Received: 29 May 2023 | Accepted: 27 February 2024

DOI: 10.1111/jvim.17039

STANDARD ARTICLE

Journal of Veterinary Internal Medicine Open Access

ACVIM American College of Veterinary Internal Medicine

Blood gases, acid-base, and metabolic alterations in calves with bronchopneumonia diagnosed via clinical signs and thoracic ultrasonography: A cross-sectional study

Antonio Boccardo[1] | Vincenzo Ferrulli[1] | Giulia Sala[2] | Donatella Scavone[1] | Saverio Paltrinieri[1] | Laura Filippone Pavesi[1] | Davide Pravettoni[1]

[1]Dipartimento di Medicina Veterinaria e Scienze Animali (DIVAS), Università degli Studi di Milano, Lodi, Italy

[2]Dipartimento di Scienze Veterinarie, Università degli Studi di Pisa, San Piero a Grado, Italy

Correspondence
Giulia Sala, Dipartimento di Scienze Veterinarie, Università degli Studi di Pisa, Via Livornese (SP-22), San Piero a Grado, 56124—Pisa, Italy.
Email: giulia.sala@unipi.it

Funding information
University of Milan

Abstract

Background: Bronchopneumonia (BP) in calves potentially causes systemic changes.

Objectives: To describe metabolic, arterial blood gas, and acid-base disorders in calves with BP diagnosed by thoracic ultrasound (TUS), Wisconsin score (WISC), and combinations of WISC and TUS.

Animals: Two hundred thirty-one dairy preweaned dairy calves from 13 dairy farms.

Methods: Cross-sectional study. Each calf sequentially underwent arterial blood gas evaluation, WISC score, venous sampling, and TUS. Calves were grouped based on a single diagnostic method and combination of WISC and 2 TUS cutoffs (≥1 cm; ≥3 cm) as healthy, upper respiratory tract infection, subclinical BP, and clinical BP.

Results: Oxygenation and acid-base variables were unaffected. Glucose concentration in TUS-affected calves was significantly lower ($P < .001$) than in healthy calves (median $\geq TUS_{1cm} = 5.2$ mmol/L 25%-75% interquartile range [IQR] 4.5-6.1, $< TUS_{1cm} = 5.9$ mmol/L IQR 5.5-6.6; $\geq TUS_{3cm} = 5.1$ mmol/L IQR 4.4-6.2, $< TUS_{3cm} = 5.8$ mmol/L IQR 5.3-6.4). Paraoxonase-1 was significantly lower ($P < .001$) in TUS-affected calves ($\geq TUS_{1cm} = 64.1$ U/mL IQR 40.8-78, $< TUS_{1cm} = 77.3$ U/mL IQR 61.9-96.5; $\geq TUS_{3cm} = 59.2$ U/mL IQR 37.7-72.4, $< TUS_{3cm} = 72.9$ U/mL IQR 53.4-95.5). None of the variables highlighted clear distinctions in WISC-detected clinical and subclinical BP calves based on the combination of WISC and TUS.

Conclusions and Clinical Importance: Clinical signs indicate minor systemic disorders compared to TUS. The abnormalities detected by ultrasonographic examination were moderate and did not deviate from normal reference ranges.

Abbreviations: A-aO_2, alveolar-arterial difference for oxygen; AG, anion gap; A_{tot}, concentration of nonvolatile weak acids; BE, base excess; BP, bronchopneumonia; Ca^{2+}, blood ionized calcium; Cl^-, blood chloride; H-H, Henderson-Hasselback; HCO_3^-, blood bicarbonate; K^+, blood potassium; ICS, intercostal spaces; IQR, interquartile range; Na^+, blood sodium; P/F ratio, Horowitz index for lung function; PaO_2, partial pressure of arterial oxygen; PCO_2, partial pressure of carbon dioxide; PON-1, paraoxonase-1; sSID, simplified strong ion difference; SID_5, strong ion difference; SIG, strong ion gap; SO_2, oxygen saturation; STP, serum total protein; TUS, thoracic ultrasonography; TUS_{1cm}, calves with a lung consolidation of ≥1 cm; TUS_{3cm}, calves with a lung consolidation of ≥3 cm; USI, unidentified strong ions; $WISC_5$, calves with a Wisconsin respiratory score ≥5.

KEYWORDS
blood gas analysis, calves, clinical signs, respiratory disease, thoracic ultrasonography

1 | INTRODUCTION

Commonly used methods for diagnosing bronchopneumonia (BP) in calves include thoracic ultrasonography (TUS) and clinical scores that indicate a respiratory tract inflammatory process.[1] Depth of lung consolidations ≥1 and ≥3 detected by TUS are considered a confirmatory test for in vivo diagnosis of a BP episode.[1,2] The Wisconsin clinical score (WISC) is commonly used to identify calves with infectious respiratory disease including BP[1] and has been correlated with a low average daily gain in positive calves.[3] However, the diagnostic accuracy and interobserver concordance of this test is modest.[1,4] Although a composite reference test for diagnosing BP in calves has not been validated and could be subject to diagnostic error,[5] recent studies have used an easy-to-obtain underfield condition TUS and clinical signs combination to classify calves into healthy, upper respiratory tract infection, subclinical BP, and clinical BP.[6,7]

Despite the extensive literature on diagnostic accuracy and the various methodologies for using TUS and clinical scores in calves, little is known about the severity of the systemic involvement in terms of blood gases, acid-base status, and metabolic disturbances of a BP episode diagnosed through TUS and WISC score.

