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Thoracoscopy pulmonary biopsy by two portals with a novel pre-tied loop ligature device in rabbits: an experimental study

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ABSTRACT: *Thoracoscopy is replacing open lung biopsies because it is less invasive, usually the technique is done using three portals and intracorporeal suture technique. This study described the feasibility and efficacy of a novel pre tied loop ligature and to propose a thoracoscopic access strategy with two portals to perform lung biopsy in patients under 5 kg. Ten rabbits were positioned in dorsal recumbency. Total thoracoscopic lung biopsy was performed using a combined transdiaphragmatic approach and a right intercostal approach. A pre tied loop ligature was placed to perform a caudal lung lobe biopsy. Insufflation of the thoracic cavity was not performed. The total surgery time was 41.4 ± 14.5 min. The procedure was carried out free of complications that prevented slippage or tightening the knot or that made it come loose after the lung biopsy; there was no serious complication during the surgical procedure. The samples obtained averaged 1x0.64x0.45 cm (Length, Width, Depth) and were considered satisfactory according to the histopathologic evaluation. Thorax radiographs taken before and after the surgeries were compared and showed no pneumothorax or hemothorax. Necropsy confirmed no knot failure occurred at the biopsy site. The use of the novel pre tied loop ligature is a safe and effective technique, avoiding problems with the limited size of the thoracic cavity in small patients.*
Key words: *endoloop, lung biopsy, small patients, thoracic surgery, transdiaphragmatic thoracoscopy and videosurgery.*

Biópsia pulmonar por toracoscopia por dois portais com uma nova ligadura de alça pré-amarrada em coelhos: um estudo experimental

RESUMO: *A toracoscopia está substituindo as biópsias pulmonares abertas por ser menos invasiva. Usualmente a técnica é feita utilizando três portais e ligaduras feitas intra corporalmente. O objetivo deste estudo foi descrever a viabilidade e eficácia de uma nova ligadura com alça pré-amarrada e propor uma estratégia de acesso toracoscópico com dois portais para realizar biópsia pulmonar em pacientes com menos de 5kg. Dez coelhos foram posicionados em decúbito dorsal. A biópsia pulmonar por toracoscopia total foi feita pela abordagem combinada transdiafragmática e abordagem intercostal direita. Uma nova ligadura pré-amarrada foi colocada para realizar uma biópsia do lobo pulmonar caudal. Não foi realizada insuflação da cavidade torácica. O tempo total de cirurgia foi de 41,4 ± 14,5 min. Os procedimentos foram realizados sem complicações que impedissem o escorregamento ou aperto do nó ou que o soltassem após a biópsia pulmonar; não houve nenhuma intercorrência grave durante o procedimento cirúrgico. As amostras coletadas tinham tamanho médio 1x0,64x0,45 cm e foram consideradas satisfatórias de acordo com a avaliação histopatológica. As radiografias de tórax feitas antes e depois das cirurgias foram comparadas e não mostraram pneumotórax ou hemotórax. A necropsia confirmou que não ocorreu falha do nó no local da biópsia. Conclui-se que uso da nova ligadura com alça pré-amarrada é uma técnica segura e eficaz, evitando problemas com o tamanho limitado da cavidade torácica em pacientes pequenos.*
Palavras-chave: *biópsia pulmonar, cirurgia torácica, endoloop, pequenos animais, toracoscopia transdiafragática e videocirurgia.*

INTRODUCTION

Thoracoscopy is a therapeutic and diagnostic tool used in small patients that offers several advantages over open thoracotomy, including a reduction in signs of postoperative pain, more rapid return to function, and fewer wound complications (MONNET et al., 2009; SCHIMIEDT, 2009). Thoracoscopy is indicated for many diagnostic and surgical procedures, and lung biopsy currently

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being the primary use of this procedure in veterinary medicine. In dogs and cats, interstitial lung diseases (ILD) are sporadically described and generally poorly characterized. Histologic examination of lung tissue by lung biopsy in patients with ILD is useful to confirm the diagnosis, select appropriate treatment, and guide prognosis in humans and veterinary patients (NORRIS et al., 2002; LIEBERMAN et al., 2017). Studies in humans and canine model warn about the technical challenges during thoracoscopic procedures to minimize rib spreading and lesion in the intercostal nerves, as well as prevent intercostal muscle tearing (LIN et al., 2013; VON HOLZEN et al., 2013; NAN et al 2015; LIU et al., 2016). In the current study, transdiaphargmatic approach offer benefits such as preventing injuries to intercostal nerves and vessels due to the bypass of the intercostal space. Additionally, a novel pre tied loop ligature does not need intercostal portal placement.

Thoracoscopic surgery has led to the development of vascular staplers that can be passed through ports. The endostaplers have been used in veterinary patients for pulmonary procedures and are generally very reliable with a low rate of complications directly attributable to the staplers (LAKSITO, 2010; MARVEL & MONNET 2013; CASE, 2016). Although, endoscopic stapling devices commonly used for thoracoscopic lung procedures in dogs and cats are too large to be safely placed into the thoracic cavity of patients less than 5kg without risk of iatrogenic damage to the surrounding anatomic structures (PONSKY & ROTHENBERG, 2008; CRONIN et al., 2019). CRONIN et al. (2019) have documented that most linear stapling devices have been designed and manufactured for human patients, so they are not optimized for use in dogs and cats; they can be bulky and awkward to place correctly during surgery, particularly in smaller patients. In addition, PONSKY & ROTHENBERG (2008) report that obtaining thoracoscopic lung biopsies with an endo stapler is proportionately large in small pediatric patients and may not even represent an improvement over mini open thoracotomy in humans.

