Field application of Lung Ultrasonography in bovine: a scoping review

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SUMMARY

Bovine respiratory disease (BRD) is considered one of the most expensive diseases in the cattle farming worldwide affecting both beef and dairy production. This disease is primarily responsible for increased veterinary costs, treatment expenses, reduced productivity, and, in severe cases, the culling of affected animals. Timely and accurate diagnosis is crucial for effective management and prevention of BRD-related losses. The difficulty in diagnosis based on clinical signs of the animal determines the need to introduce new diagnostic methods. Lung ultrasonography (LUS) has emerged as a valuable, non-invasive, and quick tool offering numerous advantages. It allows for real-time, dynamic evaluation of the lung tissue, providing detailed insights into the presence of pathological changes.

This article provides a comprehensive overview of the practical application of LUS in the diagnosis of BRD in cattle. LUS is performed using linear or convex probes, equipped with high or middle frequency transducers. The lung can be investigated from the 1st to the 10th intercostal space on the right and from the 2nd to the 10th on the left, with differences in size and age of the animal. Several alterations and artefacts can be observed in pathological conditions: comet-tails, B-lines, consolidation, fluid alveolograms and bronchograms. These signs provide valuable information on the extent and severity of lung lesions, which can be used to assign an ultrasonography score to the animal. The two ultrasound scoring systems proposed below allows to discriminate between healthy and diseased animals, guiding the decision-making process for both treatment and management strategies. Additionally, routine ultrasound screening and follow-up assessments offer valuable insights into the progression of the disease, enabling practitioners to monitor the effectiveness of treatments and make timely adjustments to therapy. Overall, LUS represents a promising diagnostic approach that enhances the ability to manage BRD in cattle efficiently, improving animal health and welfare, and minimizing economic losses in the livestock industry.

KEY WORDS

Respiratory, thoracic ultrasonography, lung lesions, BRD, artefacts, diagnosis.

INTRODUCTION

The Bovine Respiratory Disease (BRD), or Bovine Respiratory Disease Complex (BRDC), is a respiratory syndrome with multifactorial etiology caused by several microbial agents, individual factors related to the host immune response and unfavourable environmental factors, which added together result in bronchopneumonia [1].

Mixed infections in BRD are common. The main viruses isolated from cattle with respiratory syndrome are bovine herpesvirus type 1 (BHV-1), parainfluenza-3 virus (PI3), bovine viral diarrhoea virus (BVDV) and bovine respiratory syncytial virus (BRSV). Whereas, the main bacteria are *Pasteurella multocida, Mannhemia haemolytica, Histophilus somni* and *Mycoplasma bovis* [2-4]. The predisposing factors of this syndrome are well documented in literature, but the mechanism of their interaction is still being studied. The main risk factors are transport, weaning, high density of animals, environmental conditions as high dust and humidity levels [5].

Nowadays, BRD is considered a challenging problem in all types of cattle farming (veal, beef and dairy). It is characterized by a high variable incidence ranging from 10% to 75%, with veterinarians striving to maintain it below 10% to minimize its impact [6]. The economic consequences of BRD concern both direct and indirect costs. Direct costs include the use of antibiotics and anti-inflammatory drugs for treatment [7]. Additionally, BRD results in decreased weight gain and reduced milk production during lactation, with studies showing losses of 121.2 to 184.9 kg of milk per affected animal. Furthermore, the disease can delay the age at first calving by 1.75 to 3.03 months, leading to prolonged time before the animal reaches full productivity. Reproductive performance is also compromised, with increased rates of abortions and hypo-fertility. The disease heightens the risk of mortality, with affected animals exhibiting 2.85 to 6.6 times higher mortality rates, and increases the likelihood of premature culling by 2.30 times [8].

An unambiguous definition of clinical signs is still lacking to outline a common approach for BRD diagnosis. In fact, clinical signs can range from subclinical forms to fatal, involving

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upper and lower airways. Furthermore, some clinical signs as fever are not specific of BRD [9,10]. Usually, clinical scores as Wisconsin (WI) and California (CA) are used in field to perform the BRD diagnosis [11]. The WI score takes into consideration rectal temperature, cough, nasal and eye discharges, head and ear position, and faeces appearance. A score from 0 to 3 is assigned for each of the listed clinical signs according to their severity: absent or 0; mild or 1; moderate or 2; and severe or 3. The total score ranges from 0 to 18, and it is given by the sum of the individual signs' scores. Animals with at least two clinical signs with a score of 2 or more are considered affected by BRD [12].