Blood oxygenation data, L-lactate concentration, and respiratory-related Henderson-Hasselback (H-H) acid-base disturbances are objective measures of lung functions. They could be used to assess the severity of pulmonary diseases.[8,9] In addition, studies carried out on calves[10] and pigs[11] that were experimentally infected with *Chlamydia* spp. and in horses suffering from asthma syndrome,[12] suggest that lung lesions could also affect the strong ion difference (SID) and the concentration of weak nonvolatile acids (A_{TOT}) of the simplified strong ion difference (sSID) approach to the acid-base imbalances. Furthermore, a reduced blood concentration of paraoxonase-1 (PON-1) has been noted in calves aged 28 to 120 days with respiratory disease.[13] Paraoxonase-1 is a negative acute phase protein,[14] and the lower activity during inflammation might result from reduced hepatic synthesis, displacement from high-density lipoproteins, and increased peripheral consumption to counteract free radicals.[13-15]

We believe that describing the presence and the degree of abnormalities of pulmonary gas exchange, acid-base, and metabolic disarrangements that characterize the most commonly used diagnostic methods for the diagnosis of BP in calves can help clarify the ability of these tools to diagnose the physiological state of the calves and, thus the severity of the ongoing disease. Furthermore, we believe that this study could provide new insights into systemic changes during BP that should contribute to a better understanding and knowledge of the disease in preweaned dairy calves.

This study thus aimed to describe (1) blood gas status (partial pressure of arterial oxygen [PaO_2] oxygen saturation [SO_2], the alveolar-arterial difference for oxygen [A-aO_2], and Horowitz index for lung function [P/F ratio]), (2) acid-base abnormalities using the traditional H-H approach and sSID theory, (3) metabolic analytes measured by a blood gas analyzer (glucose, L-lactate, creatinine, and urea), and (4) serum PON-1 activity in preweaned dairy calves with BP detected with 2 TUS cutoffs (≥1 and ≥3 cm), WISC, and combinations of WISC and TUS.

We hypothesized that (i) lung consolidations diagnosed with TUS would demonstrate more pronounced metabolic changes than using WISC; (ii) calves with ≥3 cm lesions might have more relevant metabolic abnormalities than those with ≥1 cm lesions; and (iii) the categorization of enrolled calves into healthy, upper respiratory tract infection, subclinical BP, and clinical BP based on the combination of TUS and WISC might be characterized by a progressive severity of metabolic derangements.

2 | MATERIALS AND METHODS

Following STROBE guidelines, we conducted a cross-sectional study using a convenience sample selected from dairy farms that had used our mobile clinic from February 2021 to February 2022.

The publication of data from the ambulatory clinical activity of the Clinic for Ruminant and Swine of the University of Milan was approved by the Ethics Committee of the University of Milan (approval number 47/2017, November 28, 2017).

2.1 | Selection of the farms

The criterium for selecting the farms was a history of cough detected by the herd practitioner in preweaned calves bred in multiple pens with automatic calf feeders with no history of treatment for BP in the previous 15 days before the day of the study. This selection was performed to ensure an adequate spectrum of the disease (calves with and without BP) because a cough is associated with an increased probability of lung consolidations.[16] Enrolled farms reflected the typical midsize (milking cows ranging from 150 to 400) Po Valley milk production systems. Briefly, calves were separated from the dam immediately after birth and received 4 L of good-quality colostrum (Brix ≥22%)[17] within 6 to 8 h after birth. Calves were housed individually for up to 15 to 20 days and fed with a milk replacer before being led into multiple pens with an automated calf feeder for preweaning calves, where they remained for up to 75 to 95 days.

2.2 | Selection of the calves

On each study day, preweaned calves bred in multiple pens with automatic calf feeders were observed on each of the farms. One of the main

authors (AB or DP) observed calves for the detection of spontaneous cough. If 1 coughing calf was observed in a pen all calves within the pen were considered eligible for the study unless they showed lameness, cachexia, dehydration, diarrhea, or umbilical lesions that were considered as exclusion criteria.

A minimum of 15 to 25 preweaned calves per farm were examined. Selected animals were randomly chosen with an Android application (Randomizer, Darshan Institute of Engineering and Technology, Rajkot, India), using the list of individual ear tags provided by the farmer. Calves were assessed with the same 1-gate design protocol. Two helpers captured each calf, and arterial blood was sampled for blood gas analysis without moving the calf from the capture site. A clinical evaluation using the WISC score was then performed by the last author. The calf was then subjected to jugular vein sampling and moved to a designated corner outside the pen for TUS examination. Calves from the same herd were subjected to the clinical protocol on the same day. Each calf's ear tags, serial numbers, age, clinical score results, and ultrasound findings of each intercostal space were recorded.

2.3 | Arterial blood sampling and on-farm processing

In all cases, arterial blood was anaerobically collected from the medial intermediate auricular branch of the caudal auricular artery.[18] Hairs on the dorsal surface (in the middle position and near basal attachment) of the pinna were removed with a disposable razor. The tip of a butterfly needle (22G) was placed into the lumen of the artery until the plastic conduit was filled up to the end of the final connector. A 2.5 mL disposable heparinized (5 IU) syringe was connected until approximately 0.5 mL of blood had been aspirated. After the procedure, the needle was removed from the artery and closed with a rubber stopper. The syringe was not disconnected from the butterfly needle until the blood gas analysis had been immediately carried out on farm using an automated blood gas analyzer (EPOC Vet, Epocal Inc, Ottawa, Canada) commonly used in cattle practice[19,20] that directly measures or calculates several variables (https://www.woodleyequipment.com/docs/user_guide_english.pdf). The samples were processed following the manufacturer's guidelines. The automated blood gas analyzer was calibrated by inserting the manufacturer's card. During the calibration (approximately 70-80 seconds), each calf's rectal temperature, serial number, and the 21% inspired oxygen fraction were entered.

The device-processed variables considered for this study were the following:

- H-H acid-base model: temperature-adjusted blood pH, partial pressure of carbon dioxide (PCO_2 [mm Hg]), blood bicarbonate (HCO_3^- [mmol/L]) base excess (BE [mmol/L]).
- Blood oxygenation data: PaO_2 (mm Hg), SO_2 (%), A-aO_2 (mm Hg), and P/F ratio.
- Electrolytes: sodium (Na^+ [mmol/L]), potassium (K^+ [mmol/L]), chloride (Cl^- [mmol/L]), and ionized calcium (Ca^{2+} [mmol/L]).
- Metabolic variables: glucose (mmol/L), L-lactate (mmol/L), creatinine (mg/dL), and urea (mmol/L).

