

# Ultrasound examination for diagnosing pneumopathies in New World primates, focusing on pulmonary consolidation

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## Article

**Keywords:** respiratory condition, Callithrix sp, lobar consolidation, lung, ultrasound

**Posted Date:** May 30th, 2023

**DOI:** <https://doi.org/10.21203/rs.3.rs-2883332/v1>

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# Abstract

Pulmonary ultrasonography may be useful for early diagnosis and management of respiratory complications. The combination of air and soft tissues confirms imaging artifacts that may contribute to differentiation of healthy lung tissue from deteriorated lung tissue. Although non-human primates are often chosen as research models due to similarity to humans, there is a scarcity of data on the use of pulmonary ultrasound on these individuals. The aim of this study was to evaluate the contribution of ultrasound examinations of the thoracic region of *Callithrix* sp in diagnosing pneumopathy. Parameters were obtained from 84 *Callithrix* sp of both sexes, aged 1.6 to 15 years and weighing 222 to 684 grams, which were caught within the Mucky Project, in Itu, São Paulo. Thoracic ultrasound examinations were conducted using the LOGIQe-R7 (GE, USA), with a 12 MHz linear transducer, at four points of both antimeres. 18 individuals presented some type of pulmonary alteration. Two of the animals with pulmonary alterations died and then underwent necropsy. Histopathological analysis showed that their lung tissue was compatible with the presence of suppurative bacterial bronchopneumonia. In the light of these cases, pulmonary consolidations diagnosed through thoracic ultrasound examination can be correlated with occurrence of pneumonia.

## Introduction

Chest ultrasound examinations can be useful for early detection and management of respiratory complications, such as pneumonia, atelectasis and pleural effusion. They are of great value in both human and veterinary medicine, even for critically ill patients (Mojoli et al. 2019).

The pulmonary parenchyma has a spongiform appearance. Its porosity and pulmonary density result from a combination of elasticity and performance that enable remodeling in airspaces, insufflation, deflation, recruitment of peripheral airspace and even thickening of the interalveolar septa (Soldati et al. 2014). Bronchi, pulmonary bronchioles, ducts and alveolar sacs are components of the peripheral airspace. Their surface is protected by a thin and continuous layer of surfactant, which is responsible for organizing the bubbles that fill the distal airspaces and promote structuring and support, so that aeration of the pulmonary parenchyma occurs properly (Scarpelli 1998; Soldati et al. 2014). Any pathological or functional situation that leads to loss of porosity and lung density interferes with the relationship between air and tissue. This alteration may contribute to understanding pulmonary ultrasound findings (Soldati et al. 2014).

A healthy pulmonary parenchyma is characterized by horizontal parallel lines on ultrasound examination. The thickened pulmonary septum located in the subpleural region has very small dimensions, making it impossible to visualize on ultrasound (approximately 1 mm). However, when there is significant thickening, the beam becomes disordered and this results in a difference in acoustic impedance in the image, in comparison with the adjacent area composed of air (Lichtenstein et al. 1997).

The fluid in the pulmonary parenchyma produces an image of a small anechogenic structure. Its resolution is below that of the ultrasound beam because of the difference in acoustic impedance caused by the presence of air. This causes reverberant vertical artifacts called B-lines, which should not be found under normal conditions but, rather, in alveolo-interstitial syndromes (Lichtenstein et al. 1997).

Air bronchograms are often observed on pulmonary ultrasound and are characterized by hyperechogenic points or lines within the consolidated area. There are two types of air bronchograms: dynamic and static. Dynamic bronchograms are thus named because they involve inspiratory centrifugal movement that accompanies the patient's respiratory movement. They are related to the capacity to supply the consolidated lung tissue through the airways and, for this reason, they are often found in conditions of pneumonia. Static bronchograms are observed when there is no presence of air inside the consolidation, due to a resorption process that contributes to reducing the volume of the consolidation. This finding can be observed in late cases of atelectasis due to resorption (Lichtenstein 2010; Inchingolo et al. 2021).

Pulmonary consolidations are observed as delimited areas that are similar to the hepatic parenchyma (pulmonary hepatization), with well-defined regular or irregular outlines. Their acoustic impedance is high, due to the presence of multiple B-lines or acoustic shading above them (Soldati 2006).