Techniques for peripheral lung ligation and biopsy sampling, such as pre tied loop ligatures, stapling and vessel sealing devices have been extensively researched in cadaveric canine specimens (MARVEL & MONNET, 2013; BRÜCKNER et al., 2019). The use of a pre tied loop ligature allows the size-appropriate instrumentation and flexibility of the pre tied loop ligature allows for more discreet and size-appropriate biopsies to be taken. The specimens are also quality because of the limited traction placed on the biopsy side, since they are obtained in situ. Additionally, the pre tied loop ligature has proven to provide an air- and watertight seal in horses, exotic animals, and dogs (RELAVE et al., 2008; MEHLER, 2011, CRONIN et al., 2019).

Literature regarding the use of such minimally approaches for lung biopsy in pediatric patients and small dogs and cats is sparse. Thoracoscopic procedures in small patients are challenging (MEHLER, 2011; RADLISKY, 2014; MAYHEW et al., 2015). The present study chose rabbits' experimental models to reproduce the small size of the thoracic cavity, the smaller work area and the use of smaller instruments. The objective of this study was to describe and evaluate the use of a novel pre-tied loop ligature to perform thoracoscopic lung biopsy in rabbits.

MATERIALS AND METHODS

Pre-tied mini loop ligature

The miniature extracorporeal slipknot device was assembled by a 14G x 2" hollow needle (2.1 x 51.0 mm [diameter x length]) taken from a peripheral vascular flexible catheter kit, and a 16G x 3" Tuohy needle (1.8 x 90.0 mm [diameter x length]). The peripheral vascular needle was used as a miniature trocar and the Tuohy needle as a miniature knot pusher. Meltzer's knot was prepared with 2-metric nylon (Ethilon™; Ethicon, São Paulo, SP, Brazil) to perform peripheral lung sampling. The set Tuohy needle-slipknot can advance into the thoracic cavity through the peripheral vascular hollow needle (Figure 1).

Surgical procedure

Ten adult male New Zealand white rabbits weighing between 3.0 and 4.0 kg were used. The animals came from a producer specialized in the species. Blood samples (2ml each) were taken from the jugular vein preoperatively. The selected rabbits were declared fit for the study following clinical and haematological examination (blood counts, alanine aminotransferase, creatinine, and total protein).

Prior to the thoracoscopy technique, all rabbits underwent radiographic evaluation of the thorax in the laterolateral and ventrodorsal projections. The radiographic images were used to verify the absence of possible lung disorders.

Antibiotic prophylaxis was performed with enrofloxacin (Chemitryl® 2.5%) [5 mg/kg], administered in SC dose 120 minutes prior to the procedure. Rabbits were anesthetized using a standardized protocol. Initially, the rabbits received intramuscular pre-anesthetic medication composed of

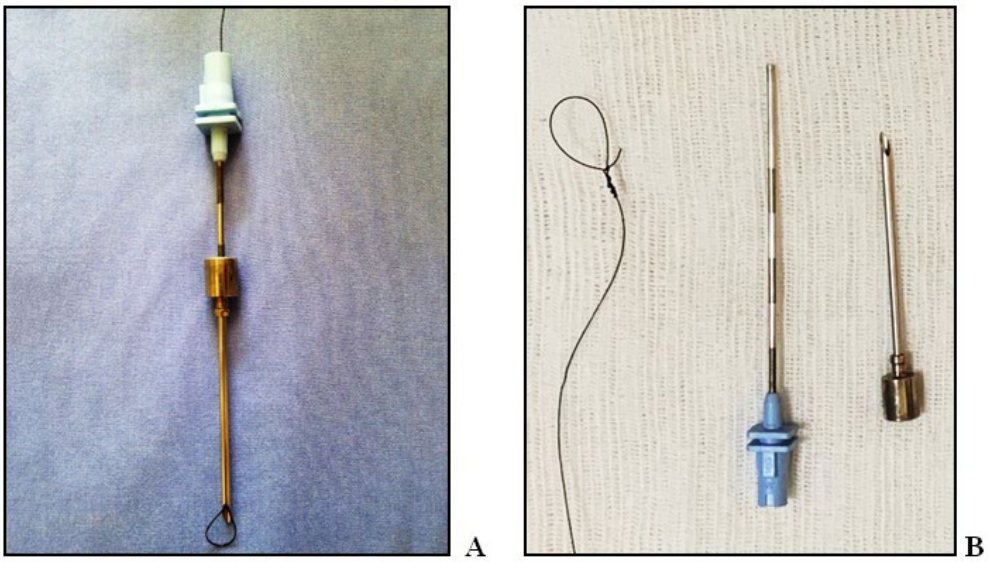

Figure 1 - A) Final setting of pre-tied loop ligature used for thoracoscopic lung biopsy in rabbits. A 14 G needle used as a 2-mm miniature port. The 16 G Tuohy needle is used as knot pusher. A small loop of the Meltzer's knot is seen as it exits the needle. Both knot and knot-pusher pass through the 14 G needle. B) Components of pre-tied loop ligature used for thoracoscopic lung biopsy in rabbits: 2-metric nylon pre-tied Meltzer's knot; knot pusher with straight tip to avoid damage to the suture thread and cannulated access needle with sharp bevel tip for percutaneous approach.