2.4 | Clinical score

Each calf was examined and assigned as healthy or as having BP, based on the WISC respiratory scoring chart. A WISC score of 0 to 3 is used in relation to the severity of 5 criteria: temperature, nasal discharge, cough, eye discharge, and ear position. Calves with a score of 5 or higher ($WISC_5$) were considered positive cases.[21]

2.5 | Blood venous sampling

After the clinical examination, each calf underwent a venous blood sampling via jugular venipuncture using a 20-gauge hypodermic needle. The blood was then stored in a sterile Vacutainer tube without an anticoagulant. Blood samples were transported to the clinic in a portable cooler (5°C), and then centrifuged at 20°C for 10 min at 900*g* within 6 h of collection.

2.6 | Thoracic ultrasonography

Bilateral TUS (intercostal spaces [ICS] 10-1 on the right and ICS 10-2 on the left) was performed—in all the calves enrolled based on the ventral landmarks described in previous studies[22]—by the first author blinded to clinical scoring results. Ultrasonography was performed using a portable unit (Esaote MyLab Five Vet, Esaote S.p.A., Genova, Italy) with a 7.5 MHz linear transducer set to a depth of 8 cm and a gain of 16 dB. Thoracic ultrasonography was interpreted in 2 different ways, and calves with a consolidation of ≥1 cm^1 (TUS_{1cm}) or with a consolidation of ≥3 cm^2 (TUS_{3cm}) were considered positive. Consolidation was defined when an echoic structure replaced the normal reverberation artifact. For each calf, the maximum depth of consolidation on TUS was recorded. Depth (cm) was calculated by manual count using the lateral grid of the ultrasound image. The measurement tool integrated into the ultrasound scanner was used in uncertain cases, or when lesions tended to approximate a cutoff measurement. Comet tail artifacts, pleural irregularities, and lesions <1 cm were not used because there is still no clarity in the literature on the diagnostic value of these lesions.

2.7 | Serum total protein, paraononase-1 assay, and simplified strong ion difference calculations

The serum obtained from the venous sample was used to measure PON-1 activity and to determine serum total protein (STP g/L) using a temperature-compensating optical refractometer. Before use, the refractometer was calibrated using distilled water. Paraoxonase-1 activity was spectrophotometrically measured using an enzymatic process[14] and

validated in cattle.[13] Briefly, 6 µL of serum was incubated at 37°C with 89 µL of distilled water and 100 µL of reaction buffer (glycine buffer 0.05 mM, pH 10.5 containing 1 mM of paraoxon-ethyl, purity >90%, and 1 mM of calcium chloride). The rate of hydrolysis of paraoxon to p-nitrophenol was measured by monitoring the increase in absorbance at 405 nm using a molar extinction coefficient of 18.050 L mol^{-1} cm^{-1}. The unit of PON-1 activity expressed as U/mL was defined as 1 nmol of p-nitrophenol formed per minute under assay conditions.

Ear tag number, age, calf serial number, STP concentration, and all data stored in the blood gas analyzer for each of the calves were manually transferred into an Excel spreadsheet.

The anion gap (AG [mmol/L]) was obtained as follows:

$$AG = (Na^+ + K^+) - (Cl^- + HCO_3^-).$$

The sSID variables were then calculated according to previously described formulas.[23] The concentration of nonvolatile weak acids (A_{TOT}, [mmol/L]) was calculated from the STP concentration (g/L) according to the experimentally determined values for calf plasma[24]:

$$A_{tot} = 0.343 \times \text{STP concentration}.$$

The measured strong ion difference was calculated from blood concentrations of Na^+, Cl^-, K^+, Ca^{2+}, and L-lactate (SID_5 [mmol/L]):

$$SID_5 = (Na^+ + K^+ + Ca^{2+}) - (Cl^- + \text{L} - \text{Lactate}).$$

Unmeasured strong ions were estimated by calculating the strong ion gap (SIG [mmol/L]):

$$SIG = \left(A_{TOT} / \left[1 + 10^{(pKa - pH)}\right]\right) - AG.$$

where pKa (7.08) is the effective dissociation constant of bovine plasma weak acids.[24]

The unidentified strong ions (USI [mmol/L]) concentration was obtained as follows:

$$USI = SIG + (\text{L} - \text{lactate}) - Ca^{2+}.$$

2.8 | Categorizations

In accordance with recent studies,[6,7] calves were categorized based on the combination of TUS and WISC as follows:

- $WISC_5/TUS1_{cm}$; healthy (WISC <5 without consolidation on TUS), upper respiratory tract infection (WISC ≥5 without consolidation on TUS), subclinical BP (WISC <5 with TUS ≥1 cm), and clinical BP (WISC ≥5 with TUS ≥1 cm).
- $WISC_5/TUS_{3cm}$; healthy (WISC <5 without consolidation on TUS), upper respiratory tract infection (WISC ≥5 without consolidation on TUS), subclinical BP (WISC <5 with TUS ≥3 cm), and clinical BP (WISC ≥5 with TUS ≥3 cm).

2.9 | Data analysis

A Wilcoxon-Mann-Whitney analysis was used to calculate the minimum number of calves using G-power v. 3.1. (Heinrich-Heine-Universität, Düsseldorf, Germany) needed to highlight BP-related metabolic differences between healthy and affected calves. An effect size of 0.5 (medium), α error of 5% (type I), a confidence interval of 95%, and a test power of 95% were used. The minimum sample size was 220 calves. Data were stored and analyzed with IBM SPSS Statistics v. 27.0 (IBM Corp. Armonk. NY). Descriptive statistics were performed, and continuous variables were reported with median and interquartile range (IQR) from the 25th to the 75th percentile because the data were not normally distributed (tested with the Shapiro-Wilk test). Categorical variables were expressed as frequencies and percentages. The statistical analysis was performed using nonparametric tests because data were not distributed normally.