Consolidations may occur in association with alveolar impregnation caused by exudate, transudate, blood, fibrin or any other air-replacement substance (Soldati 2006). In the consolidated area, loss of aeration occurs, which forms a hypoechogenic area located in a subpleural or tissue region (Staub 2017).

Consolidations may be focal, partial or lobar (Lin et al. 2020). According to the appearance and location, it is possible to differentiate pneumonia from atelectasis due to tissue resorption. In patients with atelectasis due to tissue resorption, loss of pleural slippage is observed as an early sign, which differs from the presence of dynamic air bronchograms that slide in synchrony with respiratory movements. In 98.5% of the cases, acute alveolar consolidations are in contact with the visceral pleural region. In late cases of atelectasis due to resorption, it will only be possible to visualize them when there is resorption of the gas content and a decrease in the volume of the consolidation, which is confirmed through a static bronchogram image (Lichtenstein 2010).

When bronchopneumonia progresses such that it compromises peripheral areas, loss of aeration occurs and the following ultrasound features are observed: in patients with incipient infections involving interstitial inflammation, B-line foci with unequal intervals are seen; and in cases of focal bronchopneumonia, small consolidated areas are seen in the subpleural or lobar region, in addition to larger lobar consolidations. The presence of secretions and air in the bronchial region is observed on ultrasound as hyperechogenic lines inside the consolidated area, which move together with breathing (Lichtenstein 2010; Mongodi et al. 2016).

The aim of the present study was to evaluate the contribution of ultrasound examinations of the thoracic region of animals of the species *Callithrix* sp for diagnosing pulmonary abnormalities.

# Methodology

“All procedures were conducted in accordance with the following the regulatory guidelines (ARRIVE) and standards for research involving animals, in accordance with Federal Law No. 11,794 (Arouca Law), the CONCEA resolutions, which establish procedures for the scientific use of animals in the country, and State Law No. 11,977/05, which institutes the São Paulo State Animal Protection Code, approved by institutional ethical review committees (Committee for Animal Use CEUA of Santo Amaro University and Authorization for Activities with Scientific Purposes of the Ministry of the Environment SISBIO) and conducted under the authority of the Project Licence (57/2021 and 78874-1, respectively). The consent letter for development of this study was handed over to and signed by the head of the Mucky Project, where the animals were living (<https://arriveguidelines.org/>).

This observational study used a convenience sample consisting of 84 animals of the species *Callithrix* sp (both males and females), of ages between 2 and 15 years ( $5.32 \pm 3.76$  years) and average weight  $322.8 \pm 73.2$  grams. They were living within the Mucky Project NGO in the municipality of Itu, São Paulo, Brazil, and the evaluations took place between November 2, 2021, and January 24, 2022.

Thoracic ultrasound examinations were performed using the LOGIQe – R7 device (GE, United States), with a 12-MHz linear transducer and with the aid of acoustic gel. Images were obtained through left and right parasternal windows.

The patients were sedated with isoflurane, induced via a mask, and this was maintained at a rate of 1 to 3%, using 100% oxygen, during the examination period, which had a maximum duration of 20 minutes (Carpenter and Marion 2017). Before this, a four-hour water and food fasting period was imposed. The animals remained in the supine position during the examination and were evaluated at four points in each antimere (Fig. 1).

## Results

Among the 84 primates evaluated, 18 animals presented pulmonary consolidation on thoracic ultrasonography, and this was seen in the left antimere in 11 animals and in the right antimere in seven animals (Table 1).

Among the 18 animals that presented areas of consolidation on ultrasound examination, non-aerated consolidation and presence of B-lines (Fig. 2) and consolidation with aerial bronchograms (Fig. 3) were found.

After these examinations had been concluded, two animals died: number 43, a two-year-old individual of *Callithrix jacchus*; and number 46, a fifteen-year-old individual of *Callithrix penicillata*. Both of them had shown lobar consolidation on ultrasound examination. These animals were necropsied and the findings are described below:

## Case 1

This was a two-year-old female *Callithrix jacchus* (common or white-tufted marmoset) weighing 294 grams. It was asymptomatic but presented left-lobar consolidation, diagnosed on pulmonary ultrasound. The main findings from the necropsy consisted of marked pulmonary congestion with hemorrhagic foci dispersed in all pulmonary lobes (Fig. 4a,b,c). Histopathological analysis revealed that the lungs showed marked infiltration of necrotic neutrophils in bronchiolar and bronchial lumina, interspersed between bacterial clumps (rods and cocci) and necrotic cellular remains. The alveoli exhibited marked peribronchial edema and the interstitium presented vascular congestion. The corresponding morphological diagnosis was suppurative bacterial bronchopneumonia.

