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An on-farm observational study on the prevalence and associated factors of bacteremia in preweaned dairy calves diagnosed with bronchopneumonia by thoracic ultrasonography

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Abstract

Background Bacteremia is a potential systemic complication of bronchopneumonia (BP) in dairy calves, which increases the risk of sepsis and mortality. However, data on bacteremia in farm conditions is still limited. This study investigates the prevalence of bacteremia in calves with BP on farms, examining isolated pathogens and the associations between thoracic ultrasonography (TUS) and non-endoscopic bronchoalveolar lavage (nBAL) findings.

Results The study enclosed 13 dairy farms and included 211 eligible preweaned dairy calves, of which 88 were diagnosed with BP based on a highly sensitive threshold of ≥ 1 cm for lung consolidation detected by TUS. The affected calves underwent non-endoscopic bronchoalveolar lavage (nBAL) and blood culture procedures. Blood culture results showed a positivity rate of 6.8%, identifying *Salmonella* Dublin in five cases and *Campylobacter fetus* in one case. Twenty-four (27.2%) blood samples grew presumed bacterial contaminants, while 58 (65.9%) samples had no growth. In contrast, nBAL samples revealed a 75% positivity rate, with *Pasteurella multocida* and *Mycoplasma bovis* being the most frequently identified pathogens. No associations were observed between TUS-detected lung lesions and bacteremia. Notably, BP pathogens were not identified in blood cultures, except for one instance where *Salmonella* Dublin was detected in the nBAL and blood culture.

Conclusions The study indicates a low prevalence of bacteremia in dairy calves with BP diagnosed through TUS, suggesting that recommending treatment or revisions in disease management related to potential bacteremia in these patients may not be warranted. The findings imply that lung lesions detected via TUS may occur independently of bacteremia, highlighting the value of TUS for early diagnosing and monitoring BP in field conditions.

Keywords Calves, Bronchopneumonia, Blood culture, Thoracic ultrasonography, Bronchoalveolar lavage

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Background

Suppurative bronchopneumonia (BP) is the most common form of lower tract infection of the respiratory disease complex in young dairy calves [1]. Two significant factors contribute to the occurrence of BP in calves: first, it is a multifactorial disorder with a complex interaction between infectious agents, external stressors, and the immune system that strongly influence the individual development and progression of the disease [2], secondly, the lack of a 100% accurate gold standard for the diagnosis that contributes to complicate the classification of the individual case and, consequently, the decision between conditions requiring treatment or not [3]. Inaccurate diagnosis, combined with intricate individual responses related to different herd characteristics and epidemiological pressures faced by calves, can result in extensive variation in the distribution of this disease, treatment strategies, and their effectiveness worldwide [4, 5]. Bronchopneumonia poses a significant welfare concern in the cattle industry due to the discomfort experienced by affected animals, reduced production performance, increased culling, mortality rates, and use of antimicrobials [6–9]. In recent years, data from several studies suggest that the use of thoracic ultrasonography (TUS) [10], reevaluation of the diagnostic value of some BP-related clinical signs [11, 12], and more functional on-farm techniques for lower and upper airway sampling [13, 14] could improve the diagnostic accuracy and etiologic interpretation of BP-related pathogens, thus potentially increasing therapy and disease-management accuracy.

However, significant uncertainty persists regarding the systemic involvement associated with BP episodes, which could compromise outcomes or necessitate more rigorous therapies or revisions in disease management. Although BP episodes have been linked to health disorders affecting current health status and future productivity, the pathological mechanisms underlying these abnormalities have not yet been fully elucidated. The literature reveals that the impact of this disease on the overall health of affected calves varies considerably depending on the types of animals involved and the specific contexts examined. For example, in critically ill calves, BP is an adverse prognostic indicator [15] or indicates a condition involving significant systemic implications [16]. In contrast, recent studies have shown that calves diagnosed with TUS during field BP outbreaks display only moderate metabolic disturbances and alterations in arterial blood gases and acid-base balance [17].

