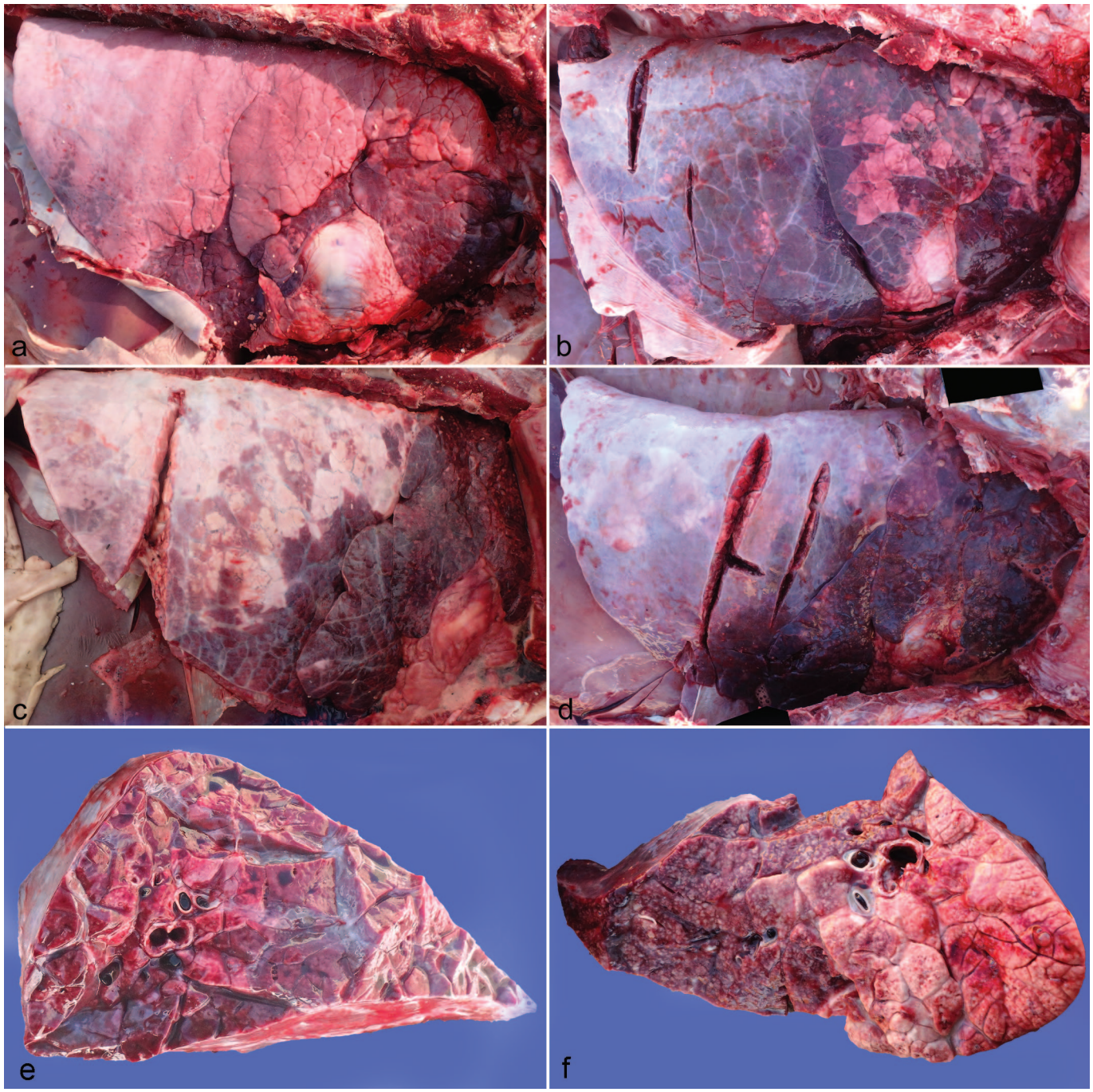

Figure 1. Gross lesions of bronchopneumonia (BP), acute interstitial pneumonia (AIP), and bronchopneumonia with interstitial pneumonia (BIP) in feedlot cattle. (a) BP. The cranioventral lung is dark red and consolidated. The caudodorsal lung is normal. (b) AIP. The lung is dark red, fails to collapse, appears overinflated, and bulges from the cut surface. The lesion in this case is diffuse in caudodorsal areas, and lobular ("checkerboard") in cranial areas. Edema expands the interlobular septa. (c, d) BIP. The caudodorsal area has lesions similar to AIP; interlobular emphysema and edema are prominent in "c." The cranioventral area is red and consolidated as for BP. (e) AIP. The cut surface has interlobular emphysema and edema; lobules are erect and separated. (f) BIP. The more caudodorsal lung (right) has lesions as described above. The more cranioventral lung (left) is red with caseonecrotic foci typical of *M. bovis* infection.

subacute or chronic lesions, including bronchiolitis obliterans in 17/18 cases (94%), alveolar or interlobular fibrosis in 16/18 cases (89%), and peribronchiolar fibrosis in 14/18 cases (77%). Foci of caseous necrosis (typical of *M. bovis* infection) were present in 9/18 cases (50%). Uncommon or absent lesions in the cranioventral lung of BIP cases included hyaline membranes (0/18), oat cells (1/18), pleuritis (2/18), and coagulative necrosis (3/18).

