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CLINICAL RESEARCH

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Development of a minimally invasive endoscopic technique for excisional biopsy of the axillary lymph nodes in dogs

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Abstract

Objective: To develop and describe a minimally invasive technique for excisional biopsy of the axillary lymph nodes in dogs.

Study design: Descriptive cadaver and clinical case series.

Animals: Four canine cadavers and three clinical patients.

Methods: A 3D computed tomographic reconstruction of the canine axilla was used to identify an optimal avenue of approach to the lymph nodes. This approach was refined using endoscopic techniques in four cadavers (six procedures) and potential surgical hazards, landmarks, and the surgical time required for excisional biopsy of the nodes was recorded. The procedure was then performed in three clinical cases.

Results: Axillary lymph node removal was achieved using an endoscopic technique with surgical times of 58 and 35 minutes in two of three clinical cases. The third case required conversion to an open approach after endoscopic identification of the node. No major complications were encountered.

Conclusion: Excisional biopsy of the axillary lymph nodes can be performed successfully using a minimally invasive technique in the dog. Further investigation in clinical cases is needed to determine the risks and complications of this procedure.

Clinical significance: Minimally invasive excisional biopsy of the axillary lymph nodes in dogs can be performed and may have a role in assisting with staging and local disease control in oncologic cases.

1 | INTRODUCTION

Neoplastic disease with metastatic potential requires staging to determine prognosis and appropriate treatment options. This process frequently involves cytological or histological assessment of lymph nodes for neoplastic infiltration.[1,2,3] Excisional biopsy and histology of lymph nodes has been shown to be more sensitive than cytology for the detection of metastatic disease and may be elected over cytological assessment in select cases.[2,3] Surgical excision and histology may also be elected over aspiration and cytology due to the survival advantage conferred by excision if the node is infiltrated. Multiple studies conducted on human patients have identified reduced risk of neoplastic recurrence[4,5] and extended median survival

Abbreviations: MIS, minimally invasive surgical; STL, stereolithography.

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times[6,7] when lymph nodes carrying metastasizing cells are surgically excised. A study investigating dogs with stage II cutaneous mast cell tumors also identified therapeutic value and improved prognosis with regional lymphadenectomy.[8]

The axillary lymphocenter consists of the axillary node and the accessory axillary node,[9,10] and may pose a particular challenge to lymphadenectomy by traditional open techniques[11] due to its location deep to the musculature of the thoracic limb and immediately adjacent to the axillary vasculature. The axillary lymph node consists of a single node in 91% of dogs, with two or even three nodes detected in the remainder,[12] and is situated medial and caudal to the shoulder joint, adjacent to the axillary artery and vein. The accessory axillary lymph node is reported as variably present, and lies caudal to the axillary node on the deep aspect of the ventral border of the latissimus dorsi muscle - this node has also been reported as a double node.[12] The axillary lymphocenter drainage area consists of the mammary glands, thoracic and cranioventral abdominal walls, and the deep structures of the thoracic limb.[10] Axillary lymphadenectomy may be indicated for neoplasia in any of these areas.

Reports of axillary lymph node identification and lymphadenectomy techniques in dogs are relatively sparse with one study reporting three cases in which only two procedures were successful in locating the nodes.[13] As the size of the dog increases, an open procedure may become progressively more challenging due to the location of the node medial to the shoulder and the limitations to retraction set by the thoracic limb musculature. A minimally invasive surgical (MIS) technique may offer advantages including improved visualization of the node for identification and dissection, reduced incision size and no need for aggressive retraction. Studies investigating minimally invasive lymphadenectomy techniques conducted in human patient populations have identified equivalent or better lymph node retrieval rates with reduced blood loss and scarring compared with open approaches.[14,15] Recognized complications include lymphedema, wound infection, hemorrhage and seroma.[15] Randomized controlled trials comparing open with minimally invasive approaches for lymphadenectomy in human patient populations have proven to be challenging due to the reluctance of patients to be assigned into the open surgery arm of these studies.[16]

The objectives of this study were to describe (1) development of an MIS technique for lymphadenectomy of the axillary and accessory axillary lymph nodes in canine cadavers, and (2) the outcomes following pilot implementation of the technique in live dogs in a clinical setting.

2 | MATERIALS AND METHODS

2.1 | Study overview

This study was conducted over the period of December 2020 to July 2021, at Cornell University Hospital for Animals. The study design was approved following ethical and scientific review for IACUC exemption (CUVCSC, Cornell University Veterinary Clinical Studies Committee Protocol ID 020221-04). The first phase of the study during which the surgical techniques were refined was conducted on four canine cadavers; these animals were research dogs that were euthanized for purposes unrelated to this study. Following refinement, the technique was implemented in three clinical cases that required lymphadenectomy for staging purposes and the results reported.