Differences between variables were analyzed first in the single scores ($WISC_5$, TUS_{1cm}, TUS_{3cm}) with the Mann-Whitney *U* test, and then in the various combinations between clinical scores and TUS with the Kruskal Wallis test. Statistical significance was set for a $P < .05$. Multiple comparisons were performed with the Bonferroni correction.

3 | RESULTS

We visited 13 dairy farms. The final sample consisted of 231 Holstein-Friesian female calves aged between 12 to 94 days old (median = 57 days; IQR 25% = 42 days; IQR 75% = 72 days). The animals considered as affected by BP or healthy using a single clinical or TUS cutoffs are as follows:

- $WISC_5$; 166 healthy calves (71.9%) and 65 BP calves (28.1%).
- TUS_{1cm}; 71 healthy calves (30.7%) and 160 BP calves (69.3%).
- TUS_{3cm}; 118 healthy calves (51.1%) and 113 BP calves (48.9%).
- The distribution of calves based on the combination of TUS and clinical scoring was:
- $WISC_5/TUS_{1cm}$; 60 healthy calves (26%), 11 upper respiratory tract infection calves (4.8%), 106 subclinical BP calves (45.8%), and 54 clinical BP calves (23.4%).
- $WISC_5/TUS_{3cm}$; 91 healthy calves (39.4%), 27 upper respiratory tract infection calves (11.7%), 75 subclinical BP calves (32.5%), and 38 clinical BP calves (16.5%).

For each group, the median values of arterial blood gas variables, acid-base status, and metabolic variables are reported in Supplementary Table 1.

TABLE 1 Differences in arterial blood gas variables, acid-base status, and metabolic variables in 231 preweaned dairy calves with or without bronchopneumonia (BP), diagnosed by the Wisconsin scoring system and 2 thoracic ultrasound cutoffs. Only variables analyzed with the Mann-Whitney test that were statistically different are reported as a median (25th and the 75th percentile).

	Reference range[23,29]	Median healthy calves	Median BP calves	*P*
$WISC_5$ (166 healthy calves; 65 BP calves)				
Blood sodium concentration	(132-152 mmol/L)	137 (136-139)	136 (135-138)	.02
Blood glucose concentration	(2.5-6 mmol/L)	5.6 (4.8-6.4)	5.4 (4.2-5.9)	.02
Blood urea concentration	(≤5 mmol/L)	2.2 (1.7-3.2)	2.6 (2.1-3.5)	.009
TUS_{1cm} (71 healthy calves; 160 BP calves)				
Alveolar-arterial difference for oxygen	(10-15 mm Hg)	12.1 (5.3-19.4)	14.4 (5.9-26.6)	.04
Blood sodium concentration	(132-152 mmol/L)	138 (137-139)	137 (135.7-138)	.02
Blood potassium concentration	(3.9-5.8 mmol/L)	4.1 (3.8-4.3)	4.2 (4-4.4)	.007
Anion gap	(9-16 mmol/L)	15.6 (13.6-17.3)	13.9 (12.4-16)	<.001
Blood glucose concentration	(2.5-6 mmol/L)	5.8 (5.5-6.6)	5.2 (4.5-6.1)	<.001
Strong ion difference (mmol/L)	(n/a)	44.6 (42.3-46.9)	43.2 (40.8-45.8)	.016
Unidentified strong ions (mmol/L)	(−2 to 0 mmol/L)	−2.8 (−4.9 to 1.1)	−0.95 (−3 to 0.9)	<.001
Strong ion gap	(−3 to 3 mmol/L)	−2.6 (−4.4 to 0.7)	−0.9 (−3 to 1.2)	<.001
Paraoxonase-1	(>63.2 U/mL)[a]	77.3 (61.9-96.5)	64.1 (40.8-78)	<.001
TUS_{3cm} (118 healthy calves; 113 BP calves)				
Blood sodium concentration (mmol/L)	(132-152 mmol/L)	137 (136-139)	137 (135-138)	<.001
Blood potassium concentration (mmol/L)	(3.9-5.8 mmol/L)	4.1 (3.9-4.3)	4.2 (4-4.4)	.04
Blood glucose concentration (mmol/L)	(2.5-6 mmol/L)	5.7 (5.2-6.3)	5.1 (4.4-6.1)	<.001
Creatinine	(0.5-1.2 mg/dL)	0.9 (0.7-1.1)	0.8 (0.7-1)	.02
Unidentified strong ions (mmol/L)	(−2 to 0 mmol/L)	−1.7 (−4.1 to 0.1)	−1 (−3.1 to 0.9)	.03
Paraoxonase-1	(>63.2 U/mL)[a]	72.9 (53.4-95.4)	59.2 (37.7-72.4)	<.001

Note: $WISC_5$ = Healthy calves defined as the total score of <5 by the Wisconsin respiratory scoring system; BP calves defined as the total score of ≥5. TUS_{1cm} = Healthy calves defined as such in the absence of lung consolidation; BP calves defined by the presence of consolidation at least 1 site with depth ≥1 cm. TUS_{3cm} = Healthy calves defined as such in the absence of lung consolidation; BP calves defined by the presence of consolidation at least 1 site with depth ≥3 cm.

[a]Age matched reference interval generated by our laboratory according to the ASVCP guidelines for the determination of de novo reference intervals.[38]

Significant differences between healthy and BP-affected calves categorized with the WISC score and TUS cutoffs are reported in Table 1. The variables that exhibited statistically significant differences between healthy and sick calves, as classified with $WISC_5$, included Na^+, blood glucose, and blood urea. When categorizing animals with TUS_{1cm}, the statistically different variables among healthy and sick calves included A-aO_2, Na^+, K^+, AG, blood glucose, SID_5, USI, SIG, and PON-1. On the contrary, when using TUS_{3cm}, the statistically different variables were Na^+, K^+, blood glucose, blood creatinine, USI, and PON-1.