In the caudodorsal lung of BIP cases, histologic findings within alveoli included intraluminal inflammatory cells in

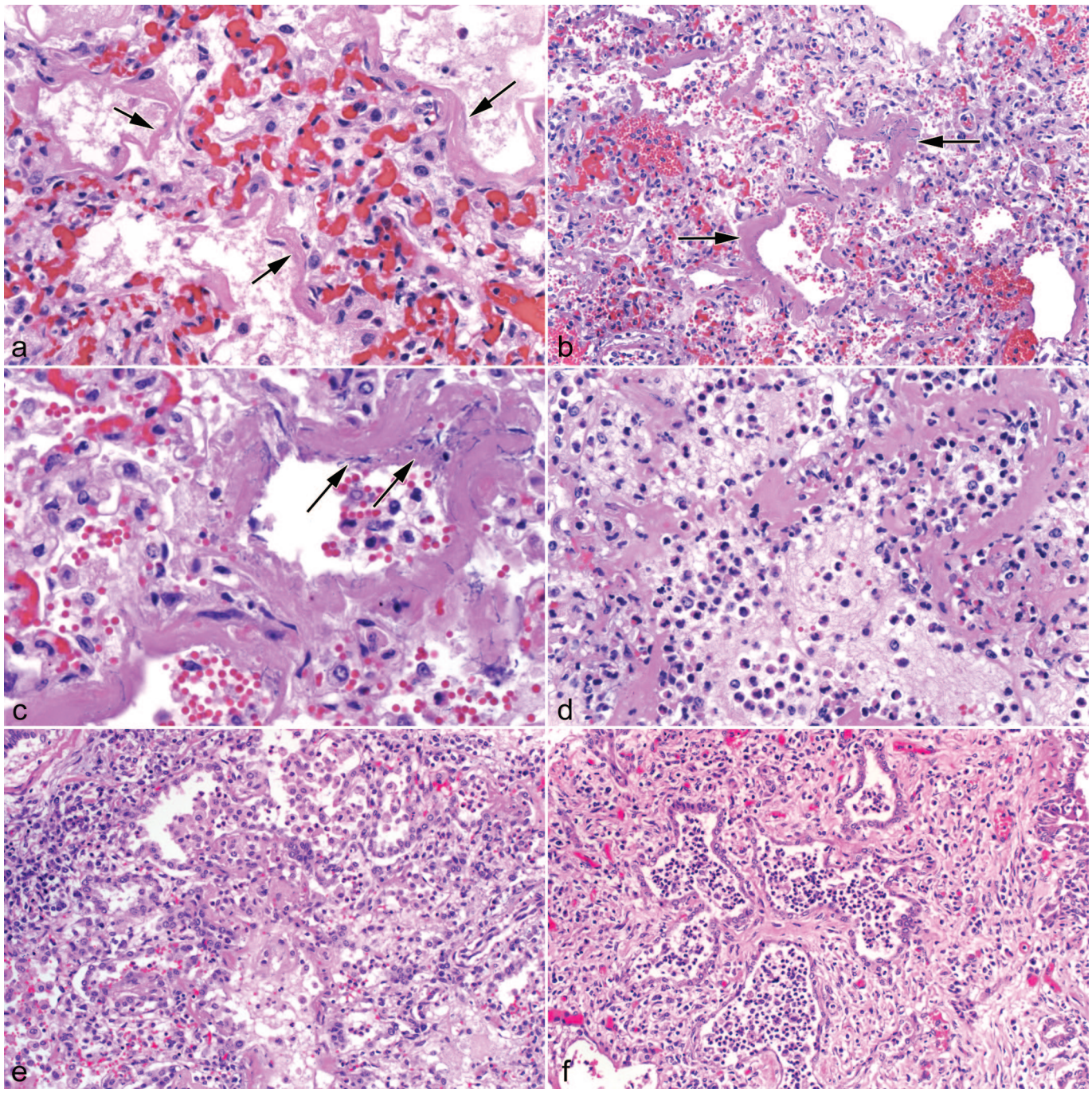

Figure 2. Bronchopneumonia with interstitial pneumonia, alveolar lesions in caudodorsal lung, feedlot cattle. (a) Alveoli are lined by thin hyaline membranes (arrows) and contain protein-rich edema but few inflammatory cells. (b–c) Alveolar walls are obscured by thick eosinophilic hyaline membranes containing basophilic stippling (interpreted as chromatin; arrows). Alveolar septa are hypercellular. (d) Alveoli contain many neutrophils and a network of fibrin and are lined by hyaline membranes. (e) Alveoli are lined by a continuous layer of cuboidal type II pneumocytes. (f) Alveolar septa are expanded by fibrous tissue containing mononuclear cells, and alveoli are lined by type II pneumocytes.

17/18 cases (94%), intraluminal fibrin in 15/18 cases (83%), interstitial hypercellularity in 15/18 cases (83%), hemorrhage in 14/18 cases (78%), hyaline membranes in 14/18 cases (78%), and type II pneumocyte proliferation in 12/18 cases (67%). The hyaline membranes in these cases formed thick bands of homogeneous eosinophilic material that lined alveolar surfaces. Bronchioles contained inflammatory cells in 15/18 cases (83%) and attenuation of bronchiolar epithelial cells (bronchiolar necrosis) in 15/18 cases (81%). In its most severe form, bronchiolar injury included not only attenuation and loss of bronchiolar epithelium, but the full thickness of the bronchiolar wall had an eosinophilic hyaline or coagulated appearance with