2.2 | Development phase

To assist in the selection of the best surgical corridor to approach the axillary node, a 3D reconstruction of the axillary anatomy was created (Figure 1). A dog receiving a CT scan of the thorax for another purpose was positioned in right lateral recumbency with the left thoracic limb abducted. The thorax was scanned at 120 kVp using automatic MAs control (SUREExposure) in 1.0 mm transverse slices after administration of IV contrast agent

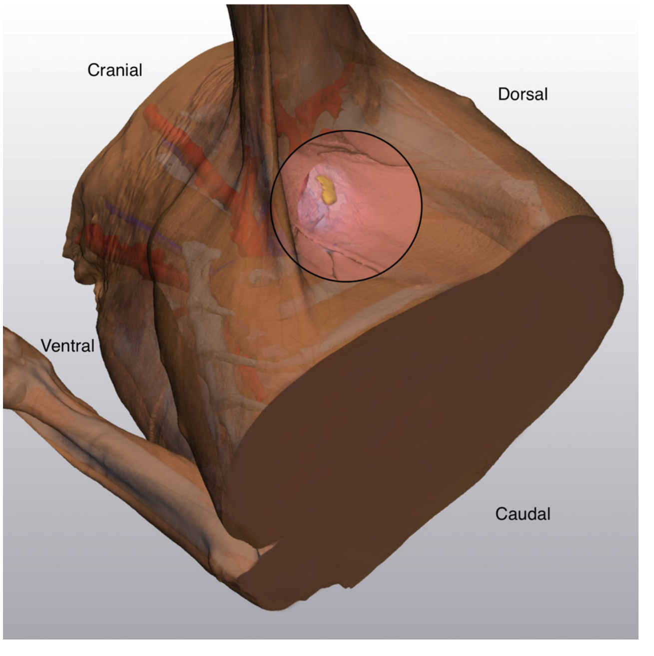

FIGURE 1 Computed tomographic 3D reconstruction of axillary anatomy.

(2 ml per kg iohexol, 350 mg/ml, Omnipaque 350, GE Healthcare, Princeton, New Jersey) using a 16-slice CT scanner (Aquilion LB, Toshiba/Canon America Medical Systems, Tustin, California). Images were reconstructed in 1.0 mm transverse slices using a soft tissue algorithm (window level 30, window width 320).

A 3D model was created from a post-contrast CT scan by anatomical segmentation of (1) the left axillary lymph node, (2) the intrathoracic and axillary region blood vessels (3) the bones, and (4) the body wall and limb musculature (Materialize Mimics, Plymouth, Michigan). Segmentation was performed using thresholding and region grow techniques.[17] The resulting layers (masks) were modified using the split mask tool to retain only the relevant anatomy. The masks were converted to parts and exported as stereolithography (STL) files. The STL files were later converted to object file format in Autodesk Meshmixer and uploaded to create a single model using Sketchfab.com (New York) where color and transparency features were subsequently applied to each layer.

Following identification of a candidate surgical corridor that would achieve an approach to both the accessory axillary and axillary nodes without muscular transection, the approach was then performed on a canine cadaver using an open technique with progressive removal and retraction of the overlying musculature to fully expose and confirm the anatomy, as shown in Figure 2. Axillary and accessory axillary lymphadenectomy was then performed on six thoracic limbs on four canine cadavers using an endoscopic technique. Two surgeons (GH and NB) performed the procedures. Endoscopic images were procured and the time to complete the lymphadenectomy recorded.

2.3 | Implementation phase

Once the authors were convinced of the feasibility and were familiar with the anatomic details of the technique, endoscopic axillary and accessory axillary lymphadenectomy were performed under general anesthesia on three clinical cases by a single surgeon (GH) following the provision of informed owner consent. For all cases, the clinical indication for lymphadenectomy was to complete staging of an oncological disease process. Perioperative analgesia was provided for all cases at the discretion of the primary clinician. Intraoperative and short-term postoperative outcomes including procedural time and complications, postoperative discomfort and lameness, and incidence of incisional complications (lymphedema, seroma) within the first three postoperative weeks were recorded.