## Case 2

This was a 15-year-old female *Callithrix penicillata* (black-tufted marmoset) weighing 392 grams. On thoracic ultrasound examination, it presented lobar consolidation; and on abdominal ultrasound examination, cholestasis. Echocardiographic examination showed concentric hypertrophy of the septum and left ventricular wall.

The necropsy revealed pulmonary congestive conditions, with hemorrhagic foci and areas of emphysema (Fig. 5a,b,c). Histopathological evaluation showed that the lungs presented marked infiltration of necrotic neutrophils in the bronchiolar and bronchial lumina, interspersed between coccoid bacterial clumps and necrotic cellular remains. The interstices presented marked vascular congestion and thickening of interalveolar septa, in addition to pulmonary alterations. Cardiomyocytes exhibited viscous nuclei and formed thick muscle fibers (hypertrophy). The morphological diagnosis for this case was suppurative bacterial bronchopneumonia and myocardial hypertrophy.

## Discussion

In veterinary medicine, the recommendation to use thoracic ultrasound is becoming more widespread, as also are studies on this topic. In a study using pigs as the animal model, thoracic ultrasound was found to be useful for managing pneumonia, especially of viral origin (Wolfram et al. 2020). In a study on dogs and cats, findings of ultrasound alterations were associated with a diagnosis of pneumonia. These alterations consisted of thickened or irregular pleura ( $p = 0.034$ ), consolidation ( $p = 0.032$ ) and absence of nodular lesion or mass ( $p < 0.001$ ) (Lin et al. 2020).

Abdominal ultrasound examinations on non-human primates, both for morphological and for hemodynamic evaluation, have already been well described in previous studies, with the aims of either diagnosing diseases or making periodic evaluations, especially with regard to evaluation of the reproductive system (Wilkinson 2008; Van Diepen et al. 2012; Zambon et al. 2018; Pissinatti et al. 2019; Wang et al. 2022).

Although use of chest ultrasound examinations for diagnosing diseases among humans is increasing, there are still no studies on non-human primates yet. The present study was the first to explore this topic and correlate ultrasound findings with diagnoses. It is known that pulmonary ultrasound is able to identify physical alterations in superficial lung tissue that correlate with histopathological findings, which often cannot be identified on chest x-rays (Soldati et al, 2020). In humans, chest ultrasound examinations have been found to have accuracy similar to that of tomography and to surpass that of chest x-rays, in patients with acute respiratory failure (Tierney et al. 2019). Moreover, this result was found to be the same as observed among children with pulmonary tuberculosis (Heuvelings et al. 2019).

Among humans with pneumonia due to SARS-CoV-2, predominance of B-lines was seen to be the main ultrasound finding, followed by the presence of lobar consolidation (Kong et al. 2021). This differed from the most common alterations seen on thoracic ultrasound examinations on the marmosets, which was the presence of lobar consolidation.

In the era of SARS-CoV-2, several studies have emerged related to the use of pulmonary ultrasound in cases of pneumonia, in addition some pictures demonstrated the sensitivity of ultrasound examination superior to chest X-ray (Staub et al. 2019; Kameda et al. 2021). These studies corroborate a study by Musolino et al. (2020) in pediatric patients with SARS-CoV-2, with symptomatic symptoms, in which pulmonary involvement was observed in all individuals evaluated, with the presence of vertical artifacts, consolidations or pleural irregularities. Therefore, pulmonary ultrasonography has gained prominence because of its ease of execution at the bedside and the clarifications about the condition of pulmonary parenchyma that it provides.