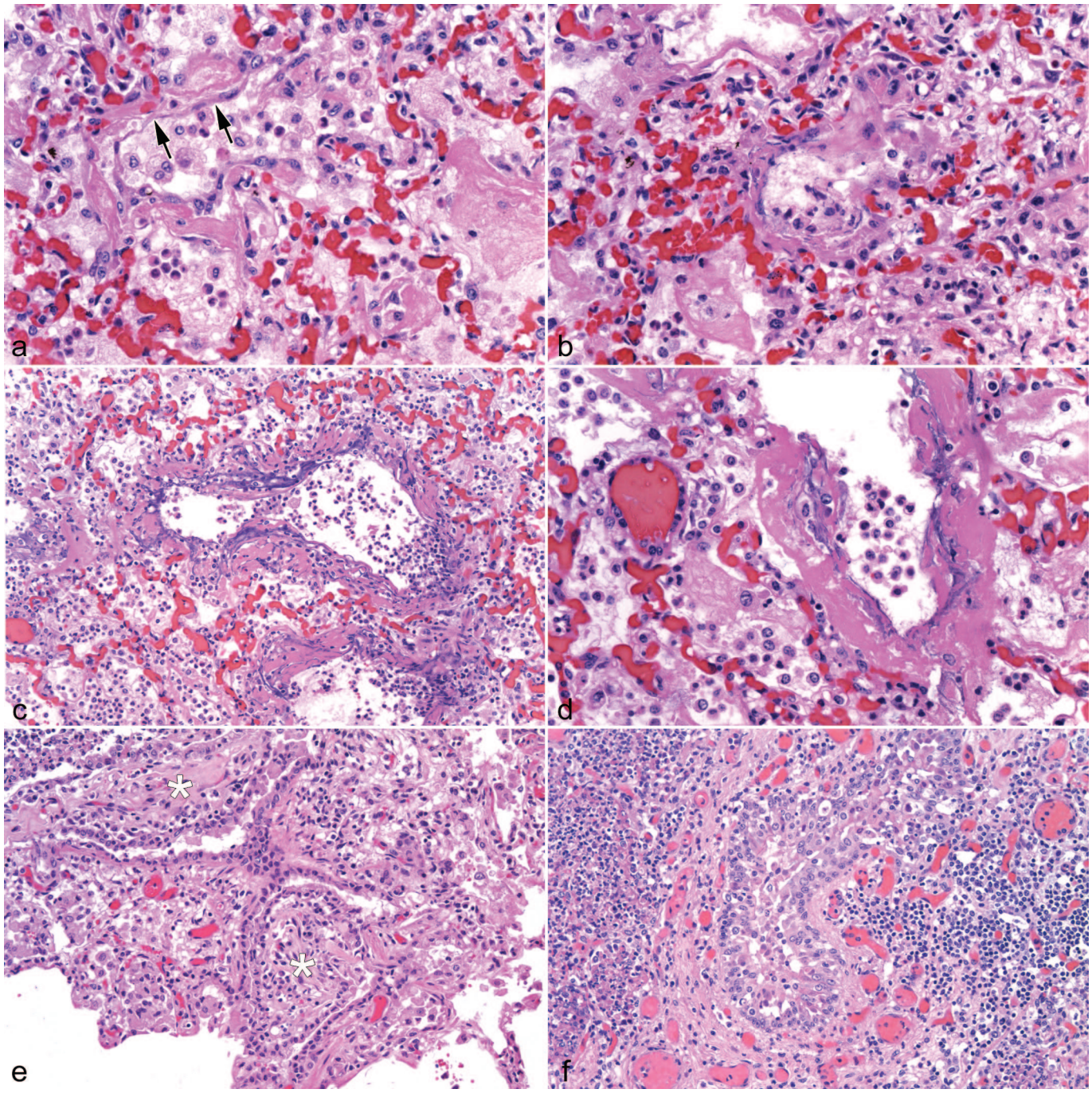

Figure 3. Bronchopneumonia with interstitial pneumonia, bronchiolar lesions in caudodorsal lung, feedlot cattle. (a) A bronchiole is lined by attenuated epithelium (arrows). Alveoli and bronchioles contain leukocytes, sloughed cells, and fibrin. (b–d) There is full-thickness necrosis of bronchiolar walls with replacement by eosinophilic hyaline material containing basophilic stippling. (e) Bronchiolitis obliterans, in which bronchiolar lumens are filled by a polyp of re-epithelialized connective tissue (asterisks). (f) Peribronchiolar fibrosis. The adjacent tissue contains many lymphocytes.

basophilic stippling (interpreted as chromatin). Some cases had subacute or chronic lesions, including bronchiolitis obliterans in 7/16 cases (44%), peribronchiolar fibrosis in 4/18 cases (22%), and alveolar or interlobular fibrosis in 2/18 cases (11%). Uncommon or absent lesions in the caudodorsal lung of BIP cases were oat cells (0/16; 0%), coagulative necrosis (1/16; 6%), and caseous necrosis (3/16; 19%).

Oat cells were less frequent in cranioventral lung of BIP compared with BP cases (6% vs 42%; $P = .008$; Fig. 4a). Other lesions did not significantly differ in frequency between these groups although peribronchiolar fibrosis (78% vs 50%; $P = .067$) and alveolar/interlobular fibrosis (89% vs 63%; $P = .054$) tended to be more frequent and coagulative necrosis less frequent (17% vs 42%; $P = .083$) in cranioventral lung of BIP

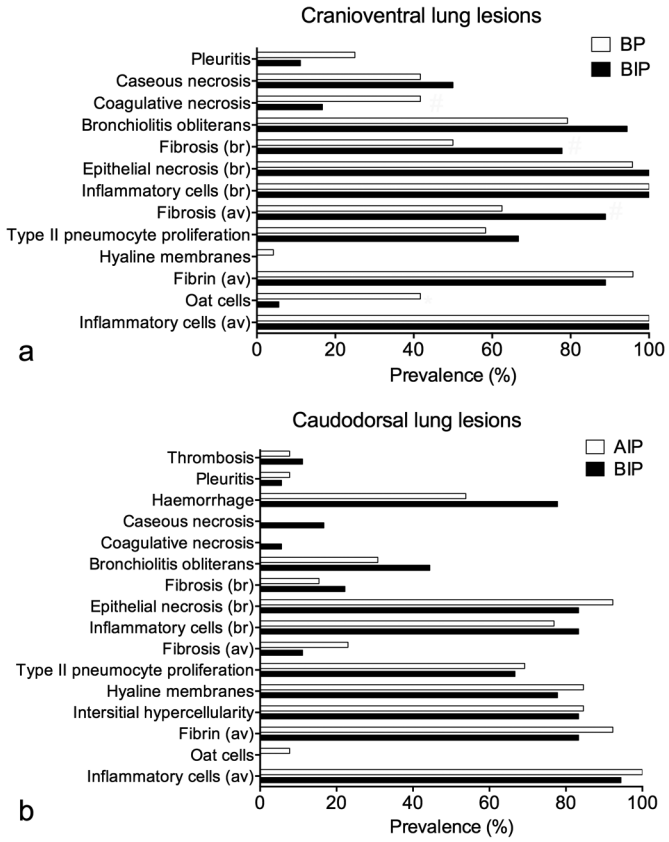

Figure 4. Frequency of histologic lesions in (a) cranioventral lung from cases of bronchopneumonia (BP, n = 24) and of bronchopneumonia with interstitial pneumonia (BIP, n = 18), and in (b) caudodorsal lung from cases of acute interstitial pneumonia (AIP, n = 13) and of BIP (n = 18). Bronchiolar and alveolar inflammatory cells in the cranioventral lung were required for diagnosis of BP and BIP, and alveolar hyaline membranes or type II pneumocytes in caudodorsal lung were required for diagnosis of AIP and BIP. br = bronchiolar; av = alveolar.