Among systematic involvement, bacteremia could be one of the most impactful systemic changes during infectious diseases. The presence of bacteria in the bloodstream can lead to sepsis, which is a life-threatening multi-organ dysfunction caused by a dysregulated host response to infection and is often associated with higher mortality [18]. Guidelines from human medicine emphasize the importance of rapid and appropriate bactericidal antimicrobial treatment to enhance survival in bacteremic patients [19]. The available body of research on calves is related to critically ill animals in hospital settings and indicates that the presence of bacteremia ranged from 31 to 40.7% [15, 20–23]. According to recent data, ≥ 1 cm-TUS-diagnosed BP may be a relevant risk factor for bacteremia, with 43.5 to 44.1% positive cases for blood culture [15]. Similarly, other studies showed that BP is one of the leading causes of secondary bacteremia for many species [24].

There is currently no data on the prevalence of bacteremia in calves with BP within a farm setting, and, in general, few studies have investigated bacteremia in calves experiencing naturally occurring diseases under field conditions. Similarly, the association between TUS lesions' severity and the possible association between the pathological agents responsible for lung infection and bacteria found through blood culture need to be addressed. By studying these associations during naturally occurring on-farm BP episodes, we aim to offer new perspectives into the systemic changes in affected calves without signs of critical illness. This will lead to a deeper understanding of the disease's impact on animal health in farm conditions where producers and practitioners are tasked with making routine decisions about initiating therapy and where BP results in significant economic and health consequences. Thus, in this study, our objectives were: (i) to determine the percentage of pre-weaned dairy calves with BP that develop bacteremia, (ii) to describe the pathogens identified in blood culture, and (iii) to investigate the association of BP detected with TUS cut-offs (≥ 1 and ≥ 3 cm) and etiological analyses from non-endoscopic bronchoalveolar lavage (nBAL) with the occurrence of bacteremia in calves from commercial dairy farms in Northern Italy.

We hypothesize that bacteremia occurs less frequently during field BP episodes than in hospital conditions. Additionally, we expect that calves with ≥ 3 cm lesions were more likely to have a higher prevalence of bacteremia than those with less severe lesions.

Methods

Study design and ethics approval

Following strengthening the reporting of observational studies in epidemiology guidelines (STROBE), we conducted an observational study without a negative control group. The study protocol received approval from the University of Milan's institutional animal welfare organization (approval number 59_2024).

Setting

This study used a convenience sample of farms that sought intervention from our mobile clinic due to reported coughing in preweaned calves within the prior 15 days. Data collection occurred across multiple farms equipped with automatic feeders between March and May 2024. The selected farms were chosen in collaboration with local practitioners based on their willingness to participate and proximity to the Clinics for Ruminant and Swine at the Department of Veterinary Medicine and Animal Science, University of Milan. These farms represented the typical milk production systems of the Po Valley, with milking herds consisting of 100–250 lactating cows. Calves were separated from their dams immediately after birth and were provided with 4 to 6 L of colostrum within 6 to 8 h. Initially, calves were housed individually for 15 to 20 days. They fed a milk replacer before being moved to group pens (each containing 10 to 20 calves) with an automated calf feeder, where they remained for 75 to 95 days until weaning.

Sample size calculation

We conducted a logistic regression analysis to determine the required number of calves for our study, focusing on the dichotomous dependent variable of bacteremia presence or absence in calves diagnosed with BP. The probability of developing bacteremia was the dependent variable in this context, as our study exclusively involved sick animals. Our sample size calculation was based on the prevalence of bacteremia in calves with BP, as reported by Pas et al. [15], which was found to be 43.5%. During the planning phase, we knew this prevalence might be higher than observed under field conditions. However, these data were the only available in the literature using ≥ 1 cm TUS-highlighted consolidation as a diagnostic criterion for BP classification. To address this potential discrepancy, we estimated that the prevalence in field conditions would be approximately half, at around 21.7%. With a type I α error probability set at 5%, a 95% confidence interval, and 80% statistical power, we calculated that a minimum of 75 calves would be necessary for our study. Given the limited data on bacteremia prevalence in calves with on-farm BP and to enhance our chances of identifying associated factors, we decided to enroll an additional 10% of calves beyond the calculated sample size. Consequently, the total number of calves enrolled was at least 83.