morphine (Dimorph®) [1 mg/kg] and acepromazine (PromAce®) [0.05 mg/kg]. Anesthetic induction was performed with isoflurane (Isoflurano, Instituto Bioquímico), using an air sealed face mask. After the animals were anesthetized, 10% spray lidocaine (Xylestesin®) was instilled into the oral cavity and, after dorsiflexion of the neck, orotracheal intubation was performed. The endotracheal tube was advanced through the larynx into the trachea; successful endotracheal intubation was confirmed by a characteristic capnogram. Capnography was useful in determining the correct placement of an endotracheal tube by detecting CO_2 in exhaled gases. The animals were maintained under spontaneous ventilation until the thorax was opened, after which assisted ventilation was initiated. Ventilation assistance was adjusted according to pulse oximetry (oxygen saturation) and capnography (end-tidal carbon dioxide [CO_2] measurement). Assessment of heart rate (HR), electrocardiogram, respiratory rate (RR) and body temperature was performed using a multiparametric monitor (DX 2023, Dixtal Biomedica) during anesthesia and surgery. We also assessed arterial blood pressure. Each rabbit was placed on a thermal mattress during surgery.

Thoracic skin was widely clipped, and rabbits were positioned in dorsal recumbency. For standardization, only the right hemithorax was approached and the right caudal lung lobe was sampled in all animals. In this study, thoracoscopy was carried out without one-lung ventilation or pleural CO_2 insufflation. The skin was aseptically prepared using chlorhexidine 2%.

The surgery procedures were performed by a main experienced surgeon (surgical residency, MSc. and PhD. degrees in the field; MCNW)" and an assistant surgeon with experience in the field. The instrument portals for transdiaphragmatic approach and intercostal approach were established based on the techniques previously described by Mayhew et al. (2012); Radlinsky (2014) and Fratini et al. (2018).

The thoracoscopic lung biopsy was performed with the following procedures:

1. The first portal was inserted at the 9th right intercostal space. A 0.5 cm small incision was made through the skin and parallel to the cranial surface of the ribs. A Halsted hemostatic forceps was inserted for intercostal musculature divulsion, pleura perforation and consequent induction of pneumothorax. A 5-mm thoracoscopic cannula was inserted dorsal to the costochondral junction in the 9th intercostal space.

2. A 4 mm - 18 cm 0° telescope was inserted at the 9th intercostal space for initial evaluation of the thoracic cavity. The endoscope was positioned to evaluate all right lung lobes and visualize the diaphragm region for video-assisted insertion of the second trocar, by the transdiaphragmatic paraxiphoid approach.
3. A cutaneous incision was made with approximately 0.5 cm of extension, longitudinally to the ribs, next to the xiphoid process (paraxiphoid). Allis hemostatic forceps were grabbing and elevating cutaneous subcutaneous and muscular tissue before cutaneous incision by a blade, to reduce the occurrence of iatrogenic lesions to the pulmonary parenchyma in the moment of insertion of the trocar. A blunt trocar-cannula was inserted directed cranially, and slightly laterally and ventrally, to avoid the mediastinum.
4. The endoscope was removed from the intercostal approach and positioned through the transdiaphragmatic approach.
5. A 5 mm - 30 cm laparoscopic Babcock (atraumatic) grasping forceps for lung clamping was inserted through the portal in the 9th intercostal space.
6. A needle portal was established under thoracoscopic guidance. The pre-tied mini loop ligature was placed to prevent injury to intercostal vessels and nerves (Figure 2A).
7. A 14 G needle was inserted through the 7th intercostal space, slightly ventral to the costochondral junction (Figure 2B).
8. The Babcock forceps was passed through the loop of the Meltzer's knot and elevate the tip of the right caudal lung lobe (Figure 2C).
9. The slipknot was advanced over the grasped peripheral lung tissue and tightened using the miniature knot-pusher (Figure 2D).
10. The pre-tied mini loop ligature device was removed, and endoscopic scissors was inserting to transect the tip of lung and severed the suture end.
11. The specimen was removed from the thoracic cavity through the instrument port.
12. The precise site of biopsy was recorded, and the lung stump was carefully evaluated for bleeding or ligature slippage once the biopsy was released (Figure 2E).

After the procedures, the portals were removed. The intercostal musculature was sutured with 3 metric polyglactin 910 (Vicryl; Ethicon) using interrupted absorbable sutures and the skin was sutured with 3 metric nylon (Ethilon; Ethicon) using simple interrupted sutures. Prior to the last suture, the lung recruitment maneuver was administered to expand the lung, remove atelectasis and improve lung function. After the last skin suture, thoracocentesis was performed on the 8th intercostal space with 23 G butterfly needle to ensure no intrathoracic air or liquid were present. No chest drains were left in postoperatively

The animals were medicated with subcutaneous tramadol hydrochloride (Tramal®) [4 mg/kg] every 8 hours for five days, subcutaneous meloxicam (Maxicam® 0.2%) [1 mg/kg] every 24 hours for two days, and subcutaneous enrofloxacin (Chemitryl® 2.5%) [5 mg/kg] every 12 hours for seven days.