The CA score takes into consideration cough, nasal discharge, eye discharge, head and ear position, breathing pattern and rectal temperature. This system is based on the absence or presence of clinical signs, not considering their severity. The absence of clinical signs corresponds to a score of 0, while pathological alterations of them receive a score greater than 0 according to the considered signs. The animal is considered as affected by BRD with a general score greater than or equal to 5 [13]. However, these clinical signs are subjective, except for rectal temperature which is the only objective measurement. Moreover, the specificity and sensitivity were found to be, respectively, 46% and 91.2% for the WI score, and 46.8% and 87.4% for the CA score. The reason is that clinical signs are not always specific of BRD, and most of them are associated to the upper respiratory tract as eye and nasal discharges. Indeed, clinical signs often do not reflect pulmonary lesions [13,14]. The low sensitivity and specificity lead to an an high risk of missed diagnosis (diseased animals that are not diagnosed and treated) and misdiagnosis (healthy animals that are treated)[15].

Consequently, the gold standard test is still considered necropsy (detection of lung gross lesions after slaughter) and identification of the infectious etiologic agents through tissue sampling and laboratory diagnostics [16].

The early diagnosis of BRD *in vivo* is the real purpose in livestock to perform an early treatment improving the drug efficacy, and to prevent the decrease in animal's welfare and productivity. Furthermore, the improvement in treatment efficacy may decrease the incidence of relapse reducing the use of antibiotics and the risk of antimicrobial resistance [17,18].

New diagnostic tools are developing to improve an early BRD diagnosis and among them, there is the lung ultrasonography. This review is focus on the use of this technique as an innovative tool for diagnosis and monitoring the pulmonary diseases in cattle.

ULTRASONOGRAPHY FUNCTIONING

Ultrasonography (US) examination is a non-invasive and safe procedure that takes little time to be performed and that can be used on field. The US is considered a diagnostic aid for bovine clinical practice in the reproductive management, and tissue heath and conformation [19-21]. During the last decades, purchasing an US scanner is more available than in the past, to due to the costs reduction and providing to the practitioner a small, portable and accessible imaging technology [22].

The US is based on the production of ultrasound waves by piezoelectric (PZE) crystals placed in the transducer of the probe. The interaction between waves and tissues is given by a return echoes that are analysed by the computer to create images of organs, tissues, and blood flows. According to the setting is possible to modulate or acquire several images: the image quality directly depends by the waves' frequency; whereas the frequency and penetration power are inversely proportional. Consequently, a greater frequency provides a better image quality but a lower depth of image [23]. Therefore, the choice of the probe frequency has to be done according to the distance from the skin to the evaluated structure: 7.5 MHz penetrates a depth of 4-6 cm, 5 MHz penetrates 10-12 cm, 3.5MHz penetrates 15-20 cm and 2.5 MHz penetrates 25-30 cm. The trans rectal probes used for bovine reproduction have a variable frequency from 3.5 to 8 MHz that, as mentioned above, are also able to reach the adequate depth to assess pleurae and lungs [24].

As concerns the image produced by the scanner, the most commonly form of US examination used in veterinary is Real-Time Brightness mode (B-mode): this returns a 2D black and white image (various shades of grey) whose brightness is positively correlated to the magnitude of the reflected echoes returning to the transducer, at the exact moment as it occurs [25]. On more distinction needs to be done, regarding the image produced by the different types of probes.

The linear probe generates a large number of parallel beams that can explore a section of rectangular shapes and size equal to the one of the transducer. As far as the convex probe, the crystals are place on a circumference and the emitted beams generate a trapezoidal scan ("truncated-cone shape), expanding the scanning area compared with the linear probe [26]. When using a convex probe, ultrasound waves are emitted in a fan-shape. mode. In this way, on the lateral parts of the fan, the US beam does not intersect the lung surface orthogonally, and the pleura line at the border of the fan is mostly fuzzy [27].

LUNG ULTRASONOGRAPHY

The application of lung ultrasonography (LUS) in cattle husbandry has been studied during the past few years in order to develop a new tool for BRD diagnosis [28]. Taking into consideration the frequency and the shape of the probes, a high frequency linear transducer (7.5 MHz) is most recommended for calves and to observe adults' pleurae [29]; while low-middle frequency convex o linear probes (2-5.5 MHz) allow to investigate adult bovine lungs [30].

Before US examination, both sides of the animals need to be prepared: trichotomy from the caudal part of scapula to the last rib and from the transverse processes of the thoracic vertebrae to the elbow is considered the best choice to have a clear field but not always necessary. A transducing agent as gel or ethylic alcohol 90% are essential to perform a LUS examination in order to reduce the air impendence to ultrasound waves [31]. LUS has to be performed systematically in a caudo-cranial direction. The probe is positioned parallel to the ribs and moved dorsum-ventrally toward the sternum. The lung extends from the 1st to the 10th intercostal space on the right and from the 2nd to the 10th on the left. The caudal lung can be investigated between 10th and 7th intercostal space (ICS); between 6th and 5th ICS for the middle lung of both sides. When the muscle masses are not yet well developed (usually animals with less than six months), the cranial component of the cranial lung lobes can be observed at the level of the 1st and 2nd intercostal spaces on the right and the 2nd and 3rd on the left [32,33].