Participants

On the enrolled farms, we examined all calves in one preweaning pen. We selected the most suitable pen for the study when multiple pens were available in larger herds. Two assistants on each farm were tasked with capturing the calves. Only purebred Holstein Friesian calves were considered eligible for the study unless they displayed signs of lameness, cachexia, dehydration, diarrhea, or umbilical disease or if they had received antimicrobial treatments within the prior 15 days, which were established as exclusion criteria. To assess the clinical conditions of the animals, the first author (AB) conducted an initial group evaluation to exclude any that showed apparent signs of disease, such as visible umbilical swellings or severe lameness indicated by an inability to bear weight. After capturing the animals, the same author checked for joint swellings, palpated the umbilical region and related intra-abdominal structures, and examined the body condition score and the spine of the scapula to assess muscular masses. Following rectal temperature assessment, an assistant stimulated defecation to evaluate fecal scores, according to Renaud et al. [25]. The calves underwent TUS and a clinical examination. Those with ultrasonographic lung consolidation lesions measuring ≥ 1 cm were included in the study for nBAL and blood culture.

Clinical measurements

To select calves for nBAL and sterile blood sampling to evaluate bacteremia, we established a sampling scheme (gate) specifically designed to target only diseased calves while excluding those unaffected by BP. Accordingly, we utilized the single-gate reverse flow design as previously described [26] employing TUS as the diagnostic reference standard.

Bilateral TUS was conducted by the first author (AB), targeting intercostal spaces (ICS) 10 – 1 on the right side and ICS 10 – 2 on the left, following the ventral landmarks outlined in previous studies [27]. The ultrasound was performed using a portable unit (Draminski Blue, Draminski Ultrasound Scanners, Ząbruk, Poland) equipped with a 7.5 MHz rectal transducer and set to a depth of 8 cm. A transducing agent consisting of 70% isopropyl alcohol was applied to each ICS. The classification of ultrasonography was based on the volume of lung tissue involved, with scores ranging from 0 to 2; 0 indicated no lesions or less than 1 cm consolidation with comet tail artifacts; 1 indicated patchy lesions or consolidation of ≥ 1 cm but < 3 cm amid normally aerated lung parenchyma or diffuse comet tail artifacts, and 2 indicated lung consolidation depth of ≥ 3 cm. For each ICS, the maximum score was recorded. The maximal depth of lung consolidation (in cm) was determined by manually counting using the ultrasound image's lateral grid. In cases of uncertainty or near-cutoff measurements, the measurement tool integrated into the ultrasound scanner was utilized. Calves with a highly sensitive threshold of ≥ 1 cm (score 1) for lung consolidation were classified as positive and underwent nBAL and blood culture procedures.

In addition to the TUS used as the sole diagnostic tool for defining a BP case in our study sample, another author (GS) performed a clinical examination utilizing the California respiratory scoring chart (CALIF). A dichotomous scoring system was employed to assess the presence or absence of six criteria: eye discharge, nasal discharge, ear droop or head tilt, cough, rectal temperature, and respiratory pattern [28]. Additionally, calves underwent thoracic auscultation (AUSC) following the sound classification system detailed in our recently published guidelines [12]. In summary, auscultation scores ranged from 0 to 4: 0 indicated normal breath sounds, 1 represented increased breath sounds, 2 denoted the presence of wheezes or crackles, 3 indicated increased bronchial sounds and pleural friction rubs characterized 4.