Samples were measured, identified, kept in a 10% buffered formalin solution and then submitted for histological analysis. The size of the pulmonary biopsy was not standardized between rabbits. All samples were evaluated by the same pathologist through the Hematoxylin-Eosin staining technique.

Postoperative assessment

The animals underwent thoracic radiography in three projections in the immediate post-operative period and on the second and fourth days after the surgery. Thorax radiographs taken before and after the surgeries were compared to verify the absence of pneumothorax or hemothorax. Thorax X-rays were evaluated comparatively by same radiologist.

Pain assessment was performed by an investigator using evaluates posture and behavior, wound palpation, and abdominal tension. The animals were closely monitored daily for rectal temperature, respiratory rate, heart rate, pupil dilation, searching behavior, interaction with the environment and standing.

Respiratory parameters were assessed daily for 15 days, including lung auscultation, respiratory rate, and color of mucosae. In the postoperative clinical evaluation of all animals, dyspnea, stridor in the upper airways, or other complications related to the respiratory tract were evaluated during the study. In addition, possible changes such as the presence of subcutaneous emphysema, seroma, local infection and dehiscence of stitches were observed.

Post mortem examination

Fifteen days after the procedure, the rabbits were prepared for euthanasia. The right auricular vein was catheterized for propofol administration to reach a deep anesthetic plane verified by apnea and total mydriasis. Then, through the same intravenous route, potassium chloride was administered until permanent cardiorespiratory arrest. The postmortem examination was made. The right hemithorax was examined for ligature failure (ligature slippage and suture breaking), iatrogenic lung damage, pleural effusion and postoperative adhesions.