The stratigraphy of the image consists of thoracic wall, fatty

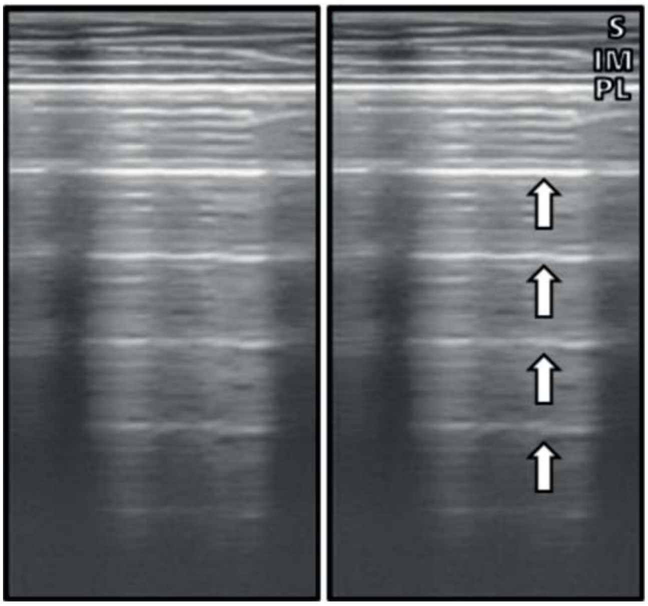

Figure 1 - Ultrasound image of a healthy lung and pleura. The stratigraphy of the image consists of skin (S), intercostal muscles (IM) on top, followed by the "pleural line" (PL). The hyperechoic lines (arrows) are artefacts so called A-lines that represent the reflection of USs beams at the transition to the normally aerated lung also called mirror effect (Resources: U.O. Ruminants, OVUD, Department MAPS, University of Padua).

tissue, intercostal muscles on top, followed by the "pleural line" [34]. Pleura is composed by two sheets: parietal and visceral ones, which are visualized as a one hyperechoic interface echoes in absence of pathological process (thick bright line). The only way to appreciate the two pleural sheets is during a real-time examination, when the sliding movement of lung (*gliding sign*) show their synchronous movement [35].

Under the pleura, there is the lung parenchyma which appears as a reverberation artefact called *A-lines*. These lines begin from the pleural line as horizontal repeated artefacts (repeated bright line). They stand for the reflection of the US beam by the air in the lung and represent a physiological situation (aerated lungs' parenchyma) (Figure 1) [36]. Consequently, a healthy lung parenchyma cannot be investigated for the presence of these artefacts.

MAIN ALTERATIONS AND LESIONS

Comet tail and B-lines

The comet-tail is a form of reverberation artefact characterized by a bright hyperechoic focus on the pleural line and a triangular acoustic enhancement posterior to the focus. Consequently, the comet tail arises from the pleural line and extend to the bottom of the screen. It occurs when USs hit an object with marked different acoustic impedance as air bubbles, small calcific structure o fibrotic tissue [37]. However, it is considered a normal and para-physiological lesion in all animals [38] (Figure 2).

B-lines are vertical reverberation artefact that extends from pleura and/or lung parenchyma to the depth of the image. They are frequently produced when there is an increase of the lung density caused by the substitution of air with a fluid as exudate, transudate and blood. The change in acoustic impedance with these underlying structures causes an important reflection of the ultrasound beam [39]. Consequently, the appearance of a B-line a vertical thick bright line single or multiple (Figure 2). When there are multiple B-lines, they can appear close to each other and can also be connected. In this case, they occur in a