Table 2. Estimated duration of histologic lesions in cranioventral and caudodorsal lung regions of beef calves.

Location	Diagnosis	Acute	Subacute	Chronic
Cranioventral	BIP (n = 18)	2 (11%)	0 (0%)	16 (89%)
	BP (n = 24)	5 (21%)	3 (13%)	16 (67%)
Caudodorsal	BIP (n = 18)	11 (61%)	3 (17%)	4 (22%)
	AIP (n = 13)	8 (62%)	2 (15%)	3 (23%)

Cases with mature fibrosis within the lung lesions were classified as chronic, cases with immature granulation tissue were classified as subacute, and cases with neither of these were classified as acute. The data show the number and percentage of cases, for each diagnosis.
Abbreviations: BIP, bronchopneumonia with interstitial pneumonia; BP, bronchopneumonia; AIP, acute interstitial pneumonia.

Table 3. Bacteria isolated from the cranioventral or caudodorsal lung from calves with BIP (n = 18), BP (n = 24), and AIP (n = 13).

	Cranioventral Lung		Caudodorsal Lung	
	BIP	BP	BIP	AIP
Mannheimia haemolytica genotype 1	0 (0%)	1 (4%)	0 (0%)	0 (0%)
Mannheimia haemolytica genotype 2	1 (6%)*	8 (33%)*	0 (0%)	1 (8%)
Bibersteinia trehalosi	4 (22%)	5 (21%)	3 (17%)	0 (0%)
Histophilus somni	7 (39%)	8 (33%)	6 (33%)	4 (31%)
Pasteurella multocida	9 (50%)	13 (54%)	8 (44%)	2 (15%)
≥1 of the above bacteria	11 (61%)	20 (83%)	12 (67%)	5 (38%)
Trueperella pyogenes	10 (56%)	10 (41%)	11 (61%)*	2 (15%)*
Escherichia coli	5 (28%)	7 (29%)	2 (11%)	2 (15%)
Mycoplasma bovis	15 (83%)	23 (96%)	15 (83%)	12 (92%)
Mycoplasma arginini	13 (72%)	20 (83%)	11 (61%)	8 (62%)
Mycoplasma alkalescens	0 (0%)	3 (13%)	1 (6%)	4 (31%)
Ureaplasma spp	11 (61%)	17 (71%)	14 (78%)	10 (77%)

The data show the number and percentage of cases for each diagnosis.
Abbreviations: BIP, bronchopneumonia with interstitial pneumonia; BP, bronchopneumonia; AIP, acute interstitial pneumonia.
*$P < .05$, BIP vs BP, or BIP vs AIP.

compared with BP cases. There were no significant differences in the frequency of any histologic lesions between BIP and AIP in caudodorsal lung tissues, although hemorrhage (78% vs 54%; $P = .15$) and caseous necrosis (17% vs 0%; $P = .12$) tended to be more common in caudodorsal lung of BIP cases (Fig. 4b).

The duration of lung lesions was estimated based on the absence or presence of immature granulation tissue or mature fibrosis. For BIP cases, cranioventral lung lesions were significantly more chronic than caudodorsal lung lesions ($P < .001$; Table 2). There were no significant differences in lesion duration between cranioventral BIP and cranioventral BP lung ($P = .17$), nor between caudodorsal BIP and caudodorsal AIP lung ($P = .99$; Table 2).

Microbiology

The bacteria most frequently isolated from the lung are shown in Table 3 and Supplemental Table S6. *M. bovis* infection was frequent in all 3 groups. *Mannheimia haemolytica* genotype 2 was less frequently isolated from cranioventral lung tissue of BIP compared with BP cases (6% vs 33%; $P = .030$). *Trueperella pyogenes* was more frequently isolated from caudodorsal lung tissue of BIP cases compared with AIP cases (61% vs 15%; $P = .010$).

Virology data are summarized in Table 4. Bovine hokovirus was the most commonly detected virus, followed by BCV, bovine adenovirus (serotypes 1–8), BHV-1, and BRSV. BPIV-3 and BVDV were not detected. The frequency of BHV-1, BPIV-3, BRSV, BCV, and BVDV was not significantly different among groups. Bovine adenovirus was more frequent in BP compared with BIP cases (45% vs 0%; $P = .014$).

Cytokine Analysis

The concentrations of IL-1β, IL-6, and IL-8 in heart blood serum were compared in BIP, BP, and AIP cases to evaluate

sepsis/cytokine storm as a possible cause of the alveolar damage (Supplemental Figure S1). There were no significant differences in concentrations of IL-1β, IL-6, or IL-8 in BIP compared with BP or AIP cases.

Surfactant Analysis

Surfactant pool sizes and function were analyzed to evaluate surfactant dysfunction as a possible cause of alveolar and bronchiolar damage in BIP. Whereas preparations of fresh and autolyzed surfactant from normal animals had similarly low total protein concentrations, protein concentrations were significantly higher in samples from BIP and BP cases ($P < .05$; Table 5, Supplemental Figure S2). Surfactant consists of surface-active large aggregate and less active small aggregate subfractions. The concentration and percentage of large aggregates was not different in BIP cases and fresh normal samples or BP cases, although autolyzed samples had a higher concentration and percentage of large aggregates (Table 5, Supplemental Figure S2).