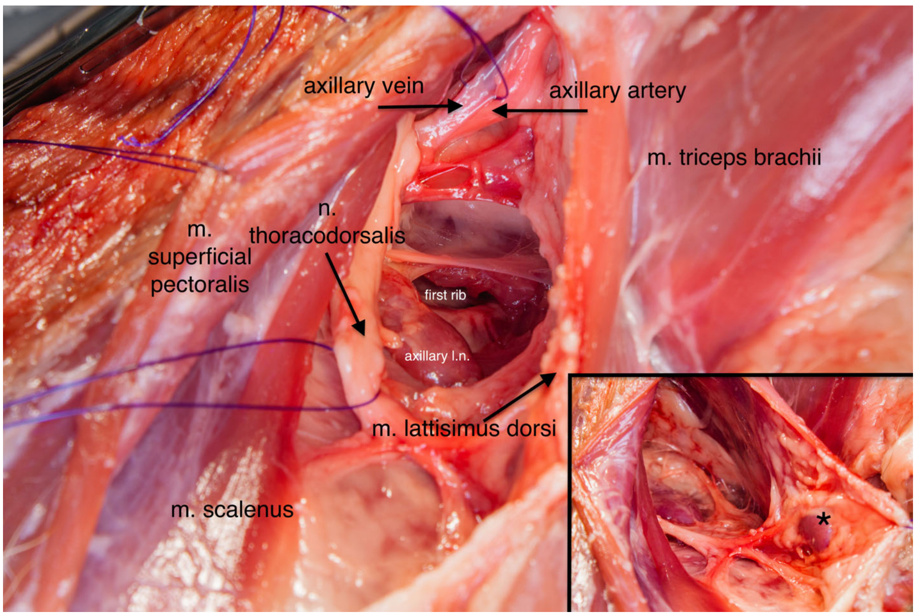

FIGURE 2 Cadaver dissection showing key landmarks of the axillary lymphocenter. The latissimus dorsi and superficial pectoral muscles have been separated and stay sutures placed to elevate and evert the musculature, shown in the near field. The accessory axillary lymph node is then visible on the ventral surface of the latissimus dorsi (inset, *). The thoracodorsal nerve bundle crosses the dissection avenue from caudodorsal to cranioventral. The axillary lymph node is then situated within a fat pad lying adjacent to the musculature of the chest wall, immediately dorsal to the thoracodorsal nerve bundle and immediately caudal to the axillary artery and vein where they exit the thoracic cavity. The first rib can be palpated just cranial to the node location.

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2.4 | Surgical technique description

The patient was prepared for aseptic surgery and positioned in lateral recumbency with operated limb positioned uppermost with the limb abducted and extended cranially. The division between the dorsal border of the m. superficial pectoralis and the ventral border of the m. latissimus dorsi was palpated and a 3 cm skin incision was made following the orientation of this division and starting at a point approximately 6 cm caudal to the caudal border of the m. triceps brachii (Figure 3). The muscular junction was separated by blunt dissection and two stay sutures were placed in the margins of these muscles. The latissimus dorsi muscle margin was elevated and reflected through the incision to expose the deep surface of the muscle belly. When present, the accessory axillary node was located adhered to the deep surface of the muscle, sometimes within a fat pad, and could be excised at this location (Figure 4). The stay sutures were then used as anchors to allow the insertion of a SILS port (Medtronic, Minneapolis, Minnesota) between the latissimus and m. superficial pectoralis margins. A 5 mm 30-degree laparoscope was then inserted and CO2 insufflation to +5 cm H_2O (3.7 mmHg) initiated. Dissection began using a blunt probe through the loose areolar fascia in a cranial direction (Figure 5 and inset), entering the space deep to the superficial pectoral and latissimus dorsi muscles, with the thoracic wall (pectoralis profundus, serrates ventralis and scalenus m) creating the floor and the axillary artery and vein and first rib acting as landmarks to the cranial limit of the dissection. Early in the dissection the thoracodorsalis n. bundle could be identified crossing the near field, and care was taken to preserve this. The axillary node could be identified within a fat pad lying adherent to the thoracic wall at the base of the axillary artery and vein, which could be seen traversing the dissection field perpendicular to the thoracic wall. Once identified, the axillary node was grasped and dissected free of the surrounding fat using a combination of a monopolar L hook and a 5 mm Ligasure device (Figure 6). The node was then retrieved with the SILS port. The surgical site was closed routinely using 3/0 PDS in a simple continuous pattern to reappose the margins of the latissimus dorsi and superficial pectoralis m., and 3/0 monocryl in the subcutaneous fat and skin.