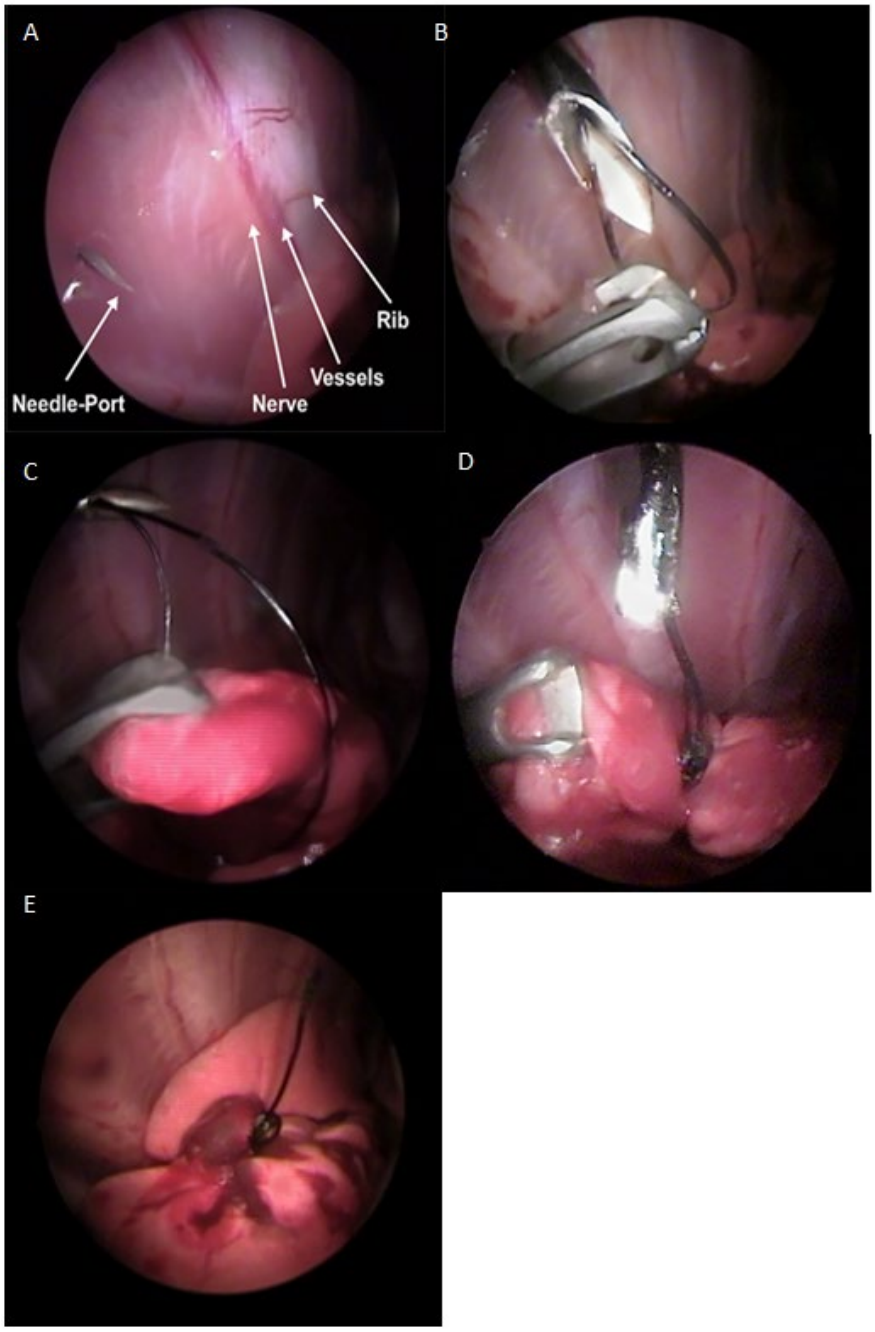

Figure 2 - A) Thoracoscopic lung biopsy in rabbits. Intraoperative view of the needle portal (mini-trocar) through the intercostal approach. Note that the novel pre-tied mini-loop ligature prevents injury to intercostal vessels and nerves. B) Thoracoscopic lung biopsy in rabbits. The 14 G needle-port in inserted through the 7th intercostal space and the Babcock forceps is advanced through the Meltzer's knot's loop over the anticipated biopsy site. C) Thoracoscopic lung biopsy in rabbits. The periphery of the right caudal lung lobe is encircled with a pre-tied loop ligature and grasped with Babcock forceps for sampling. D) Thoracoscopic lung biopsy in rabbits. The miniature knot-pusher is advanced and tightens the knot like a snare at the base of the lung tissue to be resected. E) Thoracoscopic lung biopsy in rabbits. Lung stump after lung lobe biopsy with a pre-tied loop ligature.

Lungs were collected from rabbits euthanatized in this study. Lung lobes were harvested immediately after euthanasia and separated. Entire lung sets with tracheas were harvested from rabbits. An endotracheal tube was used to intubate the right main bronchus bronchi (Figure 3). Endotracheal tube was connected to a continuous flow anesthetic machine. Lungs were slowly reinflated and pressurized to 20 cmH_2O to eliminate atelectasis and then submerged in water and assessed for leaks. Leak was defined as bubbles present on the cut surface or near the suture line. Failure mode was defined as suture breaking or suture slipping.

RESULTS

Surgical technique and pre-tied loop ligature assessment

All 10 procedures were successfully completed thoracoscopically. Mean (SD) surgical time was 41.4 ±14.5 minutes (range, 20 – 65minutes). There were no intraoperative complications associated with the biopsy technique, such as bleeding from the pulmonary parenchyma, issues preventing sliding or tightening the knot or the ligature coming loose after the lung biopsy (ligature slippages). The method of introduction of the minitrocar (14 G needle) was safe, without any case of perforation or laceration of thoracic organs.

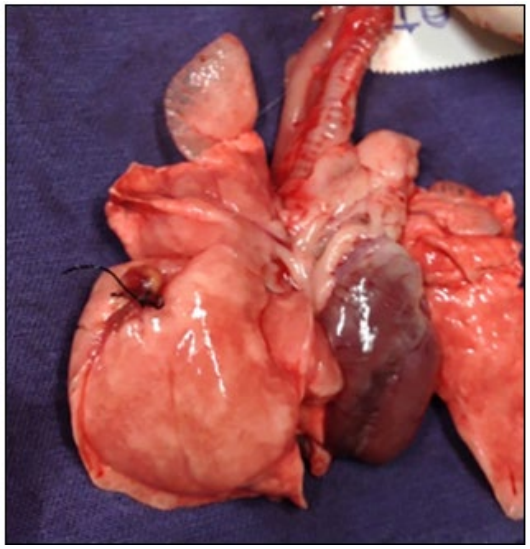

Figure 3 - Lung and trachea specimen from rabbit with the endotracheal tube in place. The lung lobe biopsied with the pre-tied loop ligature was gradually reinflated and pressurized to 20 cmH_2O and then submerged in water.

The pre-tied ligating loop fitted easily over the tissue to be resected in all ten rabbits. Lung tissue congestion distal to the knot was observed in all animals due to occlusion of blood vessels and airways. A lung stump distal to the ligature was achieved in all animals.

Histologic assessment of lung biopsy specimens

Ten biopsies were collected. The mean dimensions of the samples taken from of the right caudal lung lobe were 10.0 (±2.6) x 6.4 (±2.1) x 4.5 (±1.4) mm (length x width x height). The dimensions of the largest sample were 17x10x4mm and the dimensions of the smallest sample were 5x5x2 mm. Biopsy sizes were not standardized due inability to accurately determine the tissue sample size before tightening the ligating loop. According to the histopathology report, all samples obtained were considered satisfactory in all specimens. Main lung tissue components such as bronchioles, alveoli, blood and lymphatic vessels, and parenchyma were visualized.

Postoperative and radiographic findings

One rabbit developed a superficial wound infection at the transdiaphragmatic approach; however, the wound healed uneventfully by second intention after removal of skin sutures and wound lavage at 8 days. In the clinical evaluation of all animals, no respiratory distress, tachypnea or other clinical signs related to the respiratory tract were reported during the study. The rabbits did not present subcutaneous emphysema and seroma.

Postoperatively, thoracic radiographs results were considered normal in all animals (Figure 4). Rabbits did not show residual pneumothorax on the immediate postoperative radiography, and none presented pneumothorax on radiographic examinations after surgery. Pleural effusion was also not identified after all procedures by the same radiologist.