The functional capability of surfactant samples to lower surface tension was assessed by subjecting drops of surfactant to dynamic cycles of compression and expansion using a constrained sessile drop surfactometer. Through 20 compression-expansion cycles, surfactant from BIP cases and BP cases had significantly higher minimum surface tensions (that is, a diminished capability for lowering surface tension) compared with surfactant from normal calf lungs that were fresh (n = 6) ($P < .001$–$.014$) or autolyzed for 24 h (n = 5) ($P < .001$–$.016$) (Fig. 5). The initial minimum surface tension of the BIP and BP surfactant (cycle 0 in Fig. 5, prior to beginning compression-expansion cycles) was higher than that for fresh normal surfactant ($P = .019$) (Fig. 5).

To evaluate the contribution of inflammatory exudate proteins to the above functional abnormalities, the function of an organic extract of surfactant (which contains only surfactant lipids and surfactant proteins B and C) was compared with unextracted surfactant from BIP cases (n = 4), BP cases (n = 4), and normal cattle (n = 4). During cycles of compression and expansion (cycles 1–20 in Fig. 6), minimum surface tensions of extracted BIP and BP samples remained higher than those of extracted samples from healthy animals ($P < .0001$) (suggesting that hydrophilic proteins were not mainly responsible for this functional impairment). In contrast, although initial minimum surface tensions (cycle 0 in Fig. 6) were significantly higher for nonextracted BIP and BP samples compared with nonextracted normal samples, the initial minimum surface tensions were similar among samples of extracted surfactant from BIP cases, BP cases, and normal animals (suggesting that hydrophilic proteins were responsible for this abnormality).

Table 4. Viruses detected by polymerase chain reaction testing in lung of beef calves with BIP (n = 18), BP (n = 24), and AIP (n = 13).

	BP	BIP	AIP
Bovine herpesvirus 1	4 (17%)	1 (6%)	2 (15%)
Bovine parainfluenzavirus 3	0 (0%)	0 (0%)	0 (0%)
Bovine respiratory syncytial virus	1 (4%)	0 (0%)	1 (8%)
Bovine coronavirus	9 (38%)	4 (22%)	4 (31%)
Bovine viral diarrhea virus	0 (0%)	0 (0%)	0 (0%)
Any of the above viruses	10 (42%)	5 (28%)	5 (38%)
Bovine hokovirus[a]	17 (85%)	9 (75%)	8 (90%)
Bovine adenovirus[a]	9 (45%)*	0 (0%)*	1 (10%)

The data show the number and percentage of cases for each pathological diagnosis.
Abbreviations: BP, bronchopneumonia; BIP, bronchopneumonia with interstitial pneumonia; AIP, acute interstitial pneumonia.
[a]Number tested for hokovirus and adenovirus: 12 BIP, 20 BP, and 9 AIP. Samples were tested by polymerase chain reaction for bovine hokovirus based on results of high-throughput sequencing (see Supplemental Methods).
*$P < .05$, BP vs BIP.

Discussion

This study characterized the pathological and microbiologic features of BIP, identified the accuracy of gross diagnosis, compared BIP with BP and AIP, and tested the hypotheses that the frequency of *M. bovis* or viral infections, blood cytokine concentrations as indicators of sepsis, or pulmonary surfactant function would differ in BIP, AIP, and BP cases. The findings indicate that in BIP cases, cranioventral BP precedes caudodorsal alveolar and bronchiolar damage, blood cytokine storm does not appear to play a role, and BIP cases appear to have dysfunction of lung surfactant that is independent of protein exudation into lung fluids. The morphological and microbiologic features of BIP resembled those of the component lesions;

Table 5. Analysis of pulmonary surfactant prepared from cases of BIP (n = 4), BP (n = 8), and AIP (n = 1), and from fresh (n = 6) and autolyzed (n = 5) lungs of the same normal calves.

Sample	Total protein (mg/ml)	Total surfactant (μg/ml)	Large aggregates (μg/ml)	Small aggregates (μg/ml)	Large aggregates (%)
Fresh (n = 6)	0.12 ± 0.02*	23.4 ± 3.5	10.2 ± 1.1	10.4 ± 1.9	50.9 ± 3.0
Autolyzed (n = 5)	0.43 ± 0.06*	67.0 ± 8.9	47.9 ± 7.6*	18.7 ± 2.9	71.9 ± 2.7*
BIP (n = 4)	5.1 ± 2.4	43.6 ± 11.1	13.2 ± 5.4	25.2 ± 13.1	43.7 ± 13.3
BP (n = 8)	3.4 ± 1.0	38.5 ± 4.7	19.5 ± 2.4	17.2 ± 2.9	54.3 ± 3.8
AIP (n = 1)	12.0	170.3	72.8	97.0	45.2

The data show the mean ± SEM.
Abbreviations: BIP, bronchopneumonia with interstitial pneumonia; BP, bronchopneumonia; AIP, acute interstitial pneumonia.
*$P < .05$, different from BIP.

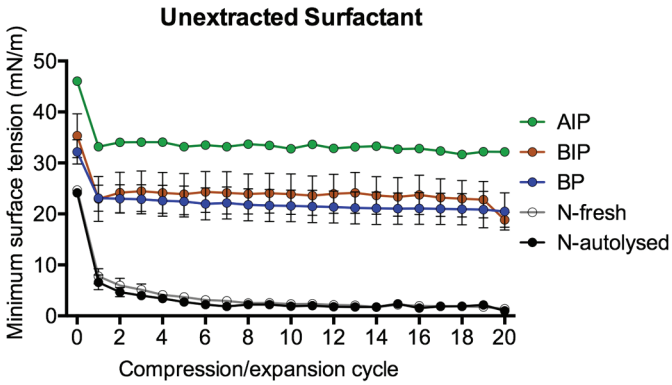

Figure 5. The functional capability of unextracted surfactant in lowering surface tension as measured by a constrained sessile drop surfactometer. The data show mean ± SEM for surfactant preparations from 4 BIP, 8 BP, and 1 AIP cases, and from fresh (N-fresh, n = 6) and autolyzed lungs (N-autolysed) of the same 5 normal calves. X = 0 represents the surface tension after 2 minutes of adsorption to the surfactometer pedestal and prior to compression/expansion cycles. Compared with surfactant preparations from normal animals, preparations from BIP and BP cases had significantly higher minimum surface tensions both initially (x = 0) and during compression/expansion cycles (x = 1 to 20). BIP, bronchopneumonia with interstitial pneumonia; BP, bronchopneumonia; AIP, acute interstitial pneumonia.