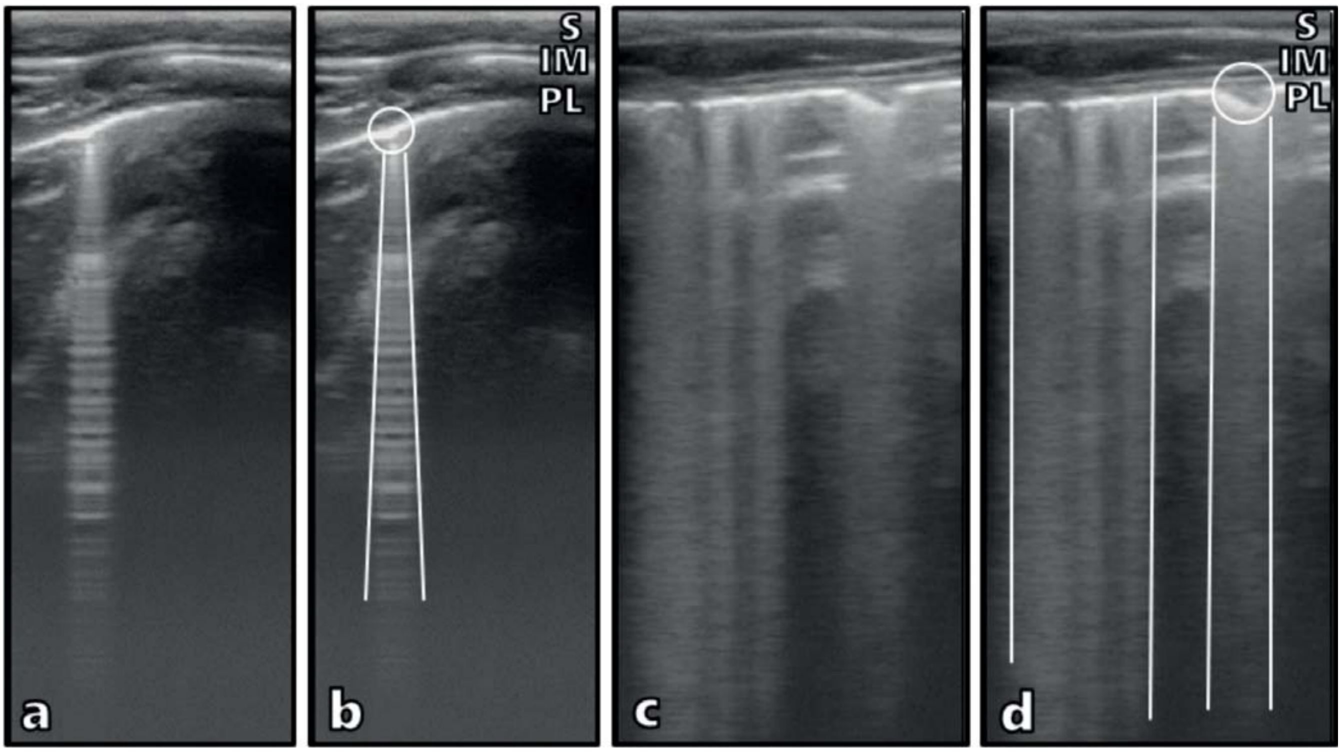

Figure 2 - Comet-Tail arising from the parietal pleura. The circle points out the pleura focus while the white lines the following acoustic enhancement (**a-b**).
Example of B-lines artefacts beginning from the pleura (white lines) related to a pleura lesion (white circle) (**c-d**) (Resources: U.O. Ruminants, OVUD, Department MAPS, University of Padua).

cascading, diffuse, confluent or bright white appearance [27,40].

The presence of B-lines should alert as they assume a high probability of an early stage of a pathology. The most common lesions associated to B-line are pleurisy or fluid alveolograms and/or bronchograms. Consequently, when a B-line is observed, it is suggested to investigate the artefacts in order to find possible lesions [39].

Pleurisy and pleural effusion

The fluid accumulation process between the parietal and visceral pleura is called pleural effusion. It can occur by an inflammation of pleura itself (pleurisy) or of a pathological process of the surrounding parenchyma (infection or other inflammatory conditions). When a fluid is present, the two pleura are progressively separated with the inter-pleura space filled by a liquid-like content (Figure 3). Usually, the pleural effusion is more common in the ventral portions of the thorax. The nature of liquid could be determined anechoic or in order of scale of grey. Generally, transudate shows anechoic fluid (black image), while an exudate is more echoic (grey image) because of cellular and highlighted with fibrin presence [35,41]. In cases of fibrinous pleurisy, it is also possible to distinguish septa and fibrin inside the collection (Figure 3). They appear like echogenic, stretched and floating structures in the fluid located in the pleural cavity [42].

Lung consolidation

Lung consolidation (LC) indicates an area of increase in lung parenchymal fading (non-aerated lung), for the presence of an exudate, increased cellular component or other product that replaces alveolar air and makes the lung appear as solid (Figure 4) [43]. Many physiological or pathological structures can be distinguished in a state of consolidation. It is always suggested to move the probe to avoid operator errors lead to possible artefacts. If the lesion dos not persist moving the probe what was observed was an artefact [44].

Consolidation can occur without or with fluid accumulation. Typically, consolidation without significant fluid accumulation occurs in the early stages of infection or inflammation. In the affected regions, it is possible distinguish the bronco-aerograms. Broncho-aerograms could be observed as anechoic structure of few millimetres of diameter with a posterior bight line (enhancement artefact) and reverberation artefacts [43].