Table 2 reports significant differences found in blood variables between healthy, upper respiratory tract infection, subclinical BP, and clinical BP calves categorized with the combination of WISC and TUS cutoffs and the results of multiple combinations. The analysis conducted with the Kruskal-Wallis test reveals that with the classification using $WISC_5 + TUS_{1cm}$, the variables that exhibited statistically significant differences were Na^+, K^+, AG, blood glucose, blood urea, USI, SIG, and PON-1. However, with the classification using $WISC_5 + TUS_{3cm}$, only the variables Na^+, blood glucose, blood urea, and PON-1 showed statistically significant differences.

4 | DISCUSSION

A major finding of this study indicates that BP detected by the TUS and WISC scoring system was characterized by disturbances that mostly had clinically irrelevant effects on measured variables. Blood glucose concentration, SIG, USI, and PON-1 were the only biochemical variables associated with lung consolidation diagnosed by TUS. In $WISC_5/TUS_{1cm}$ and $WISC_5/TUS_{3cm}$ categorizations, there was a clear difference between calves with and without lung consolidations detected by TUS, although, the abnormalities found were usually not beyond the normal reference ranges (see Table 2). Overall, these results suggest that metabolic changes during an episode of BP diagnosed with WISC score or by TUS set at ≥1 or ≥3 cm were less critical than other disease conditions characterized by severe disarrangements, such as abdominal surgical emergencies and diarrhea in calves[23,25] or severe mastitis and acute diarrhea in goats,[26] which were characterized by clinicopathologic imbalances and alterations in acid-base status exceeding the reference ranges. Our results agree with previous observations,[10] which showed that in calves with BP the overall effects on base-acid balance were of mild to moderate magnitude.

TABLE 2 Results of the Kruskal Wallis test and multiple comparisons of arterial blood gas variables, acid-base status, and metabolic variables between combinations of the Wisconsin scoring system and 2 thoracic ultrasound cutoffs to detect bronchopneumonia (BP) in 231 preweaned Holstein-Friesian calves. Only variables that were statistically different ($P < .05$) are reported as a median (25th and the 75th percentile).

Categorizations	Blood sodium (mmol/L)	Blood potassium (mmol/L)	Anion gap (mmol/L)	Blood glucose (mmol/L)	Blood urea (mmol/L)	Unidentified strong ions (mmol/L)	Strong ion gap (mmol/L)	Paraoxonase-1 (U/mL)
$WISC_5/TUS_{1cm}$								
Kruskal Wallis test	$P < .001$	$P = .03$	$P = .006$	$P < .001$	$P = .02$	$P = .002$	$P = .01$	$P < .001$
Healthy $WISC_{5-}$ TUS_{1cm-} (60 calves)	138 (137-139)[a]	4 (3.8-4.2)[a]	15.6 (13.7-17.3)[a]	5.9 (5.4-6.6)[a]	2.3 (1.6-3.2)[ab]	−2.7 (−4.9 to −1.1)[a]	−2.6 (−4.4 to −0.7)[a]	77.2 (61.9-102.2)[a]
Upper respiratory tract infection $WISC_{5+}$ TUS_{1cm-} (11 calves)	137 (136-138)[ab]	4.1 (3.9-4.3)[ab]	16.1 (12-17.4)[ab]	5.7 (5.5-6.3)[ab]	2.1 (2-3.3)[ab]	−3.5 (−5.3 to −0.3)[ab]	−3.1 (−4.7 to 0.5)[ab]	81.4 (58.8-95.1)[ab]
Subclinical BP $WISC_{5-}$ TUS_{1cm+} (106 calves)	137 (136-138) [b]	4.2 (4-4.4)[b]	13.8 (12.6-16)[b]	5.3 (4.6-6.3)[b]	2.2 (1.8-3.2)[a]	−1 (−2.7 to 0.6)[b]	−0.7 (−2.9 to 0.8)[b]	58.7 (39.1-75.4)[b]
Clinical BP $WISC_{5+}$ TUS_{1cm+} (54 calves)	136 (135-138) [b]	4.1 (4-4.4)[ab]	14.6 (12.2-16)[b]	5.2 (4.1-5.9)[b]	2.7 (2.3-3.2)[b]	−1.1 (−3.4 to 1.5)[b]	−1.3 (−3.2 to 1.6)[b]	64.9 (46-83.5)[ab]
$WISC_5/TUS_{3cm}$								
Kruskal Wallis test	$P = .001$			$P < .001$	$P = .02$			$P < .001$
Healthy $WISC_{5-}$ TUS_{3cm-} (91 calves)	138 (137-139)[a]	/	/	5.7 (5.1-6.5)[a]	2.2 (1.6-3.2)[a]	/	/	73.5 (52.1-94.4)[a]
Upper respiratory tract infection $WISC_{5+}$ TUS_{3cm-} (37 calves)	137 (136-138)[ab]	/	/	5.8 (5.5-6.2)[a]	2.4 (2-3.3)[ab]	/	/	70.6 (58.1-97.9)[a]
Subclinical BP $WISC_{5-}$ $TUS3_{cm+}$ (75 calves)	137 (135-138)[b]	/	/	5.2 (4.5-6.4)[b]	2.3 (1.8-3.6)[ab]	/	/	57.1 (36.8-70.3)[b]
Clinical BP $WISC_{5+}$ TUS_{3cm+} (38 calves)	136 (134.5-138)[b]	/	/	5 (3.7-5.5)[b]	2.7 (2.4-4)[b]	/	/	62.5 (39.9-75.5)[ab]

Note: Categories with different superscript letters (a, b) were statistically different from each other ($P < .05$). In contrast, those with the same superscript letters were not statistically different from each other ($P > .05$). $WISC_{5+}$ = positive, defined as the total score of ≥5 by the Wisconsin respiratory scoring system; $WISC_{5-}$ = negative, defined as the total score of <5. TUS_{1cm+}= positive, defined by the presence of consolidation at least 1 site with depth ≥1 cm by thoracic ultrasound; TUS_{1cm-} = negative, represented by the absence of consolidations with depth ≥1 cm by thoracic ultrasound. TUS_{3cm+} = positive, defined by the presence of consolidation at least 1 site with depth ≥3 cm by thoracic ultrasound; TUS_{3cm-} = negative, represented by the absence of consolidations with depth ≥3 cm by thoracic ultrasound.