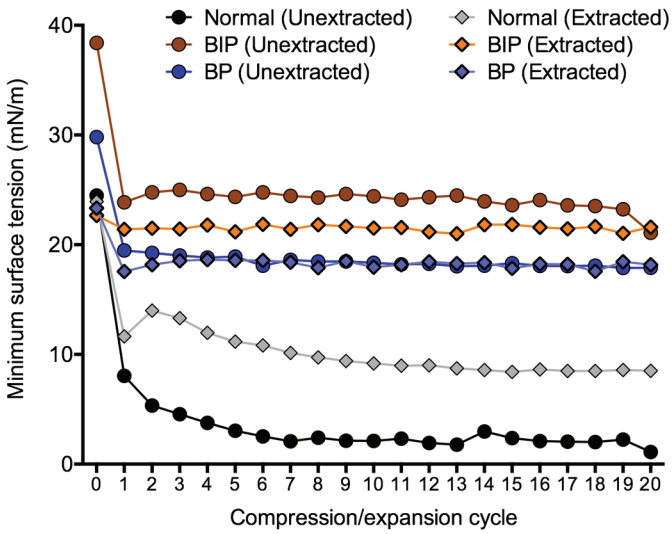

Figure 6. Comparison of minimum surface tensions in unextracted surfactant preparations and in the same preparations following organic extraction to remove hydrophilic proteins (extracted). The data show means from 4 BIP cases, 4 BP cases, and 4 normal calves (error bars are omitted for clarity but are shown in Supplemental Figure S3). During compression/expansion cycles 1–20, minimum surface tensions are higher for both unextracted and extracted surfactant from BIP and BP cases compared with normal animals ($P < .0001$). The initial minimum surface tensions (x = 0) are higher for unextracted surfactant from BIP and BP cases compared with normal animals, whereas initial minimum surface tensions are similar for extracted surfactant from BIP and BP cases and from normal animals. BIP, bronchopneumonia with interstitial pneumonia; BP, bronchopneumonia.

however, the findings of a separate study[11] indicate that BIP is a distinct condition rather than chance concurrence of BP and AIP (namely, a higher concurrence of lesions than expected by chance, and differences between BIP and AIP in the sex predominance, seasonality, timing relative to entry to the feedlot, and prior clinical illnesses[11]).

Compared with histopathology, gross diagnosis of BIP by a single experienced feedlot veterinarian reviewing postmortem photographs had a high sensitivity (83%) and moderate specificity (73%). The reliance on postmortem photographs is advantageous for population surveillance but might contribute to the moderate number of false-positive BIP diagnoses because lung texture cannot be evaluated.

We considered whether increased respiratory effort or respiratory rate leading to alveolar wall stress or excessive negative intra-alveolar pressures might be a reason for subsequent development of alveolar and bronchiolar damage (i.e. interstitial lung disease). However, a similar proportion of the lung was grossly affected by bronchopneumonia in BIP and BP cases (53% and 45% respectively), suggesting that the extent of BP was not the sole determinant of whether interstitial lung damage developed.

Based on histopathology of BIP cases, the cranioventral lesions of bronchopneumonia were more chronic than the caudodorsal lesions of alveolar and bronchiolar damage. Relevant to the pathogenesis, this implies that BIP represents calves with chronic bronchopneumonia that die when acute alveolar and bronchiolar damage (i.e. interstitial lung disease) compromises function of the remaining lung.

Gross and histologic lesions in the cranioventral lung of BIP cases were similar to those of BP cases. The exceptions were that BIP cases had a lower frequency of oat cells and coagulative necrosis and a greater frequency of bronchiolar, alveolar, and interlobular fibrosis compared with BP cases. In a separate study, we found that BIP cases (compared with BP cases) had a longer interval from first illness to death and more instances of clinically recognizable illness.[11] Thus, these differences in histologic lesions probably reflect a more chronic bronchopneumonia in BIP than in BP cases, or lung that is more typically infected with lower virulence or secondary bacterial pathogens. The similarity of cranioventral lung lesions in BIP and BP also highlights the diagnostic value in histologically examining ≥2 sections of caudodorsal lung to detect the alveolar and bronchiolar damage.

Consistent histologic lesions in the caudodorsal lung of BIP cases were morphologically similar to those of AIP cases and included alveolar hyaline membranes and/or type II pneumocyte proliferation (required for the diagnosis), alveolar edema and fibrin, bronchiolar necrosis, bronchiolitis obliterans, patchy or multifocal alveolar hemorrhage, mononuclear cell infiltrates in alveolar septa, and neutrophils and other leukocytes within bronchioles and alveoli. Such severe and frequent bronchiolar damage seems inconsistent with sepsis or cytokine storm and more compatible with toxic or viral causes. The full-thickness damage to bronchiolar walls and the alveolar hemorrhages are features not often seen in other interstitial lung diseases of

domestic animals (personal observation, JLC), but the implications with respect to cause and pathogenesis are currently unknown.

The findings of chronic bronchopneumonia associated with more acute alveolar and bronchiolar damage may suggest that chronic BP affects the metabolism or excretion of a lung toxicant. For example, inflammatory mediators affect cellular expression of cytochrome P450 enzymes and prostaglandin H synthetase that metabolize 3-methylindole to 3-methyleneindolenine.[4,6,7,12,13,23] Thus, further studies may investigate whether cattle with chronic BP have an enzyme expression profile that favors the production of 3-methylindole metabolites (or metabolites of other feed-derived toxicants) that are lung-damaging rather than inert or easily excreted.