3 | RESULTS

3.1 | Cadaver outcomes

The mean bodyweight of the cadavers ($n = 4$) employed was 10.2 kg (95% CI: 8.6–11.8). The mean time to removal

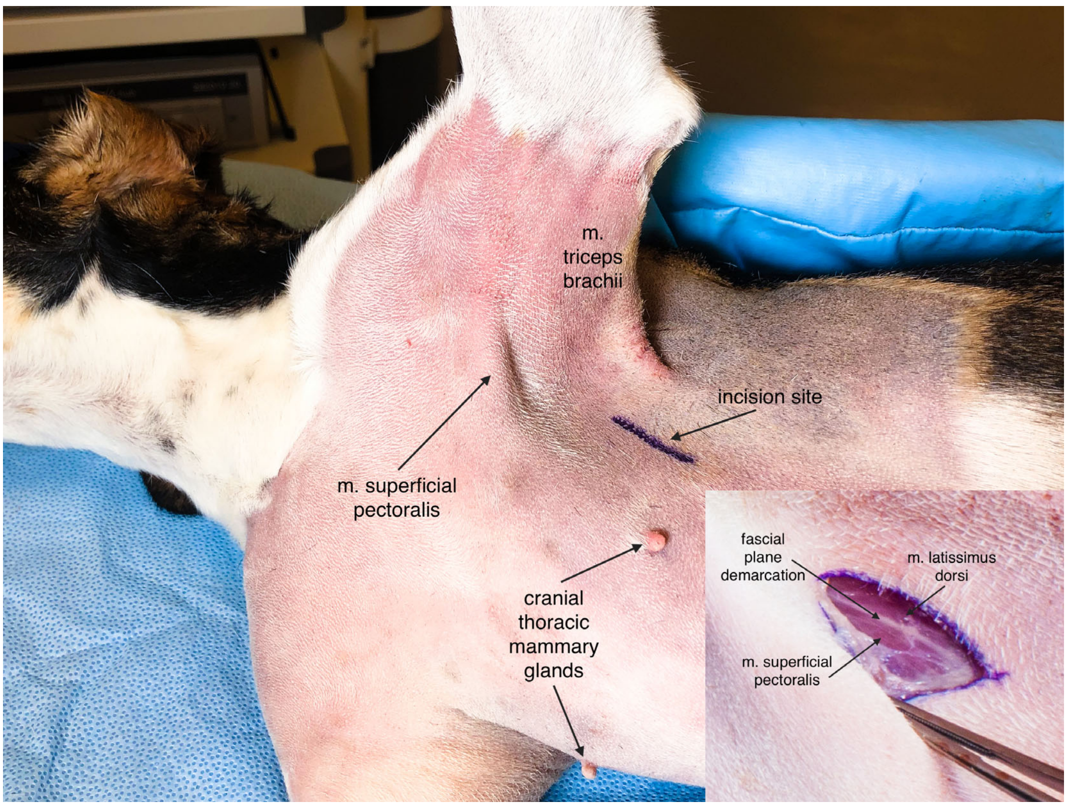

FIGURE 3 Initial patient positioning and incision location for a minimally invasive surgical (MIS) approach. Inset image shows the division between the superficial pectoral and latissimus muscle.

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of the accessory axillary lymph node over six cadaveric procedure was 5.1 minutes (95% CI: 2.3–8.0) and a single node was found in all six limbs. The mean time to removal of the axillary lymph node was 33 minutes (95% CI: 18.1–48.1), and a double node was found in one limb with a single node in the remaining five.