Fluid alveolograms and bronchograms are the expression of lung parenchyma substituted by fluid (transudate or exudate). Fluid alveolograms or bronchograms are anechoic to hypoechoic areas with posterior B-lines artefacts. However, the fluid alveologram is immediately under the pleura line, while the fluid bronchogram is inside the lung parenchyma. (Figure 5) [35,45,46].

The hepatisation describes a condition of lung consolidation, where the US texture of the tissue is similar to the liver as in condition of pneumonia or atelectasis [35,45]. It is indicative of a chronic lesion or a viral infection resulting in lymphocyte proliferation. Within the hepatisation it is possible distinguish the blood vessel as multiple small anechoic circle without posterior bight line [41].

The evidence of small multifocal masses randomly distributed throughout the lung are representative of granulomatous dis-

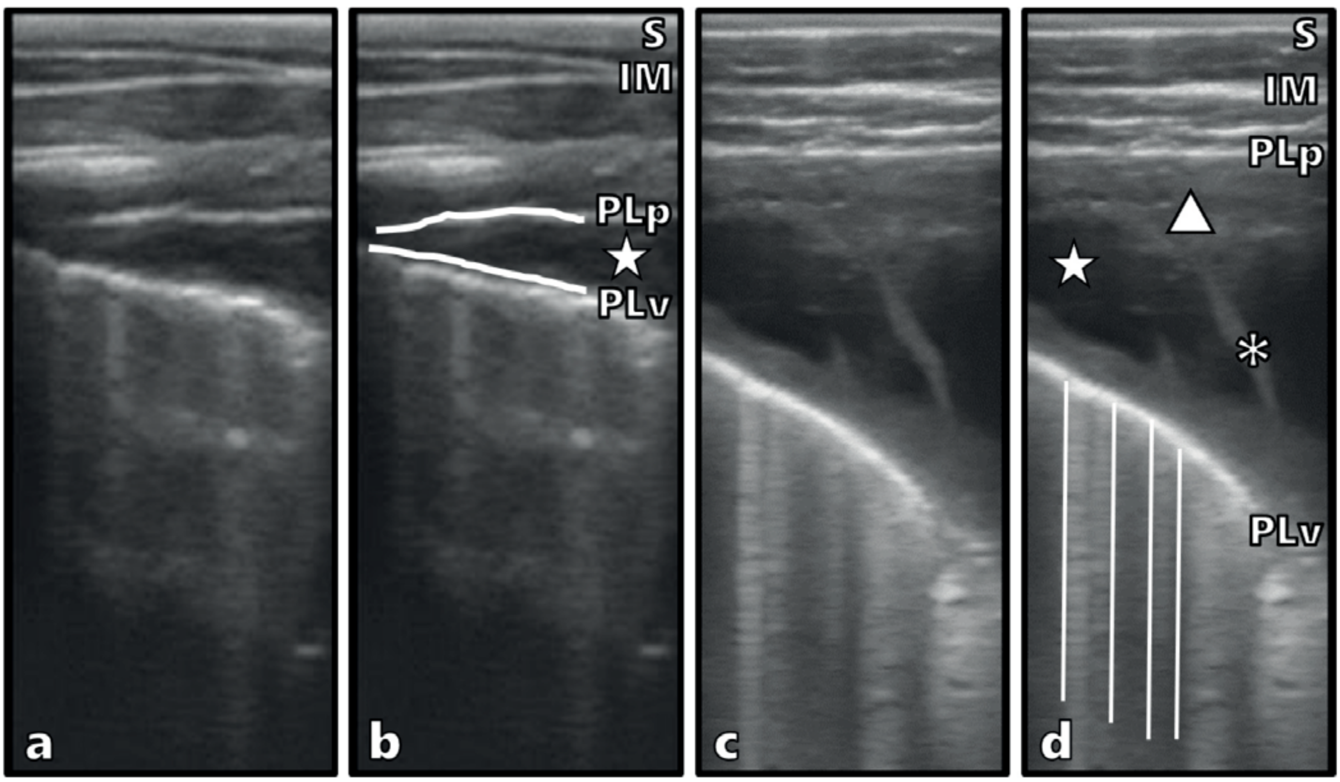

Figure 3 - Lung ultrasonography of a cow with moderate pleural effusion. The parietal and visceral pleura are progressively separated (PLp and PLv respectively) and the space between them is filled by an anechoic to hypoechoic fluid (star) (**a-b**).
Animal affected by a severe pleurisy with fibrinous effusion. The parietal (PLp) and visceral (PLv) pleura are progressively separated. The space between them is filled by anechoic fluid (star) to hypoechoic deposits (triangle). A hypoechoic septa of fibrin (asterisk) with a fluctuating movement in real-time image link the hypoechoic deposits (fibrin) on the PLp and PLv. Under the PLv there are B-lines artefacts (white lines) (**c-d**).
(Resources: U.O. Ruminants, OVUD, Department MAPS, University of Padua).