Non-endoscopic bronchoalveolar lavage and blood samples

Calves exhibiting lung consolidation of ≥ 1 cm underwent nBAL using a commercially available kit (Easy Lavage Basis Set, iVet® Innovation Tierwohl). The procedure was performed on standing animals without sedation. First, the nostrils were sanitized with 70% alcohol and dried with a paper towel. A curved nasotracheal tube with an end-site guarded valve was carefully inserted through the nasal cavity, larynx, and into the trachea until the cough reflex was elicited. The calf's head was slightly extended throughout the procedure, and by manually palpating the area, the larynx was positioned dorsally to minimize the risk of swallowing the catheter. The tube was gently advanced into the trachea to the deepest point possible without further insertion. A dedicated catheter was placed until it reached the final valve site (noting slight resistance). At this juncture, the catheter was pushed outside the valve, and a 50 mL syringe, pre-filled with 20 mL of sterile saline [13], was attached for the injection and aspiration of the sample. The validity of the sample was confirmed by the presence of a characteristic foam layer, ensuring that at least 30% of the injected fluid was retrieved. A new sterilized curved tube and catheter were used for each calf. If the re-aspirated sample was less than 30%, an additional 20 mL aliquot was administered. In cases where the curved catheter was swallowed, it was promptly reinserted into the pharynx until it reached the trachea. Samples were immediately transferred into sterile containers, transported in refrigerated bags, and cultured within 4 h following collection.

Two blood samples (morning and afternoon; [20]) were collected from each calf and sent to the laboratory for culture—the first sample was taken after nBAL in the morning and the second during the afternoon of the same study day. Before sampling, the area's hair was clipped with a cordless clipper and then shaved using a disposable razor. The site was subjected to three vigorous scrubbings with povidone-iodine detergent and three with 70% isopropyl alcohol. Jugular venipuncture was performed using a 21G needle and a 20 mL syringe to collect a 10 mL sample. The calves remained standing throughout the procedure and were restrained with a halter. An experienced assistant extended the calf's head to minimize movement during the sampling and facilitate access to the jugular vein. A second needle was employed to inject 5 mL of blood into a commercially available specific vial (Signal Blood Culture System, Thermo Fisher Scientific, Waltham, USA), following disinfection of the rubber stopper with 70% isopropyl alcohol. All procedures were conducted with sterile gloves following the manufacturer's instructions.

Sample handling, microbiological analysis, and interpretation

Signal Blood Culture bottles were incubated at 37 °C, with daily checks for positive results throughout a 7-day incubation period, following the manufacturer's instructions. Whole blood samples, positive blood-culture media, and nBAL fluids were aseptically plated onto various growth media, including blood agar, MacConkey agar, and brain heart infusion agar. These samples were then incubated under microaerophilic (10% CO_2), aerobic, and anaerobic conditions at 37 ± 2 °C, with analyses performed after 24, 48, and 72 h. Bacterial species isolated from the cultures were identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) through the VITEK® system (bioMérieux SA, Marcy-l'Étoile, France). Additionally, all samples were examined for *Mycoplasma* spp. by aseptically plating each sample onto PPLO agar and incubating at 37 ± 2 °C with 10% CO_2 for 7 days. A broth enrichment was also done by inoculating each positive nBAL sample into mammal Mycoplasma liquid medium (Mycoplasma Experience®, Reigate, UK); after a 14-day incubation at 37 ± 1 °C with 5% CO_2, the enriched samples were processed as described in the literature [29]. All Mycoplasmas isolated from bacteriological culture (direct plating and/or broth enrichment) were confirmed with molecular methods, as described below. Molecular analyses were conducted on nBAL samples to detect several pathogens, including Bovine coronavirus (BCoV), Bovine respiratory syncytial virus (BRSV), Bovine viral diarrhea (BVD), Bovine parainfluenza type 3 (BPI-3), Bovine herpesvirus type 1 (BoHV-1), and *Mycoplasma bovis*. RNA and DNA were extracted from each sample using the BioSprint 96 One-For-All Vet Kit (Indical Bioscience GmbH, Leipzig, Germany) on a KingFisher Apex Purification System (Thermo Fisher Scientific, Waltham, USA), following the manufacturer's instructions. One-step real-time RT-PCR analyses were performed to identify BCoV, BRSV, BVD, and BPI-3, adhering to protocols outlined in previous

studies [30–33]. For BoHV-1 detection, a Real-Time PCR assay, as detailed in the literature [34], was utilized. All Real-Time PCR/RT-PCR reactions were conducted on a CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, USA). Based on established methodologies [35], *Mycoplasma bovis* was identified using end-point PCR on a Mastercycler Nexus X2 (Eppendorf SE, Hamburg, Germany). The resulting amplification products were analyzed through capillary electrophoresis using a QIAxcel Advanced Instrument (Qiagen, Hilden, Germany) and the QIAxcel ScreenGel Software (Qiagen, Hilden, Germany). Additionally, all *Mycoplasma* spp. cultures isolated during bacteriological analyses were identified as either *Mycoplasma bovis* or *Mycoplasma arginini* via 16 S rRNA-PCR-DGGE, following established literature [36].