In a study on newborn humans with inflammatory bronchial disease, single or multiple consolidations of variable size, with diameters smaller than 1 cm, were observed in subpleural or pleural locations. Changes to pleural integrity and presence of variable quantities of B-lines were also detected (La Regina et al. 2021). No ultrasound evaluations on newborn non-human primates have ever been conducted, but in the present study it was observed that the mean age of the animals with consolidations was 3.9 years, i.e. relatively young animals, except for the 15-year-old animal that died (case 2), which was already an elderly individual (Fig. 5).

In a study on human patients without any history of inflammatory or infectious disease who underwent mechanical ventilation, sequenced pulmonary ultrasound was performed to evaluate the accuracy of the method for diagnosing pulmonary consolidations. The characteristics acquired through mechanical ventilation procedure were the following: areas of homogeneous appearance with absence or only slight presence of bronchograms; bronchograms with pleural effusion; bronchograms of heterogeneous appearance with the presence of dynamic aeration; bronchograms of heterogeneous appearance with static air bronchograms; and bronchograms of heterogeneous appearance with fluid bronchograms. Sensitivity of 100% and specificity of 60% was found in cases of consolidations with dynamic or static aeration (Berlet et al. 2015). These results demonstrate the importance of evaluation of non-human

primates by means of ultrasonography, especially when these animals are in captivity and in contact with humans.

A meta-analysis conducted by Staub et al. (2018) showed that presence of consolidations may form useful confirmatory evidence for pneumonia, but that may not be the best diagnostic sign for ruling out this disease. In the present study, two patients with consolidations seen on ultrasound examination presented confirmation of pneumonia in postmortem examinations.

The histological similarities of the respiratory tract between humans and non-human primates demonstrate that the same pathophysiological processes occur during respiratory diseases (Lemaitre et al. 2021). Nonetheless, even though many studies have used non-human primates as models, such as for monitoring immune responses and pharmacological tests and even for infectious disease research (Walker et al. 2019; Hobbs and Reid 2021; Roubidoux and Schultz-Cherry 2021), the use of thoracic ultrasound for investigating respiratory diseases in these animals is not a subject that has been much addressed so far. Thus, the present study demonstrates the use of pulmonary ultrasound examination as a tool for diagnosing respiratory diseases, often in individuals that are still asymptomatic or that have nonspecific signs. Thus, this examination should be included as an instrument within health screening for these species and can contribute to early diagnosis, thus collaborating with their conservation.

Studies on non-human primates with SARS-CoV-2 infection have explored radiography and chest tomography as diagnostic and patient monitoring methods. However, ultrasound was not addressed as a method. Monitoring based on tomography as an investigative method can hinder early diagnosis, since this is an examination that requires general anesthesia for these animals and has a high cost (Maaskant et al. 2021; Stammes et al. 2022).

Ultrasound examination presents limitations regarding the device, probe and operator. Nonetheless, pulmonary ultrasound is more sensitive because it is able to identify small alterations that are not detectable on thoracic radiographic examination (Staub et al 2018; Hansell et al. 2021; Pérez 2021).

## Conclusion

Chest ultrasound can become a routine examination method, given that it is noninvasive, and it is a useful tool for diagnosing lung disorders. In the light of the cases presented here, pulmonary consolidations diagnosed through thoracic ultrasound examination on *Callithrix* sp can be correlated with occurrence of pneumonia.

## Declarations

### Acknowledgements

We would like to thank the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES)-Programa de Suporte à Pós-Graduação de Instituições de Ensino Particulares (PROSUP) - Brazil for

## Financial Support.

All the team members from Project Mucky, Itu – São Paulo – Brazil, for allowing us to assess your precious non human primates

We would like to express our very great appreciation to Dr. Leonardo Dourado -Pathologic anatomy- Belo Horizonte – Minas Gerais – Brazil, for his valuable and constructive suggestions during the development of this research work. His willingness to give his time so generously has been very much appreciated

## Statements & Declarations

### Funding

“The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.”

### Competing Interests

“The authors have no relevant financial or non-financial interests to disclose”.

### Author Contributions

“All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Jéssica Amancio Martins, Melina de Souza Castilho Balbuena, Soraya Kezam Málaga and Cidéli de Paula Coelho. The first draft of the manuscript was written by Jéssica Amancio Martins and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.”