The microbiologic findings in cranioventral and caudodorsal lung of BIP cases were similar to those of BP and AIP, respectively. Prior gross observations suggested *M. bovis* as a cause of the cranioventral bronchopneumonia in many BIP cases. However, *M. bovis* was a frequent isolate from BIP, BP, and AIP cases, and histologic lesions of caseous necrosis were similarly frequent in BIP and BP cases. Thus, at the late stage of disease at which these animals were examined, the findings do not support a unique causal role of *M. bovis* in the development of BIP. *M. haemolytica* genotype 2 was less frequent in BIP compared with BP cases, consistent with the lower frequency of oat cells and coagulative necrosis. Since this is the *M. haemolytica* genotype most frequently associated with acute respiratory disease,[17] this finding is consistent with speculation that development of BIP might require calves to survive acute BP (such as that caused by *M. haemolytica*) and develop chronic pneumonia (such as that perpetuated by *M. bovis* or *T. pyogenes*) that increases the risk of later interstitial lung disease.

The frequencies of BHV-1, BRSV, and BCV were similar in BIP vs BP or AIP cases, and BPI3V and BVDV were not detected. Bovine adenovirus, which is not proven to cause clinically significant natural respiratory disease, was more frequent in BP cases compared with BIP cases; this might reflect that BP cases tended to die earlier in the feeding period than BIP cases. Bovine hokovirus was identified using high-throughput sequencing of lung samples from 3 BIP and 3 BP cases (data not shown), and follow-up testing of all cases by qPCR identified a high frequency of bovine hokovirus infection, but no difference among study groups; lung of healthy animals was not tested in this study. Bovine hokovirus has not been associated with clinical disease.[21]

IL-1β, IL-6, and IL-8 were measured in the heart blood serum to investigate whether sepsis might contribute to alveolar damage (i.e. interstitial lung disease) in BIP cases. These were not significantly different between BIP cases and BP or AIP cases, although caution is warranted because only a single time point was examined (i.e. the time of postmortem examination) and we did not extensively validate the assays for use on postmortem heart blood. Nonetheless, these data and the presence of severe bronchiolar necrosis suggest that the alveolar damage is not the result of sepsis. Further work is needed to determine whether the causes and pathogenesis are similar or heterogeneous across BIP cases. In addition to toxicants, infectious agents, and sepsis, other potential mechanisms of alveolar and bronchiolar damage in BIP might include physical damage from increased respiratory rate or excessive negative intra-alveolar pressure, hyperthermia, hypersensitivity reaction or trained immunity to an existing pathogen, and surfactant dysfunction.

Alterations in surfactant composition and function are consistently recognized in humans with acute respiratory distress syndrome,[18] and the histologic correlate of this syndrome—diffuse alveolar damage—is observed in cattle with BIP and AIP. Surfactant reduces the surface tension at the alveolar surface, thereby maintaining alveolar stability. Pathological processes that alter surfactant composition or function can result in elevated alveolar surface tensions and compromised alveolar stability with alveolar collapse, reduced lung compliance, and impaired gas exchange.[9] Surfactant is a complex mixture of phospholipids and proteins that are synthesized and secreted by alveolar type II pneumocytes to form a surface-active film.[14] Two main subfractions of surfactant are recognized: large aggregates are the functional surface-active component, and small aggregates are thought to represent surfactant components that have been degraded during the respiratory cycle.

Surfactant preparations from calves with BIP or BP had significantly higher total protein concentrations compared with that of healthy calves. This likely represents exudation of plasma proteins into air spaces resulting from either damage to alveolar epithelium or inflammation-induced vascular permeability.

Surfactant preparations from postmortem bronchoalveolar lavage fluid samples of diseased animals had impaired function, compared with preparations from lung of healthy slaughter calves that were prepared freshly or after 24 hours of autolysis. Because protein is known to impair surfactant function,[26] we compared surfactant function of unextracted surfactant preparations with those subjected to organic extraction. This extraction purifies the phosopholipid components and hydrophobic surfactant proteins, but removes hydrophilic proteins such as those in plasma and inflammatory exudates. Some of the impaired ability of surfactant to reduce surface tensions was attributed to increased protein levels, specifically the initial adsorption phase for BIP and BP samples. However, during compression/expansion cycles of BIP and BP surfactant preparations, minimum surface tensions remained high after organic extraction. These observations indicate that, in BIP and BP cases, compromised surfactant function during expansion and compression of the surfactant film was not entirely a consequence of interference by serum proteins. A minor detrimental effect of organic extraction on normal surfactant function was also noted and is attributed to removal of surfactant protein A (a hydrophilic protein that augments surfactant function).

Thus, surfactant compromise in BIP seems to involve both interference by inflammatory proteins and an uncharacterized abnormality during compression/expansion that is not attributed to protein contamination. It is not known if this surfactant dysfunction is a cause of the alveolar and bronchiolar damage in BIP (similar to the diffuse alveolar damage that occurs in premature animals with hyaline membrane disease) or a

consequence of the disease (such as from damage to the type II pneumocytes that synthesize and recycle surfactant components). Nonetheless, it is plausible that surfactant dysfunction might contribute to respiratory difficulty and death in cattle with BIP.

Conclusions

This study establishes BIP as a unique condition of feedlot cattle that is characterized by chronic BP in the cranioventral lung and acute alveolar and bronchiolar injury in the caudodorsal lung. Gross diagnosis is the basis for routine diagnosis of BIP in feedlot populations but with only moderate specificity; some gross BIP diagnoses were shown by histopathology to be BP or AIP. Thus, definitive diagnosis of BIP should be based on histologic examination of multiple samples from cranioventral and caudodorsal lung regions. The histologic lesions and microbiologic findings in the cranioventral lung of BIP were similar to those in chronic BP cases, and differences between the groups were attributed to a more chronic disease process involving less virulent or secondary bacteria in BIP cases. *M. bovis* infection was common in BIP cases but was not significantly different than that in BP or AIP. The study findings did not support a virus as the main cause of BIP. Analysis of cytokine concentrations in the heart blood serum of BIP cases did not suggest that the alveolar damage in BIP cases was caused by sepsis. Surfactant dysfunction during expansion/compression cycles was identified in BIP cases and was not entirely attributed to exudation of proteins into the lung fluids. It is unknown whether surfactant dysfunction causes alveolar and bronchiolar damage in BIP cases or whether it is a consequence of the lung injury. In summary, this study described the pathological and microbiologic features of BIP, a condition long recognized by feedlot veterinarians. The cause and pathogenesis remains uncertain, but this study forms a basis for investigating whether chronic bronchopneumonia alters the metabolism of a pneumotoxicant to cause the acute interstitial lung lesions characteristic of this disease.