Results from blood cultures were evaluated to distinguish between true pathogenic bacteria and potential integumentary contaminants following two recent studies on calves by Garcia et al. [37] and Pas et al. [22]. True pathogens were identified as bacteria from the *Enterobacteriaceae* family, significant bovine pathogens related to bacterial BP, and pathogens associated with other diseases that exhibit recognized characteristics of causing bacteremia. Common integumentary contaminants previously documented include species from *Staphylococcus*, *Streptococcus*, *Bacillus*, *Micrococcus*, *and Clostridium*, classified as putative integumentary contaminants [22, 37, 38].

Statistical analysis

Statistical analyses were performed with IBM SPSS Statistics v. 29.0.2.0 (IBM Corp. Armonk. NY). Age was reported as the average ± standard deviation (SD) because it is normally distributed (Shapiro-Wilk test). The other categorical variables collected were expressed as frequencies and percentages. Associations between the presence/absence of bacteremia and collected variables were tested with a chi-square test. Statistical significance was set for a $P < 0.05$.

Table 1 Results of bacteriological and virological analyses of Bronchoalveolar lavage samples (nBAL) from calves diagnosed with Bronchopneumonia via thoracic ultrasonography. Eighty-eight evaluations were conducted on calves with lesions > 1 cm, and 66 tests returned positive results

Pathogen	nBAL positive samples ($n = 66$)
Mycoplasma bovis	41
Pasteurella multocida	32
Bovine coronavirus	22
Mannheimia haemolytica	15
Trueperella pyogenes	7
Moraxella spp.	5
Salmonella Dublin	2
Bovine parainfluenza 3 virus	2
Bovine respiratory syncytial virus	1
Bovine viral diarrhea virus	1

Results

During our study, we visited 13 dairy farms and conducted a complete TUS on 211 eligible calves. One hundred and nineteen calves were excluded due to a TUS score 0. Additionally, four enrolled calves were excluded from the nBAL process due to severe behavioral reactions, which included a tendency to fall on the ground during catheter insertion in the nostrils. To prevent unnecessary injury and ensure the welfare of these calves, we decided not to proceed with nBAL in these cases. Ultimately, our final sample consisted of 88 Holstein-Friesian calves with a mean age of 59.51 ± 17.32 days. Among these, 64 (72.7%) were female, while 24 (27.3%) were male. Comprehensive clinical data—including age, farm origin, TUS, clinical findings for all eligible calves, and results from nBAL and blood cultures for those included in the final analysis—can be found in Supplementary Table 1.

Blood culture results revealed that 6 calves (6.8%) tested positive, 58 negatives (65.9%), and 24 (27.2%) tested positive by bacteria assumed to be contaminants.

Among the positive samples, 5 were identified as Salmonella Dublin, 3 of which originated from the same farm, and 1 sample was identified as *Campylobacter fetus*. In the nBAL analysis, 66 samples (75%) were positive, showing coinfections, while 22 (25%) were negative. Pathogens detected in nBAL samples are summarized in Table 1.

Ultrasound findings indicated that 1 (16.7%) had lesions measuring between 1 and 3 cm among the calves with positive blood cultures, while 5 (83.3%) had lesions larger than 3 cm. In contrast, among the calves with negative blood cultures, 19 (23.2%) had lesions between 1 and 3 cm, and 63 (76.8%) had lesions larger than 3 cm. The statistical analysis revealed that positive blood culture results were only associated with the isolation of *Salmonella* Dublin in nBAL (P 0.014). No significant associations were found between blood culture results and primary BRD pathogens ($P = 0.910$) or secondary BRD pathogens ($P = 0.629$), with the differentiation between primary and secondary agents evaluated according to Pardon and Buczinski [3].