### Ethics approval

“This study was performed *in accordance with the* following the regulatory guidelines and standards for research involving animals, in accordance with Federal Law No. 11,794 (Arouca Law), the CONCEA resolutions, which establish procedures for the scientific use of animals in the country, and State Law No. 11,977/05, which institutes the São Paulo State Animal Protection Code, Ethics Committee of University Santo Amaro (Date:29/10/2021/No57) and SISBIO 78874-1”

### Data Availability

“The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.”

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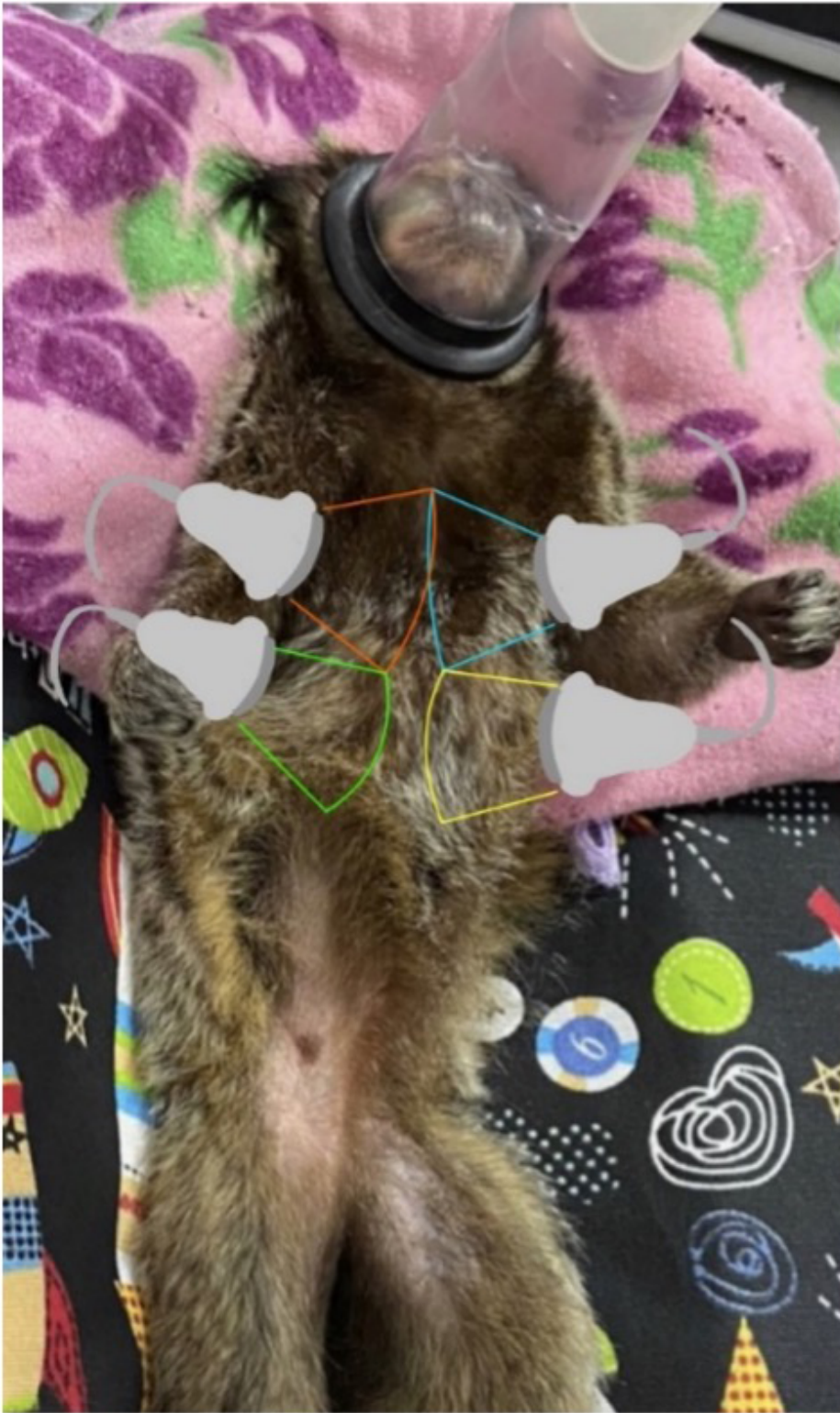
## Table