Necropsy and leak test by submersion in water

The macroscopic findings during necropsy were adhesion of the biopsy site to the mediastinum membrane in three rabbits (Figure 5). No adhesion was identified in the other seven animals. In all animals, the knot was properly positioned with no signs of ligature slippages and a 2 to 5 mm margin of lung tissue distal to the ligature was observed. None of the animals had signs of lesions to the lung parenchyma associated with the suture applied with a knot pusher.

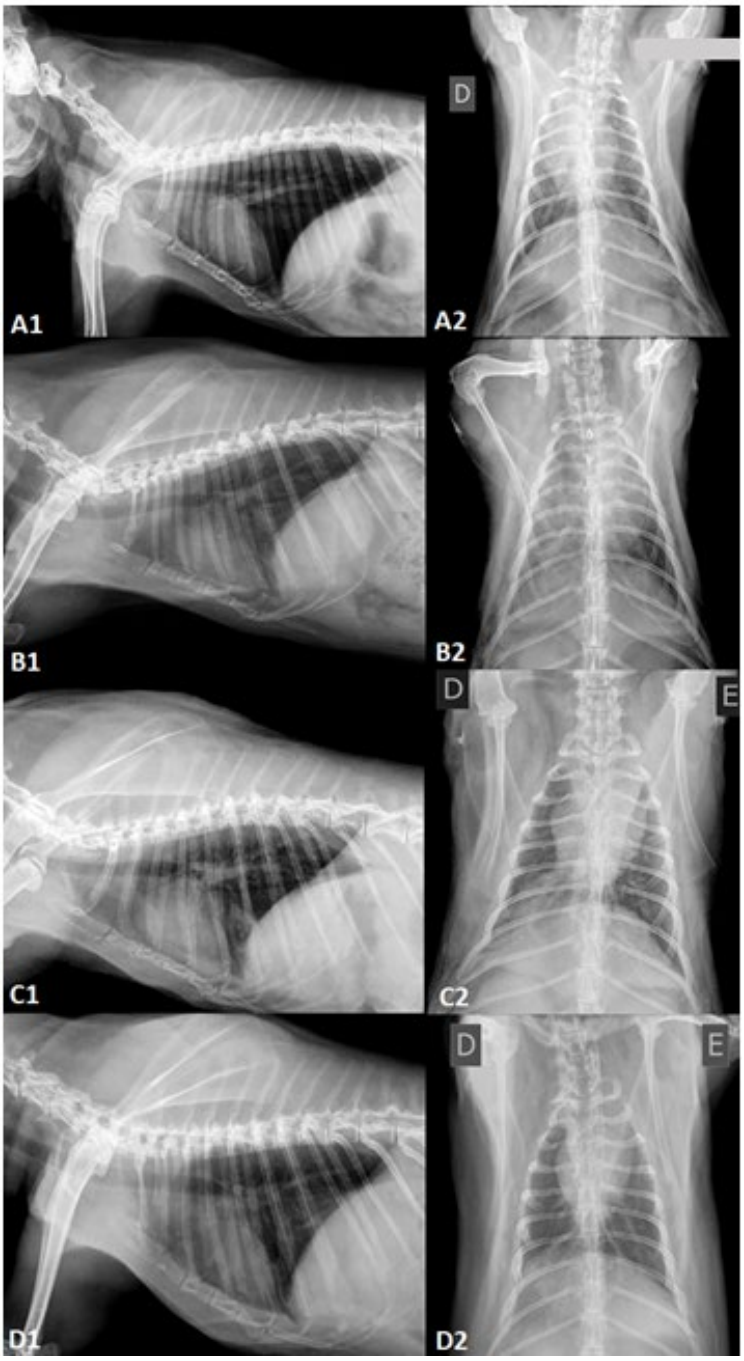

Figure 4 - Thoracic radiographs before and after thoracoscopic lung lobe biopsy in a rabbit. (A1 and A2) Preoperative lateral and ventrodorsal thoracic radiographs showing no evidence of lung disease. (B1 and B2) Immediate postoperative thoracic radiographs after right caudal lung lobe biopsy. Lateral (C1) and ventrodorsal thoracic radiographs (C2) were taken 2 days after surgery. Four days after surgical procedure, no remarkable changes compared to the preoperative radiographs were observed in the lateral view (D1) and ventrodorsal view (D2). Thoracic radiographs analysis detected no pneumothorax and pleural effusion.