Table 2 summarizes TUS findings of calves that tested positive or negative for blood culture.

Supplementary File 2 provides details of the associations between the presence/absence of bacteremia and clinical variables (CALIF and AUSC scores) tested with a chi-square test.

Table 2 Differences in TUS score in calves positive and negative of blood culture. Categorical variables were expressed as frequencies and percentages, and the chi-square test was utilized for underlined differences. Statistical significance was established at $P < 0.05$

Parameters	Score	Blood culture positive calves (*n.* 6)	Blood culture negative calves (*n.* 82)	*P*
TUS	1	1 (16.7%)	19 (23.2%)	0.901
	2	5 (83.3%)	63 (76.8%)	

TUS: thoracic ultrasonography. TUS; thoracic ultrasonography. Score 1 indicated lesions or consolidation depth of ≥ 1 cm but < 3 cm; 2 indicated lung consolidation depth of ≥ 3 cm

Discussion

Our study showed a low prevalence (6.8%, 6/88) of bacteremia in calves diagnosed with BP ultrasonographically on commercial dairy farms. This finding indicates that recommending treatment or revisions in disease management to address potential bacteremia in these patients may not be justified. These results are consistent with those of Garcia et al. [37], who reported a bacteremia prevalence of 9.2% in field diarrheic calves. On the other hand, they differ significantly from the prevalence data observed in hospitalized calves exhibiting ≥ 1 cm lung lesions highlighted by TUS and presenting in critically ill conditions [15, 23]. Such discrepancies may arise because hospitalized calves often display a more advanced and severe stage of the disease. Moreover, critically ill patients may harbor undetected comorbidities, along with TUS-detected lung lesions, or have confirmed conditions such as omphalitis or enteritis that significantly elevate the risk of bacteremia. Another possible explanation for these results may be related to our population's etiology of the infectious disease process. *Enterobacteriaceae* are recognized as the most frequently identified causative agents of bacteremia in calves. In contrast, bacterial agents commonly associated with BP, such as *Pasteurellaceae*, are encountered infrequently, and their role in bacteremia is relatively rare compared to intestinal Gram-negative bacteria [21, 24]. Conversely, other etiological agents typically linked to BP, like *Histophilus somni*, which were not detected in our study sample, appear to have a greater propensity to enter systemic circulation due to the production of specific metalloproteinases capable of disrupting the alveolar-capillary barrier [39]. In addition to the limited ability of classic BP etiological agents to induce bacteremia, another explanation for the low levels of bacteremia observed in our study may relate to the inclusion criteria for the calves. While we focused on lung lesions measuring ≥ 3 cm, hypothesizing that these larger lesions were more likely to correspond with bacteremia compared to those measuring ≥ 1 cm, it is plausible that the calves included in the study had pathological conditions that were insufficient to significantly disrupt the alveolar-capillary barrier, preventing the etiological agents of disease from penetrating systemic circulation.

Interestingly, while Mycoplasma bovis was our study's most frequently identified isolate from nBAL samples, it was not isolated from blood cultures. Although the hematogenous pneumonia-arthritis syndrome linked to these pathogens has been recognized for a long time [40], isolating them from the bloodstream presents several challenges. Firstly, *Mycoplasmataceae* can invade host cells, allowing their entry into the circulatory system, particularly within erythrocytes and mononuclear cells [41]. As a result, detecting these intracellular bacteria in the bloodstream necessitates saponins or other lytic agents in the culture media [42]. Secondly, the growth of these pathogens is inhibited by sodium polyanethanol sulfonate, a commonly used anticoagulant in blood culture systems [43]. Finally, the initial positivity of Signal blood culture systems depends on detecting gases produced during the growth and replication of microorganisms in the blood. In this context, *Mycoplasmataceae* may not produce sufficient gas levels to exceed the detection threshold of the instrument due to their small cell mass [44]. Therefore, the consistent negativity of *Mycoplasma bovis* in blood samples—despite its frequent identification in nBAL samples from our study—can likely be attributed to the use of sodium polyanethanol sulfonate in our Signal blood culture bottles, the absence of lytic agents, and the restriction of cultures to those samples that were gas-productive positive.