Table 1: Data from animals with pulmonary alteration on thoracic ultrasound

Animal number	Age (years)	Weight (grams)	Chest US alteration	Antimere
4	5	348	consolidation	left
16	6	358	consolidation	left
17	2	222	consolidation	right
26	2	292	consolidation	right
27	9	380	consolidation	right
28	1.6	304	consolidation	right
32	2	348	consolidation + B-line	left
40	6	374	consolidation + B-line	left
43	2	294	consolidation	left
44	2	256	consolidation	right
46	15	392	consolidation	right
49	2	368	consolidation	left
71	7	306	consolidation	left
74	4	350	consolidation	left
75	7	684	consolidation	left
78	2	292	consolidation + B-line	left
79	6	404	consolidation + B-line	right
83	2	312	consolidation	left

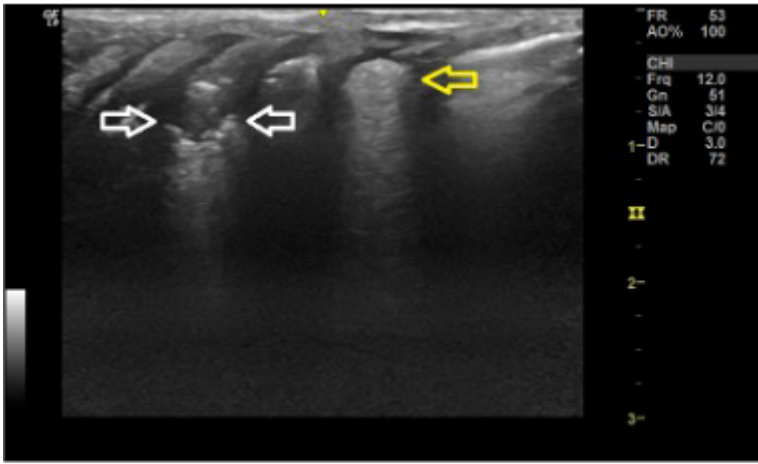
Source: author (2022)

## Figures



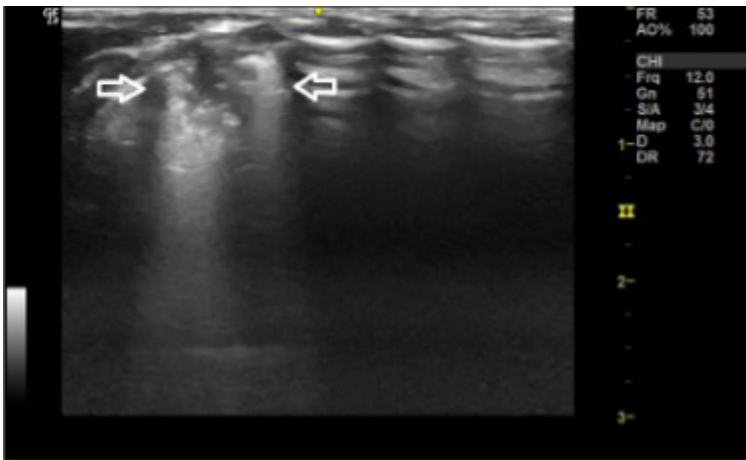
**Figure 1**

image showing the points evaluated using the chest transducer, on *Callithrix*sp.