Increasing severity of the studied variables based on classifying calves into healthy, upper respiratory tract infection, subclinical BP, and clinical BP was not detected. A statistical difference was found in blood glucose concentration, PON-1, SIG, and USI among calves with or without an ultrasonographic lesions. However, we found no progressive alterations among the TUS-positive calves with concurrent clinical signs. Similarly, our results indicated that the abnormalities found in the 2 ultrasonographic cutoffs investigated showed no marked increase in severity in ≥3 cm lesions compared with ≥1 cm lesions.

Although the diagnosis of BP revealed metabolic changes that were generally not substantial, the fact that there were few or no differences between healthy and diseased calves is interesting.

A comparison of our results with those of other studies[27] confirms that blood glucose concentrations tend to be lower in calves with clinical BP than in healthy calves. Relative hypoglycemia in affected calves was associated with secondary bacterial infection.[28] Regarding the evaluation of the combination of WISC and TUS, the decrease in glucose concentration was significant in calves with ultrasonographic lesions compared with those without such lesions. However, no significant difference was found between calves with clinical BP and those with subclinical BP.

Evaluating SIG and USI represents the logical extension of the AG concept of the traditional H-H approach.[29] In disease conditions characterized by pronounced metabolic changes, negative values for the calculated USI and SIG indicate the presence of unidentified anions often correlate with negative outcomes of the disease.[23,25,30,31] Median USI and SIG values in our study were within the normal limits for healthy and diseased calves diagnosed with TUS and in groups based on categorizing TUS and WISC score. The statistical differences were found in calves with ultrasonographic lung consolidations, which paradoxically had higher mean values than healthy calves. One possible explanation for this result could be related to the increase in the A_{TOT} value because of hyperglobulinemia, which is typical of the spontaneous immune response during the postexposure phase of the causative agent of BP.[10]

Paraoxonase-1 was lower in calves diagnosed with BP using TUS. This result agrees with previous findings showing low PON-1 activity in calves with aspiration pneumonia.[15] Our data indicate that PON-1 was lower using a ≥3 cm cutoff than ≥1 cm. This result appeared most evident among the variables analyzed in our study. Similar findings were observed in other species,[32] in which acute phase proteins were positively correlated with the increased severity of pneumonia assessed by thoracic radiography. As observed for sSID variables and blood glucose concentration, when the combined classification between TUS and clinical signs was considered, no statistically significant differences were observed between calves with clinical and subclinical BP. This highlights that the severity of the inflammatory process and the subsequent oxidative reactions were greater in calves diagnosed with TUS and that the presence or absence of clinical signs was not of particular importance in discriminating the severity of the ongoing inflammatory process.

Another finding of this study was that none of the classifications used corresponded to alterations in H-H acid-base respiratory variables, L-lactate concentration, and among all oxygenation variables used in this study, only A-aO_2 was slightly different between healthy and diseased calves diagnosed using TUS_{1cm}. Nevertheless, the median values of the diseased animals remained within normal ranges, making this statistical significance biologically negligible. Likewise, SID_5 and A_{TOT} also showed marginal importance.

Studies suggest that blood oxygenation values and respiratory acidosis represent good predictors of BP's severity.[8,9,33,34] A series of events related to the severity of lung disease, such as hypercapnia accompanied by increased HCO_3^- concentration, hyperalbuminemia, hypernatremia, or hypochloremia change the metabolic aspect of the sSID approach.[12] It is difficult to compare our results with those of other studies because the latter were performed using experimental inoculation[8,10] or calves with chronic BP often affected by life-threatening conditions.[33,34] The discrepancy between our results and those of other previously published studies could be attributed to the disparity in the nature and distribution of lung lesions induced by the experimental inoculation of the pathogens and the chronic conditions of the disease. For example, the inoculation of the respiratory syncytial virus caused lesions in approximately 24% of the lung mass, which severely affected gas exchanges during the acute phase of the postinfection disease.[10] In beef calves, L-lactate concentration higher than 4 to 5 mmol/L is a good predictor of death and was used to assess BP severity.[9,35] Similarly, the concentration of L-lactate is higher in calves exposed to the highest intrabronchial dose of *Chlamydia psittaci*, highlighting that the increase in this metabolite occurs in severe infection. The discrepancy between our results and those of other published studies could be attributed to the naturally occurring infection of our study sample with presumably less impactful pathogens that consequently might have involved a smaller lung mass in the pathological process. These findings are similar to those of other studies,[36] which failed to detect hypoxemia and hypercapnia after endoscopic inoculation of *Mannheimia haemolytica*.