The study presents another interesting finding regarding blood samples that tested positive solely for *Salmonella* Dublin and *Campylobacter fetus*. In recent years, an increased prevalence of *Salmonella* Dublin has been observed on dairy farms [45]. This strain has emerged as one of the most identified isolates of *Salmonella* spp. on dairy farms in the United States, Germany, and the United Kingdom [46–48]. In Italy, S. Dublin ranks second only to S. Typhimurium, representing 25.3% of total *Salmonella* spp. isolates in dairy farms [49]. This bacterium can lead to lifelong infections, characterized by intermittent bacteremia, often found in asymptomatic carriers among infected calves [50]. It is important to note that *Salmonella* spp. is well-established as an invasive enteric pathogen, particularly in bacteremic calves from which it is frequently isolated [15, 22, 23, 37, 51]. Furthermore, *Salmonella* Dublin has been identified as a significant contributor to pneumonia in young calves [50]. Our findings indicate that a bacteremic calf demonstrated the presence of *Salmonella* Dublin in the nBAL results. Interpreting these results should be approached with caution. Although the nBAL procedure utilized a specialized catheter to reduce contamination from external sources and upper airway and pharyngeal contamination, this factor cannot be entirely eliminated. Therefore,

additional investigations are necessary to determine whether *Salmonella* Dublin acts as a primary or secondary causative agent of bacterial pneumonia in pre-weaning dairy calves.

The single positive result for *Campylobacter fetus* aligns with the findings of Garcia et al. [37] regarding dairy calves suffering from neonatal diarrhea. In contrast to *Enterobacteriaceae*, bacteremia caused by *Campylobacter* spp. appears to be considerably less common. Recent research indicates that *Campylobacter fetus* has not been detected in blood samples following experimental intrauterine infections [52]. The authors suggest that bacteremia caused by *Campylobacter fetus* in cattle is more likely to occur in those experiencing episodes of immunosuppression or suffering from extragenital infections, similar to observations in sheep, which seem to be more susceptible to *Campylobacter fetus* bacteremia following oro-fecal infection.

Overall, the findings of this study suggest that diagnosing BP through TUS under field conditions did not reveal a considerable prevalence of bacteremia or any association between the presence of ultrasound lesions and the common pathogens linked to the disease. This outcome is consistent with other authors, indicating that TUS can provide valuable diagnostic information for BP by recognizing lesions before they lead to systemic functional damage [17], thus improving treatment responses [53]. The ability to detect ultrasound lesions for evaluating lung disorders in the absence of concurrent bacteremia markedly expands the diagnostic utility of this tool. Our study also revealed that contamination of blood samples in cattle occurred frequently, even when aseptic techniques were implemented. We found that contamination rates reached 27.2%, while other studies have reported frequencies ranging from 7.5% to over 50% [22, 37] influenced by the methods used to assess contamination and the various study settings. Consequently, it is essential to recognize that this issue is challenging to eliminate. As such, evaluating the positivity or non-positivity of a blood sample in bovines necessitates carefully considering the results.