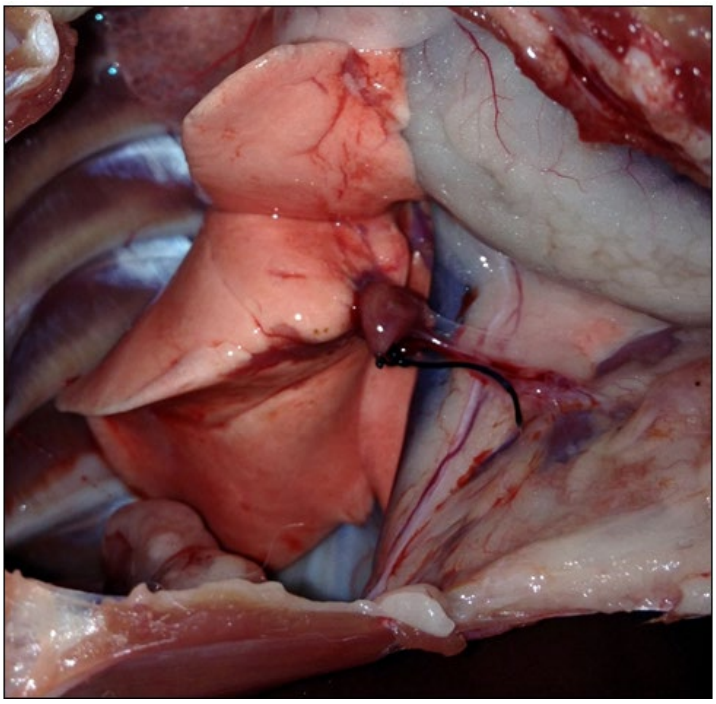

Figure 5 - Macroscopic findings in rabbit hemithorax after lung lobe biopsy performed with the pre-tied loop ligature through thoracoscopy. Adhesion of the lung biopsy site to the mediastinum membrane can be seen.

At the visually test inspecting for leaks, no air leakage occurred from the lung stump at pressure 20 cmH_2O. No leaks were observed from the suture line in all lungs. In addition, there was no slip or breakage of the knot during the test.

DISCUSSION

This study described the feasibility and efficacy of a novel pre tied loop ligature and proposed a thoracoscopic access strategy with two portals to perform lung biopsy in patients less than 5 kg. Thoracoscopy is replacing open lung biopsies because it is less invasive. Recently, minimally invasive approaches that is different from the conventional three ports thoracoscopic technique is gradually becoming of great interest in the diagnosis of interstitial lung diseases in dogs and cats. A novel pre tied loop ligature described in the present study has potential advantages, such as decrease intercostal approaches, which prevent signs of pain attributable to the spread of the ribs and prevent intercostal muscle tearing. Additionally, paraxiphoid transdiaphragmatic approach causes no lesion in the intercostal nerves and reduces postoperative pain, as previously documented by ZIELIŃSKI et al. (2018) and CHEN et al. (2020).

Thoracoscopic lung biopsy technique using biopsy forceps has been used in several species including dogs and cats with harvested samples being representative of the patient's condition. Nevertheless; although. not reported in some case reports, this inexpensive technique may require specific equipment like electrosurgery to control hemorrhage or air leakage from the biopsy site. Likewise, use of electrosurgery increases tissue thermal damage and may cause artifacts in the biopsy sample (RELAVE et al., 2008; SANTINI et al., 2008; BRÜCKNER et al., 2019). In addition, according to several authors, the most serious complication of lung biopsy or wedge resection with the thoracoscopic technique is bleeding (RELAVE et al., 2008; NAN et al., 2015; RADLINSKY, 2015). The most common complication after biopsy/wedge resection

of the lung is air leak. Possible consequences are prolonged postoperative air leak, pneumothorax requiring suction with permanent negative pressure, pleural effusion, failed thoracoscopic approach with conversion to open surgery, prolonged hospital stay, or complications requiring intensive treatment in humans (CHAKRABARTI et al., 2009; FIBLA et al., 2012; LUO et al., 2013; WIENER et al., 2013; LIEBERMAN, 2017). Considering these concerns, we favored a hemostatic technique that would avoid air leakage and so used a ligating loop technique as reported in humans, horses, pigs and dogs (RELAVE et al., 2008; OIZUMI et al. 2017; CRONIN et al., 2019).

Lung biopsy can be performed using loop ligatures, intracorporeally tied ligatures, or endo stapling equipment. Endo-staplers are far from the most used device in thoracoscopic lung surgery in the veterinary practice worldwide. However, wider access incisions are may be necessary; devices are expensive and can be cumbersome in small patients. Stapler cartridge length can also make deployment difficult in the thoracic cavity of smaller animals (PONSKY & ROTHENBERG 2008; WORMSER et al, 2014; BLEAKLEY et al., 2015; CRONIN et al., 2019). Consequently, other methods for pulmonary parenchymal sealing have been investigated. Vessel-sealing device was developed to seal the walls of blood vessels; although, some studies have tested the safety and effectiveness for peripheral lung biopsy in healthy and cadaveric dogs (MAYHEW et al., 2012; MARVEL & MONNET 2013; BRÜCKNER et al., 2019). Suture techniques are the safest and most predictable techniques to collect lung biopsies in dogs (PRAMATWINAI et al., 2006; CRONIN et al., 2019). In the present study, we developed a novel pre-tied loop ligature, which enabled instrumentation appropriate to the small size of the thoracic cavity, forming an adequate seal, in addition to being less invasive than the stapler technique and less expensive.

In the current study, thoracoscopic procedure without the additional insufflation of CO_2, was the chosen option. A recent review emphasizes that thoracoscopic procedures in cats are done within the rigid thorax and insufflation is not required (RADLINKSKY, 2014; RADLINSKY, 2015). The intrathoracic inflation with carbon dioxide in thoracoscopic procedures is not essential and promotes small distension of the chest cavity. In addition, it can cause an increase in blood pressure and compromise circulatory dynamics (LANDRENEAU et al., 1994; DALY et al., 2002; RADLINSKY, 2015). Additionally, one-lung ventilation and endobronchial blockade are not standard for thoracoscopy. One-lung ventilation may be necessary for pneumolobectomy, but is not usually used to decrease pulmonary trauma, because of its consequences with ventilation. Also, when one-lung ventilation is essential, it requires intensive monitoring and specialized equipment (MAYHEW & FRIEDBERG 2008; MAYHEW et al., 2015). Furthermore, performing biopsies on ventilated lungs may reflect a more realistic clinical situation for testing lung biopsies using suture technique (RELAVE et al., 2008).