Our results represent a snapshot of a study sample's health status over a specific period. Given that it is a cross-sectional study it is potentially subject to bias, particularly because of the absence of a temporal relationship between putative etiologic exposures and the outcomes under investigation. Adding incident cases evaluated over an extended period could have yielded different results. In addition, the lack of knowledge of the pathogens responsible for the disease could have biased the results of this study because of the possible infection of low-pathogenic viruses and bacteria that might have caused modest changes in the variables investigated. Selection and information bias could have distorted the results, which is common when convenience samples are used. We tried to mitigate these limitations by adopting validated methods to measure metabolic variables from calves classified by standardized methods of clinical scoring and TUS examination performed by experienced veterinarians. In addition, using a 1-gate design, which usually includes doubtful cases returning results generally closer to what would be expected in a practical context,[37] could have mitigated the selection bias. Unfortunately, the 1-gate design did not enable us to have a uniform number of calves within the categorizations used. This was especially true for the calves of the upper respiratory tract infection group, which were in

lower numbers than the other groups. Therefore, the results from these calves need to be interpreted with caution.

In conclusion, the categorization of calves based on ultrasound lesions, rather than on clinical signs alone, appears to be more effective in describing disease severity based on impaired glucose metabolism, relative changes in metabolic compounds of the on sSID approach, and decreased PON-1 activity because of inflammation of lung disease. These results highlight that clinical signs were indicative of minor systemic disorders when considered alone for diagnosis. The disorders did not cause further exacerbation in the presence of a concomitant ultrasound lesion, probably because they often indicate milder inflammatory phenomena involving the upper airways,[37] which are unlikely to be associated with pneumonia.[16] It is crucial to consider that the changes assessed in this study were moderate. The absence of disturbances related to hypoxemia, respiratory acidosis, and increased anaerobic metabolism highlights that ultrasonic lesions of 1 to ≥3 cm were unable to produce significant systemic changes.

ACKNOWLEDGMENT

The authors acknowledge the support of the APC central fund of the University of Milan.

CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Residual blood samples collected for clinical screening of ill animals were employed following the University of Milan's ethics committee guidelines to re-use collected samples (approval number 2/16). All calves included in the study were managed according to standard protocols for diagnosing bronchopneumonia in compliance with the professional ethics of veterinarians and the standards for protecting calves. The publication of data from the routine extramural clinical activity of the Ruminant and Swine Clinic of the University of Milan was approved by the Ethics Committee of the University of Milan (approval number 47/2017, November 28, 2017).

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

ORCID

Antonio Boccardo https://orcid.org/0000-0003-0051-6990
Giulia Sala https://orcid.org/0000-0002-8847-5531
Saverio Paltrinieri https://orcid.org/0000-0001-7117-7987
Davide Pravettoni https://orcid.org/0000-0003-3338-6053

REFERENCES

1. Buczinski S, Ollivett TL, Dendukuri N. Bayesian estimation of the accuracy of the calf respiratory scoring chart and ultrasonography for the diagnosis of bovine respiratory disease in pre-weaned dairy calves. *Prev Vet Med*. 2015;119(3–4):227-231.
2. Berman J, Francoz D, Dufour S, Buczinski S. Bayesian estimation of sensitivity and specificity of systematic thoracic ultrasound exam for diagnosis of bovine respiratory disease in pre-weaned calves. *Prev Vet Med*. 2019;162:38-45.
3. Cramer MC, Ollivett TL. Growth of preweaned, group-housed dairy calves diagnosed with respiratory disease using clinical respiratory scoring and thoracic ultrasound-a cohort study. *J Dairy Sci*. 2019;102(5):4322-4331.
4. Buczinski S, Faure C, Jolivet S, Abdallah A. Evaluation of inter-observer agreement when using a clinical respiratory scoring system in pre-weaned dairy calves. *N Z Vet J*. 2016;64(4):243-247.
5. Schiller I, van Smeden M, Hadgu A, Libman M, Reitsma JB, Dendukuri N. Bias due to composite reference standards in diagnostic accuracy studies. *Stat Med*. 2016;35(9):1454-1470.
6. Jourquin S, Bokma J, De Cremer L, et al. Randomized field trial comparing the efficacy of florfenicol and oxytetracycline in a natural outbreak of calf pneumonia using lung reaeration as a cure criterion. *J Vet Intern Med*. 2022;36(2):820-828.
7. Denis-Robichaud J, Cléroux MÈT, Buczinski S, et al. Assessment of the evolution of the proportion of respiratory and enteric pathogens and diseases in pre-weaned unvaccinated dairy heifers from Québec. *Can Bov Pract*. 2021;55(2):140-152.
8. Ellis J, Waldner C, Gow S, Jackson M. Relationship of the extent of pulmonary lesions to the partial pressure of oxygen and the lactate concentration in arterial blood in calves experimentally infected with bovine respiratory syncytial virus. *Can J Vet Res*. 2013;77(3):205-210.
9. Buczinski S, Rademacher RD, Tripp HM, Edmonds M, Johnson EG, Dufour S. Assessment of L-lactatemia as a predictor of respiratory disease recognition and severity in feedlot steers. *Prev Vet Med*. 2015;118(4):306-318.
10. Ostermann C, Linde S, Siegling-Vlitakis C, et al. Evaluation of pulmonary dysfunctions and acid-base imbalances induced by *Chlamydia psittaci* in a bovine model of respiratory infection. *Multidiscip Respir Med*. 2014;9(1):1-16.
11. Reinhold P, Hartmann H, Constable PD. Characterisation of acid-base abnormalities in pigs experimentally infected with *Chlamydia suis*. *Vet J*. 2010;184(2):212-218.
12. Niedzwiedz A, Slawuta P, Zak A, et al. Changes in the SID_{Actual} and $SID_{Effective}$ values in the course of respiratory acidosis in horses with symptomatic severe equine asthma—an experimental study. *J Equine Vet*. 2019;78:107-111.
13. Giordano A, Veronesi MC, Rossi G, et al. Serum paraoxonase-1 activity in neonatal calves: age related variations and comparison between healthy and sick animals. *Vet J*. 2013;197(2):499-501.
14. Feingold KR, Memon RA, Moser AH, Grunfeld C. Paraoxonase activity in the serum and hepatic mRNA levels decrease during the acute phase response. *Atherosclerosis*. 1998;139(2):307-315.
15. Akyüz E, Merhan O, Aydın U, et al. Neopterin, procalcitonin, total sialic acid, paraoxonase-1 and selected haematological indices in calves with aspiration pneumonia. *Acta Vet Brno*. 2022;91(2):115-124.
16. Lowie T, Van Leenen K, Jourquin S, et al. Differences in the association of cough and other clinical signs with ultrasonographic lung consolidation in dairy, veal, and beef calves. *J Dairy Sci*. 2022;105:6111-6124.
17. Bielmann V, Gillan J, Perkins NR, Skidmore AL, Godden S, Leslie KE. An evaluation of Brix refractometry instruments for measurement of colostrum quality in dairy cattle. *J Dairy Sci*. 2010;93(8):3713-3721.
18. Bleul U, Lejeune B, Schwantag S, Kähn W. Blood gas and acid-base analysis of arterial blood in 57 newborn calves. *Vet Rec*. 2007;161(20):688-691.
19. Coenen MC, Gille L, Eppe J, et al. Blood inflammatory, hydro-electrolytes and acid-base changes in Belgian blue cows developing