This study had several limitations. The investigation was conducted on a selection of dairy farms known explicitly for their respiratory issues, chosen for convenience. Given the limited information available on the epidemiology of bacteremia in pre-weaning dairy calves, we intentionally focused our study on a setting with a higher incidence of lung lesions. We aimed to clarify the potential outcomes of bacteremia across various scenarios of more frequent and potentially more severe BP conditions. We implemented strictly defined eligibility criteria to prevent overrepresenting calves with severe pathological conditions and employed a single-gate reverse flow design across all participating farms. This allowed us to sample cases from a uniform source population, ensuring that the individuals shared similar characteristics regarding pathogen exposure. As a result, our findings have applicability to a broader context and reduce the risk of overrepresenting patients with advanced disease or those unaffected by the condition being investigated. This methodology enabled us to present results that more accurately reflect the circumstances faced by BP-affected calves in field settings. Another critical limitation could be related to the detection of bacteremia. Diagnosing bacteremia requires intensive monitoring techniques that involve serial blood sampling; however, this approach is impractical in field conditions. For our research, we utilized the double daily blood sampling method described by Fecteau et al. [20]. While this method may have its diagnostic limitations, it allowed us to investigate this condition in a farm setting. Moreover, this observational study lacked a negative control group, which constrains our ability to interpret blood culture results in calves without TUS-detectable lung lesions. The primary objective of this study was to evaluate the prevalence of bacteremia and its relationship with the severity of ultrasound lesions and clinical signs; consequently, a control group of healthy animals was not considered necessary during the design phase. It is essential to recognize that positive results for *Salmonella* Dublin and *Campylobacter fetus* may also occur in calves without lung lesions; however, the absence of all expected findings suggests that including a control group would not have added significant value for BP-affected calves. Our observational approach, however, constrains our understanding of the temporal progression of lung lesions within our study sample. A longitudinal ultrasound evaluation of lung lesions and serial blood cultures could have provided different insights, especially if we had included participants with confirmed chronic lung conditions, who may be more prone to developing bacteremia. Furthermore, although the management characteristics of the enrolled farms were relatively similar, we did not have access to the calves' clinical histories before their grouping in the multiple pens. Consequently, we were unaware of critical factors such as the prevalence of neonatal diseases, passive immune levels among the calves, or any prior antimicrobial treatments during the neonatal phase, all of which could have influenced the pathological manifestations of the respiratory issues addressed in our study. Additionally, given the low prevalence observed in our research (6.8%), the sample size may have affected the results.

In conclusion, the prevalence of bacteremia in dairy calves with lung lesions detected through TUS was 6.8% (6 out of 88), significantly lower than previously reported estimates from hospital settings. Thoracic ultrasonography lesions categorized as ≥1 cm and ≥3 cm were not

linked to bacteremia in this study sample. Additionally, no classical BP pathogens were identified in the blood samples. These findings indicate that the lung lesions detected via TUS may occur independently of concurrent bacteremia, underscoring the potential of early TUS diagnostics in preweaning dairy calves. Further research is necessary to explore blood cultures in calves with chronic or more severe bronchopneumonia through longitudinal studies utilizing TUS. Moreover, establishing precise epidemiological data on *Salmonella* Dublin pneumonia in dairy calves would be beneficial.

Abbreviations

AUSC	Thoracic auscultation score
BCoV	Bovine coronavirus
BoHV-1	Bovine herpesvirus type 1
BP	Bronchopneumonia
BPI-3	Bovine parainfluenza type 3
BRSV	Bovine respiratory syncytial virus
BVD	Bovine viral diarrhea
CALIF	California respiratory scoring system
DNA	Deoxyribose nucleic acid
ICS	Intercostal space
IQR	Interquartile range
MALDI-TOF	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
nBAL	Non-endoscopic bronchoalveolar lavage
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
SD	Standard deviation
TUS	Thoracic ultrasonography

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12917-025-04707-x.

Supplementary Material 1: **Supplementary File 1.** Complete clinical data for all 211 calves enrolled in the study. The file includes age, herd origin, TUS and clinical findings for all eligible calves, and nBAL and blood culture results for those included in the final analysis.

Supplementary Material 2: **Supplementary File 2.** Associations between the presence/absence of bacteremia and clinical variables tested with a chi-square test. Statistical significance was set for a $P < 0.05$.

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Author contributions
AB conceived and designed the study, performed thoracic ultrasonography and drafted the manuscript. GS and DP analyzed the data and performed the clinical evaluation of the enrolled calves. LFP performed bronchoalveolar lavage and blood sampling. AMM, MO, SR, AG, LS, CFM, SC and MC processed and analyzed the biological samples in the laboratory. AMM drafted the microbiology section of the paper. All authors read and approved the final manuscript.

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Data availability
All data generated or analyzed during this study are included in this published article and in additional files.

Declarations

Ethics approval
The study protocol received approval from the University of Milan's institutional animal welfare organization (approval number 59_2024).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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