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References

1. Ayroud M, Popp JD, VanderKop MA, et al. Characterization of acute interstitial pneumonia in cattle in southern Alberta feedyards. *Can Vet J*. 2000;**41**(7):547–554.
2. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol*. 1959;**37**(8):911–917.
3. Curtis RA, Thomson RG, Sandals WC. Atypical interstitial pneumonia in cattle. *Can Vet J*. 1979;**20**(5):141–142.
4. de Jong LM, Jiskoot W, Swen JJ, et al. Distinct effects of inflammation on cytochrome P450 regulation and drug metabolism: lessons from experimental models and a potential role for pharmacogenetics. *Genes*. 2020;**11**(12):E1509.
5. Doster AR. Bovine atypical interstitial pneumonia. *Vet Clin North Am Food Anim Pract*. 2010;**26**(2):395–407.
6. Formosa PJ, Bray TM. Evidence for metabolism of 3-methylindole by prostaglandin H synthase and mixed-function oxidases in goat lung and liver microsomes. *Biochem Pharmacol*. 1988;**37**(22):4359–4366.
7. Formosa PJ, Bray TM, Kubow S. Metabolism of 3-methylindole by prostaglandin H synthase in ram seminal vesicles. *Can J Physiol Pharmacol*. 1988;**66**(12):1524–1530.
8. Fulton RW, Blood KS, Panciera RJ, et al. Lung pathology and infectious agents in fatal feedlot pneumonias and relationship with mortality, disease onset, and treatments. *J Vet Diagn Invest*. 2009;**21**(4):464–477.
9. Günther A, Ruppert C, Schmidt R, et al. Surfactant alteration and replacement in acute respiratory distress syndrome. *Respir Res*. 2001;**2**(6):353–364.
10. Haydock LA, Fenton RK, Sergejewich L, et al. Acute interstitial pneumonia and the biology of 3-methylindole in feedlot cattle. *Anim Health Res Rev*. 2022;**23**(1):72–81.
11. Haydock LA, Fenton RK, Smerek D, et al. Bronchopneumonia with interstitial pneumonia in feedlot cattle: epidemiologic characteristics of affected animals. *Vet Pathol*. 2023;**60**(2):226–234.
12. Kuhn MJ, Mavangira V, Sordillo LM. Invited review: cytochrome P450 enzyme involvement in health and inflammatory-based diseases of dairy cattle. *J Dairy Sci*. 2021;**104**(2):1276–1290.
13. Lanza DL, Yost GS. Selective dehydrogenation/oxygenation of 3-methylindole by cytochrome p450 enzymes. *Drug Metab Dispos*. 2001;**29**(7):950–953.
14. Lewis JF, Veldhuizen RAW. The future of surfactant therapy during ALI/ARDS. *Semin Respir Crit Care Med*. 2006;**27**(4):377–388.
15. 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.
16. Loneragan GH, Gould DH, Mason GL, et al. Association of 3-methyleneindolenine, a toxic metabolite of 3-methylindole, with acute interstitial pneumonia in feedlot cattle. *Am J Vet Res*. 2001;**62**(10):1525–1530.
17. Loy JD, Clawson ML. Rapid typing of Mannheimia haemolytica major genotypes 1 and 2 using MALDI-TOF mass spectrometry. *J Microbiol Methods*. 2017;**136**:30–33.
18. Matthay MA, Zemans RL, Zimmerman GA, et al. Acute respiratory distress syndrome. *Nat Rev Dis Primers*. 2019;**5**(1):18.
19. Milos S, Hiansen JQ, Banaschewski B, et al. The effect of diet-induced serum hypercholesterolemia on the surfactant system and the development of lung injury. *Biochem Biophys Rep*. 2016;**7**:180–187.
20. Milos S, Khazaee R, McCaig LA, et al. Impact of ventilation-induced lung injury on the structure and function of lamellar bodies. *Am J Physiol Lung Cell Mol Physiol*. 2017;**313**(3):L524–L533.

21. Mitra N, Cernicchiaro N, Torres S, et al. Metagenomic characterization of the virome associated with bovine respiratory disease in feedlot cattle identified novel viruses and suggests an etiologic role for influenza D virus. *J Gen Virol*. 2016;**97**(8):1771–1784.
22. Panciera RJ, Confer AW. Pathogenesis and pathology of bovine pneumonia. *Vet Clin North Am Food Anim Pract*. 2010;**26**(2):191–214.
23. Sorden SD, Kerr RW, Janzen ED. Interstitial pneumonia in feedlot cattle: concurrent lesions and lack of immunohistochemical evidence for bovine respiratory syncytial virus infection. *J Vet Diagn Invest*. 2000;**12**(6):510–517.
24. Stanford K, McAllister TA, Ayroud M, et al. Effect of dietary melengestrol acetate on the incidence of acute interstitial pneumonia in feedlot heifers. *Can J Vet Res*. 2006;**70**(3):218–225.
25. Woolums AR. Feedlot acute interstitial pneumonia. *Vet Clin North Am Food Anim Pract*. 2015;**31**(3):381–389.
26. Zuo YY, Tadayyon SM, Keating E, et al. Atomic force microscopy studies of functional and dysfunctional pulmonary surfactant films, II: albumin-inhibited pulmonary surfactant films and the effect of SP-A. *Biophys J*. 2008;**95**(6):2779–2791.