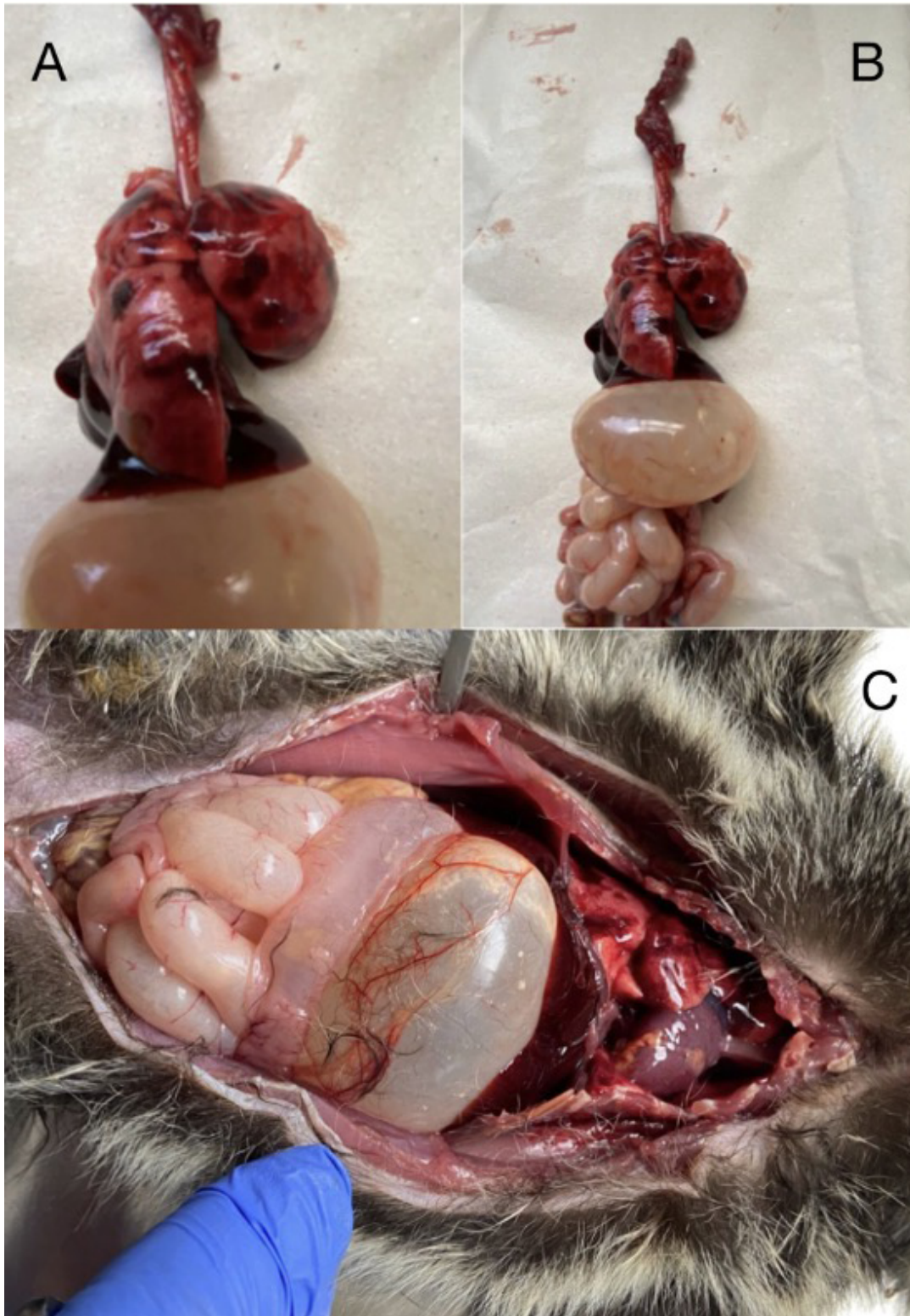
**Figure 2**

Ultrasound image of non-aerated lobar consolidation, shown by a delimited hypoechoic area, with no aeration inside (white arrow tip) and presence of B-lines promoting a vertical reverberation artifact caudally to the consolidation (yellow arrow).



**Figure 3**

Ultrasound image of lobar consolidation with dynamic air bronchograms, represented by a hypoechoic area of poorly defined outline, with the presence of multiple hyperechoic vertical lines inside this area.



**Figure 4**

A: Congested and swollen lungs with hemorrhagic foci dispersed throughout the pulmonary parenchyma; gastric distension with presence of air and liquid inside. Fig 4B: Pulmonary congestion and hemorrhagic foci dispersed in pulmonary lobes; stomach distended due to fluid and air content, with distension of intestinal loops. Fig 4C: Thoracic cavity showing generalized congestive impairment of the lungs, with

hemorrhagic foci scattered across the parenchyma of the pulmonary fields; and, in the abdominal cavity, presence of loops and stomach distended due to air and fluid.



**Figure 5**

A and B: Congested pulmonary lobes, with hemorrhagic foci in the parenchyma; stomach and intestinal loops distended due to gas; and hepatomegaly. Fig 5C: Presence of multifocal emphysematous lesions in the pulmonary field.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementarydata.pdf](#)