parietal fibrinous peritonitis or generalised peritonitis after caesarean section. *Vet Sci*. 2022;9(3):134.
20. Ro Y, Choi W, Hong L, Kim E, Choe E, Kim D. Comparison of the bovine blood gas parameters produced with three types of portable blood gas analyzers. *J Vet Sci*. 2022;23(4):e60.
21. McGuirk SM, Peek SF. Timely diagnosis of dairy calf respiratory disease using a standardized scoring system. *Anim Health Res Rev*. 2014; 15:145-147.
22. Ollivett TL, Caswell JL, Nydam DV, et al. Thoracic ultrasonography and bronchoalveolar lavage fluid analysis in Holstein calves with subclinical lung lesions. *J Vet Intern Med*. 2015;29(6):1728-1734.
23. Trefz FM, Lausch C, Rieger A, et al. Acid-base imbalances and the association of blood-gas variables, electrolytes, and biochemical analytes with outcome in hospitalized calves undergoing abdominal surgery. *J Vet Intern Med*. 2023;37(2):740-756.
24. Constable PD, Stämpfli HR, Navetat H, Berchtold J, Schelcher F. Use of a quantitative strong ion approach to determine the mechanism for acid-base abnormalities in sick calves with or without diarrhea. *J Vet Intern Med*. 2005;19(4):581-589.
25. Trefz FM, Constable PD, Lorenz I. Quantitative physicochemical analysis of acid-base balance and clinical utility of anion gap and strong ion gap in 806 neonatal calves with diarrhea. *J Vet Intern Med*. 2015; 29(2):678-687.
26. Gomez DE, Bedford S, Darby S, Palmisano M, MacKay RJ, Renaud DL. Acid-base disorders in sick goats and their association with mortality: a simplified strong ion difference approach. *J Vet Intern Med*. 2020;34(6):2776-2786.
27. Blakebrough-Hall C, Dona A, D'occhio MJ, et al. Diagnosis of bovine respiratory disease in feedlot cattle using blood 1H NMR metabolomics. *Sci Rep*. 2020;10(1):1-12.
28. Santos-Rivera M, Fitzkee NC, Hill RA, et al. NMR-based metabolomics of plasma from dairy calves infected with 2 primary causal agents of bovine respiratory disease (BRD). *Sci Rep*. 2023;13(1): 2671.
29. Constable PD. Clinical assessment of acid-base status: comparison of the Henderson-Hasselbalch and strong ion approaches. *Vet Clin Pathol*. 2000;29(4):115-128.
30. Gomez DE, Lofstedt J, Arroyo LG, et al. Association of unmeasured strong ions with outcome of hospitalized beef and dairy diarrheic calves. *Can Vet J*. 2017;58(10):1086-1092.
31. Moviat MA, Pickkers P, Ruitenbeek W, et al. The nature of unmeasured anions in critically ill patients. *Crit Care*. 2008;12(2):1.
32. Menard J, Porter I, Lerer A, Robbins S, Johnson PJ, Goggs R. Serial evaluation of thoracic radiographs and acute phase proteins in dogs with pneumonia. *J Vet Intern Med*. 2022;36:1430-1443.
33. Nagy O, Seidel H, Paulikova I, et al. Use of blood gases and lactic acid analyses in diagnosis and prognosis of respiratory diseases in calves. *Bull Vet Inst Pulawy*. 2006;50(2):149.
34. Šoltésová H, Nagy O, Tóthová C, et al. Blood gases, acid-base status and plasma lactate concentrations in calves with respiratory diseases. *Acta Vet Beogr*. 2015;65(1):111-124.
35. Coghe J, Uystepruyst CH, Bureau F, et al. Validation and prognostic value of plasma lactate measurement in bovine respiratory disease. *Vet J*. 2000;160(2):139-146.
36. Hanzlicek GA, White BJ, Mosier D, Renter DG, Anderson DE. Serial evaluation of physiologic, pathological, and behavioral changes related to disease progression of experimentally induced *Mannheimia haemolytica* pneumonia in postweaned calves. *Am J Vet Res*. 2010; 71(3):359-369.
37. Buczinski S, Pardon B. Bovine respiratory disease diagnosis: what progress has been made in clinical diagnosis? *Vet Clin North Am Food Anim Pract*. 2020;36:399-423.
38. Friedrichs KR, Harr KE, Freeman KP, et al. ASVCP reference interval guidelines: determination of de novo reference intervals in veterinary species and other related topics. *Vet Clin Pathol*. 2012;41(4):441-453.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Boccardo A, Ferrulli V, Sala G, et al. Blood gases, acid-base, and metabolic alterations in calves with bronchopneumonia diagnosed via clinical signs and thoracic ultrasonography: A cross-sectional study. *J Vet Intern Med*. 2024;1-9. doi:10.1111/jvim.17039