RELAVE et al. (2008) reported that biopsies collected in equine lungs had a complication rate of 31%, including slippage of the loop ligature or bleeding from the pulmonary parenchyma. Although, the successful use of a pre-tied ligating loop for resection of tissue for diagnosis or treatment has been reported in various clinical scenarios in humans, horses, dogs and cats (PONSKY & ROTHENBERG 2008; CUDDY et al. 2013; DA CONCEIÇÃO et al. 2018; CRONIN et al. 2019). In the present study, we not observed slippage or bleeding from the pulmonary parenchyma after pre-tied loop ligature applied and biopsy collected. Probably the most important factor that contributed to not slipping the ligature was the amount of tissue distal to the ligature (between the ligation and the cut surface of the lung). Some authors recommend 5 mm of lung tissue distal to the ligature; although, these studies used larger animals (RELAVE et al. 2008; CASE, 2016; CRONIN et al. 2019). In the present experiment, a 2 to 5 mm lung tissue distal to the ligature seems to be sufficient to prevent ligature slippage after lung biopsy in small patients.

Inability to accurately determine the tissue sample size before tightening the ligating loop was considered a disadvantage of this technique. Nevertheless, the size and quality of the material collected in the present study, obtained by pre tied mini loop ligature, were considered satisfactory, even the smaller samples were adequate for histologic analysis. The results of this study corroborated previous studies that performed lung lobe biopsy using a flexible endoscope in dogs (WU et al., 2014; LIN et al., 2013; LU et al., 2015). Ideal ligature placement was dependent on our ability to manipulate the lung with the forceps and ability to manipulate the loop inside the thorax. Good positioning of the portals was essential and reduced some of these problems. Those findings corroborated the observation of PONSKY & ROTHENBERG (2008) in humans, SCOTT et al. (2019) in dogs, and SINGH et al. (2019) in canine cadaver model.

Radiographic examination is considered an effective method to diagnose pneumothorax (RELAVE

et al., 2008). Radiographically, pneumothorax and hemothorax were not detected after procedures; this fact can support that pre-tied loop ligature provided an adequate air and vascular seal in rabbits undergoing lung biopsy. Wound infection occurred but was not correlation with the pre-tied loop ligature. No other major postoperative complications occurred. These data showed that thoracoscopic lung biopsy with pre-tied loop ligature results in low morbidity rates. These findings are in line with previous studies (MONNET 2009; RADLINSKY, 2015).

At the necropsy, none of the samples leaked at 20 cmH_2O. BORGES et al. (2011) recommend inflating the rabbit's lungs to an alveolar pressure of about 15 cmH_2O. The maximum physiologic airway pressure in dogs is considered to be 20 cmH_2O (BRÜCKNER et al., 2019). In a recent study, which evaluated the vessel sealing device for peripheral lung biopsy in canine lung cadaveric lobes, concluded that none of the samples leaked below 25 cmH_2O, which is well above the physiological airway pressure in dogs (BRÜCKNER et al., 2019). CRONIN et al. (2019) reported that none of the pre-tied ligature loop (PLL) lobectomies leaked up to a pressure of 80 mm Hg in cadaveric canine lung lobe. In another study, which compared thoracoscopic lung biopsy techniques in dogs, as a result, there was a leak at 20 cmH_2O in sample with endoscopic staple and vessel sealant device, while the leak did not occur in the pre-tied loop ligature group (MARVEL & MONNET 2013). Our pressure test and results corroborated the recommendations of these previous studies.

In the present study, adhesions in the thoracic cavity were the most common findings on gross assessment. Postoperative adhesions were also reported in other studies (WEN et al., 2013; LU et al., 2015; NAN et al., 2016). Those results suggestedintrathoracic adhesions may occur regardless of pneumostasis and hemostasis technique used. One of the most important consequences of adhesion formations in the pleural space is increased risk on further thoracic surgery (LU et al., 2015; YIN et al., 2015).

There are some limitations in our study. Biopsies were collected in animals without pulmonary pathology, which may have optimized the results of the pre-tied loop ligature ability to obtain good pneumostasis and hemostasis**.** Another limitation included that the thoracic conformation differences between rabbits, canines and felines, despite the similar body weight. Additionally, the limitations of the technique included an inability to predetermine the size of the biopsy and difficulty in the accuracy of the remaining lung stump size. Finally, additional constraint includes only right caudal lung lobe was biopsied and lung biopsy for other lobes were not made; thus, further research is warranted.

CONCLUSION

We concluded that the thoracoscopic technique proposed in this study, with the pre-tied ligature developed, is executable, safe and allowed sampling lung specimens with minimal complications. The equipment is easy to manipulate and is effective in the small thoracic cavity, avoiding problems with the limited size in patients less than 5 kg.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

This study was approved by the Animal Ethics and Use Committee of the Faculdade de Ciências Agrárias e Veterinárias da Universidade Estadual Paulista (CEUA-FCAV-UNESP protocol no. 12,900/15). The study was carried out in accordance to the animal experimentation guidelines of CONCEA.

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