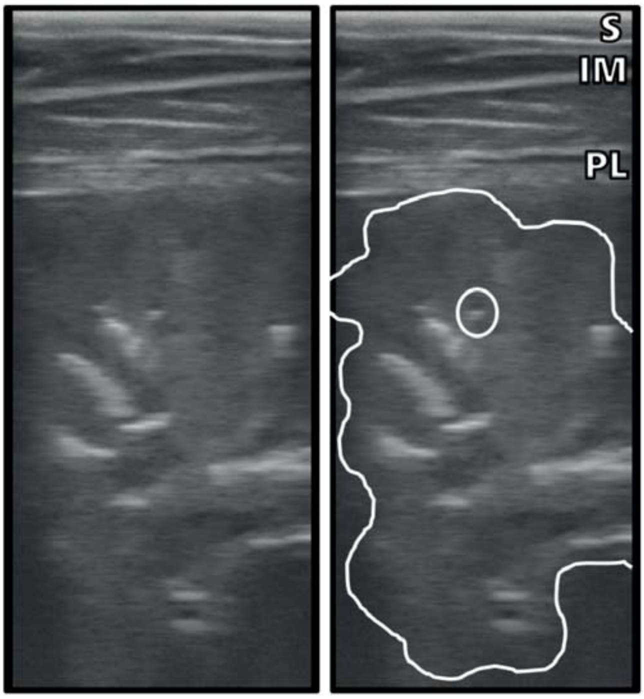

Figure 4 - The lung consolidation is characterized by a homogeneous hypoechoic texture liver-like (white line) with blood vessel inside (white circle) (Resources: U.O. Ruminants, OVUD, Department MAPS, University of Padua).

ease, fungal pneumonia, or metastatic neoplasia. These masses are mostly homogeneous and hypoechoic compared with the surrounding normal lung, but may be isoechoic [44].

Ultrasonographic scores

The US scoring system was developed by Ollivett and Buczinski (2016) on the basis of LC. The aim of this score was to rapidly screen calves and perform a diagnosis of BRD. The score was assigned according to lung lesions:

- 0: no lesions, aerated lung;
- 1: aerated lung with diffuse pleural comet-tail artefacts, without consolidation;
- 2: lobular or patchy consolidations;
- 3: lobar consolidation that affects only one lobe;
- 4: lobar consolidation that affects two lobes;
- 5: lobar consolidation that affects three or more lobes.

They demonstrated that US scores 0 to 1 are representative of a lung healthy status and scores greater than or equal to 3 are considered significant of a bacterial bronchopneumonia condition. In addition, the presence of extensive LC is correlated to a higher mortality and a greater risk of being culled [28].
Many studies tried to define a threshold about the dimension of LC to detect active pneumonia. According to Berman and Francoz, a lesion of 3 cm in the lung cranial lobe is indicative for diagnosing BRD with a good specificity and sensibility. Using this threshold, this diagnostic approach was accurate for identifying diseased animals (sensitivity [Se]=0.89) and healthy animals (specificity [Sp]=0.95) [47].
More recently, the cut-off of 1 cm consolidation has been considered to diagnose BRD. In this case, the estimated Se of LUS was 79.4% and the Sp was 93.9%. The sensibility of this threshold is lower because small single pulmonary lesions are commonly detected in calves. They do not necessarily represent an acute condition of BRD [28].
Fiore et al. introduced an Ultrasound-Lung Lesion Score (US-LLS) based on the evaluation of LUS findings of the six lung areas: caudal (10th-7th intercostal space), middle (6th-5th ICS), and cranial (4th-3rd ICS) of both lung sides. The total score was calculated according to the different lesions of each area [15]:

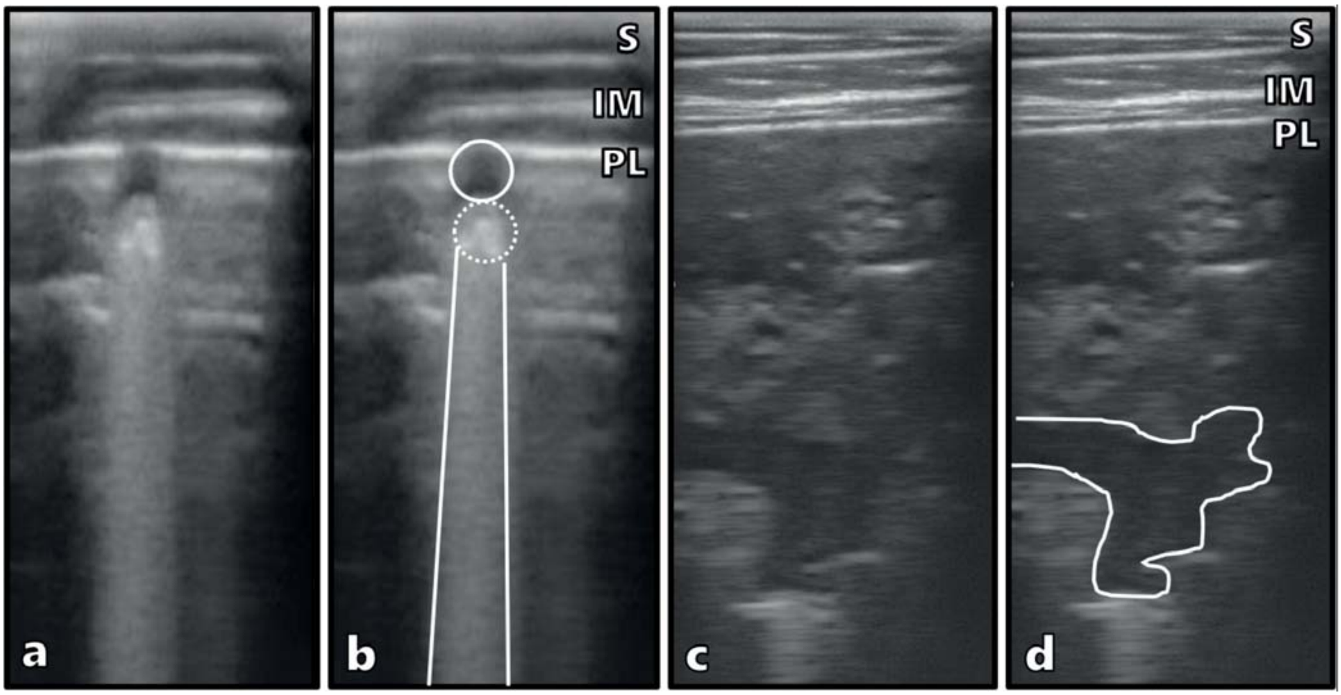

Figure 5 - Sub-pleura lesion characterized by an anechoic (continuous white circle) and hyperechoic (white dashed circle) components. These two components constitute a fluid alveologram followed by a B-line artefact (white lines) (**a-b**).
Example of fluid bronchogram (white line). It corresponds to distended bronchi filled with an anechoic fluid followed by a B-line within a marked lung consolidation (**c-d**) (Resources: U.O. Ruminants, OVUD, Department MAPS, University of Padua).

0: healthy lung;
1: presence of comet tails;
2: spot of lobular consolidation;
3: lobar consolidation:
4: lobar consolidation and comet tails;
5: fluid alveologram/bronchograms;
6: fluid alveologram/bronchogram and comet tails;
8: lobar consolidation and fluid alveologram/bronchograms;
9: lobar consolidation, fluid alveologram/bronchogram, and comet tails;
11: pleurisy.

They demonstrated that a US-LLS > 10,5 is indicative for diagnosing BRD [48].
The main difference between the two scoring systems is the attention given to individual lesions. The first one is based on the presence or absence of lesions (consolidation), and on the different involvement of the lung lobes. The second one, on the other hand, focuses on the type of lesion allowing to identify the type of alteration present. This allows the diagnosis to be directed toward
acute rather than chronic conditions.

Applications of Lung Ultrasonography in field

Preforming periodic ultrasound scans on groups of animals can provide a very useful information and help the farmer making management decisions. LUS has the potential to predict the outcomes in animals suffering from respiratory diseases within 1 month after examination based on the number of consolidated sites as well as their depth [38]. For this reason, LUS offers advantages in monitoring animals in different situations.
Ultrasound monitoring is advisable in any condition in which the animal is exposed to stressors. As mentioned before, the main stressors favouring the onset of BRD are long transport timed, poor ventilation, crowding (in particular for beef cattle), environmental changes, weaning, handling practices, exposure to pathogens in the environment, and more others [6,49].
In addition, its use is recommended if there is any doubt about the animal's state of health. Classifying animals into categories such as acutely or chronically affected by respiratory disease, or presumed healthy might be preferable, especially regarding the prudent use of antibiotics [50]. Conditions such as pleurisy, alveolograms and bronchograms are indicative of an acute state of disease. Whereas an appearance of hepatisation is more related to a chronic or viral condition [51]. The identification of sick animals is useful to provide immediate separation from the herd. Isolated boxes for sick animals are recommended. They provide a low-density environment, less stress and allow closer observation, monitoring and treatment while waiting for virologic and bacteriological examination results [5].
The recommended monitoring times differ depending on the category of animal.
Concerning beef cattle, the period most at high-risk is from weaning upon feedlot arrival. Conditions such as long transports and change of housing-system are the main predisposing factors [52]. Indeed, an increase in lung consolidation during this period is associated with a reduction in daily growth [53]. A close monitoring of animals' health is essential in the first month after arrival since the timing of BRD onset is highly variable (as early as three days post-arrival or as late as one, two, three, or even six weeks afterward) [50,54,55]. The animal can later be rechecked when handling lanes are used and it is already contained. Any condition involving a change of environment (change of box or group, introduction of new animals) can be a risk factor that needs careful monitoring [56].
As for calves, a distinction must be made between veal calves and dairy calves. The BRD prevalence in pre-weaned dairy calves varies from 12% to 23% in the dairy industry and from 14% to 61% in veal calves [57]. In veal calves, LUS is recommended especially at the arrival and within the first 2 months of rearing. They are highly susceptible to respiratory infections due to close contact, transportation stress and developing immune system. T
he onset of BRD cases affects the growth and welfare of these animals [58]. By identifying lesions at an early stage, which might otherwise go undetected, treatment can be timely and more effective. Studies on veal calves, such as the one by Lowie et al. (2024), indicate that lung consolidations larger than 1 cm at arrival significantly increase the risk of BRD outbreaks. These areas could be representative of active (treatment required) or inactive bronchopneumonia [54]. While animals with consolidations exceeding 5 cm are at a higher risk of mortality or culling [53]. Thus, improving the efficiency in detecting active bronchopneumonia would lead to a reduction in the use of antimicrobials and a reduction of economic losses [59].
In dairy calves, the most effective times to perform LUS are: within the first 2-3 weeks, 6-8 weeks (around weaning) and during post-weaning stressful periods. Weaning is a stressful transition that can weaken calf's immunity and exposes it to an increased risk of contracting BRD [55].
The US score should always be contextualized along with the animal's clinical signs. The general conditions of the animal indicate whether it needs to be treated or not. The severity and numerosity of the clinical signs within the herd should also be considered. If, despite a high ultrasound score, the animal's health status is good, it is advisable to monitor the animal once a week [60].
When the score is accompanied by severe clinical signs treatment is indicated. Antimicrobials are indicated in the treatment of cases of BRD involving primary or secondary bacterial infection. However, symptoms of diseases can be reduced by the administration of anti-inflammatory drugs [61]. Other therapeutic agents such as bronchodilators, antihistamines, mucolytics, immunomodulators and diuretics have been investigated experimentally for the treatment of BRD [62].
Cranial and middle areas on both sides of the lung are commonly the most affected by BRD. Their monitoring in the days following treatment shows an improvement already after 3 days. Similarly, the consolidation areas (hepatisation and fluid alveolograms) usually are reduced after 1.5 and 3 days after the treatment. In several cases, areas of pulmonary consolidation remain despite the therapy. It is confirmed that the treatment is more effective in acute conditions of the disease [63]. Indeed, conditions of consolidation (hepatisation) may be the result of chronic or viral infection, which don't respond to antibiotic treatment [64].
Regarding periodic monitoring, ultrasonography is considered a practical tool also to assess the evolution of lung lesion and animal recovery [47,65]. LUS allows tracking of progression or regression of lung damage, providing evidences of treatment success [66].
As a final point, it is important for veterinarians performing LUS in cattle to gain practise, following the scanning technique and applying the lesion identification methods described

above. Findings from this study stress that adequate training is essential for optimizing the diagnostic capabilities thus reinforcing its effectiveness as a valuable tool for the diagnosis and management of BRD in cattle [67].

CONCLUSIONS

BRD is still a challenging problem for farmers and veterinarians, whose primary goal is monitoring and detecting it in young animals before clinical onset. As mentioned before, physical examination is inadequate alone with the need to introduce different diagnosis methods. Ultrasonography is an available, quickly, non-invasive tool already used by veterinarians which needs to be improve also in respiratory diseases. Lung ultrasonography (LUS) can be used on the individual or at herd level: on single animal it gives a prognosis index based on lobe consolidation, helping in taking decisions about therapies or culling; at the herd level, LUS supplies a representative image of the epidemiologic situation of the farm in order to monitor prevalence and severity of BRD, and to introduce management chances to reduce its incidence such as the introduction of a prophylactic procedure.

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