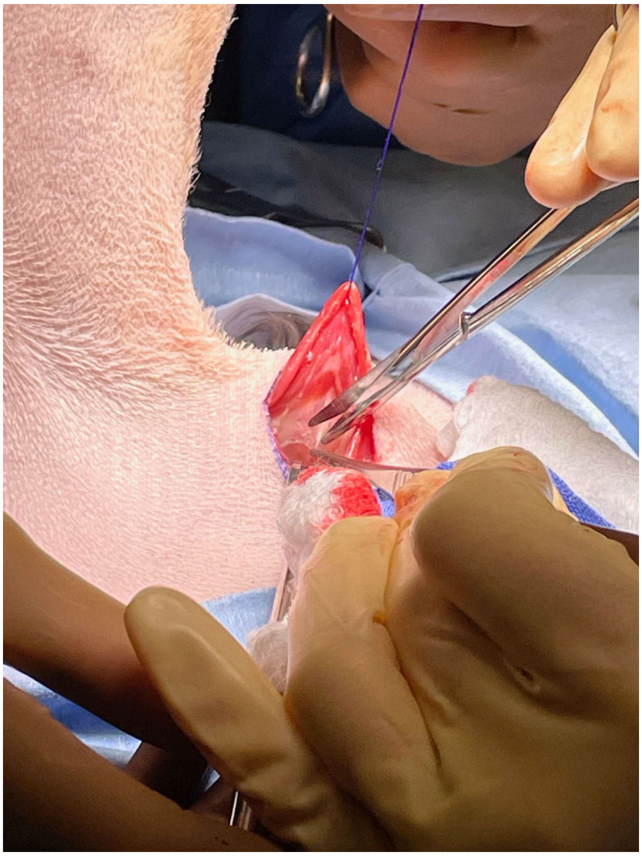

FIGURE 4 Dissection of the accessory axillary lymph node from the deep surface of the everted latissimus dorsi m via the initial SILS incision.

3.2 | Clinical case 1

A 12-year-old FS mixed breed (22.6 kg) was presented for assessment and treatment of recurrence of a mass on P2/3 of the second digit of the left thoracic limb. The mass had been previously excised 6 months prior to the visit. Histopathological diagnosis at that time was acanthocytoma; however, the dog had persistently licked at the area over the subsequent months, with increasing pain, swelling and inflammation unresponsive to antibiotic therapy. FNA of the area during a recheck appointment was suggestive but not definitive for a sarcoma. Thoracic radiographs were unremarkable. Ultrasound of the axillary lymph node revealed it to be enlarged at 2.4 cm (normal <1.6 cm[12]) in the longest dimension however ultrasound guided FNA of the node was nondiagnostic. Digit amputation at the metacarpophalangeal joint in combination with excisional biopsy of the axillary and accessory axillary lymph node was performed. The surgical time for identification, excision and closure for two axillary nodes and one accessory axillary node was 58 minutes. No intraoperative complications occurred. Postoperatively, the dog received routine analgesic management (methadone 0.1 mg/kg IV every 4 h as needed, meloxicam 0.1 mg/kg IV every 24 h). Moderate lameness was noted during the evening post procedure which persisted into the following morning, although improved.

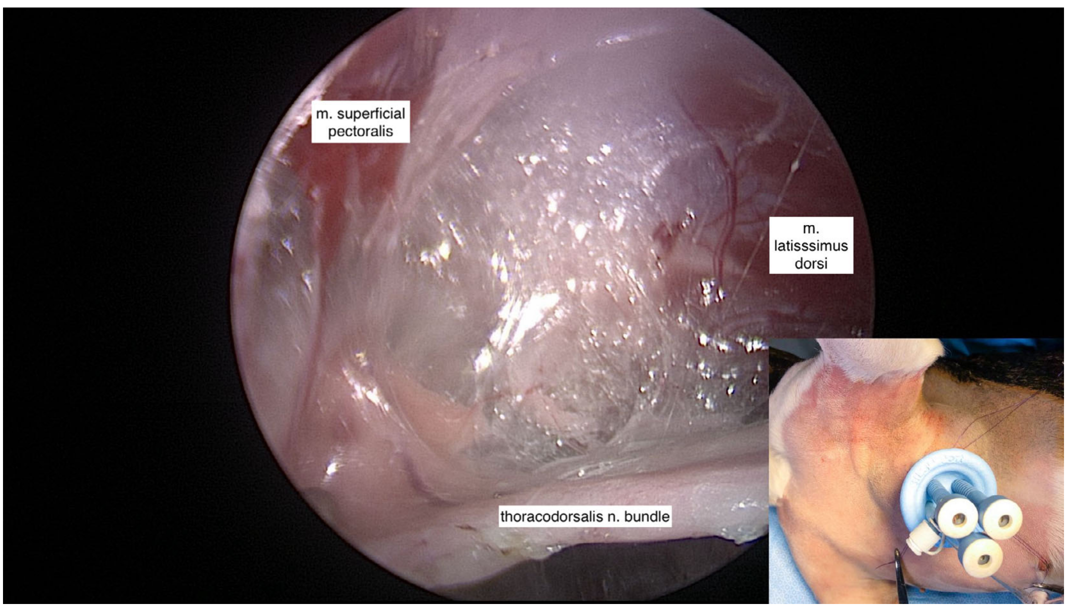

FIGURE 5 Endoscopic dissection of the initial loose areolar fascia in the axillary space following insertion of a SILS port, inset image shows SILS position after insufflation.

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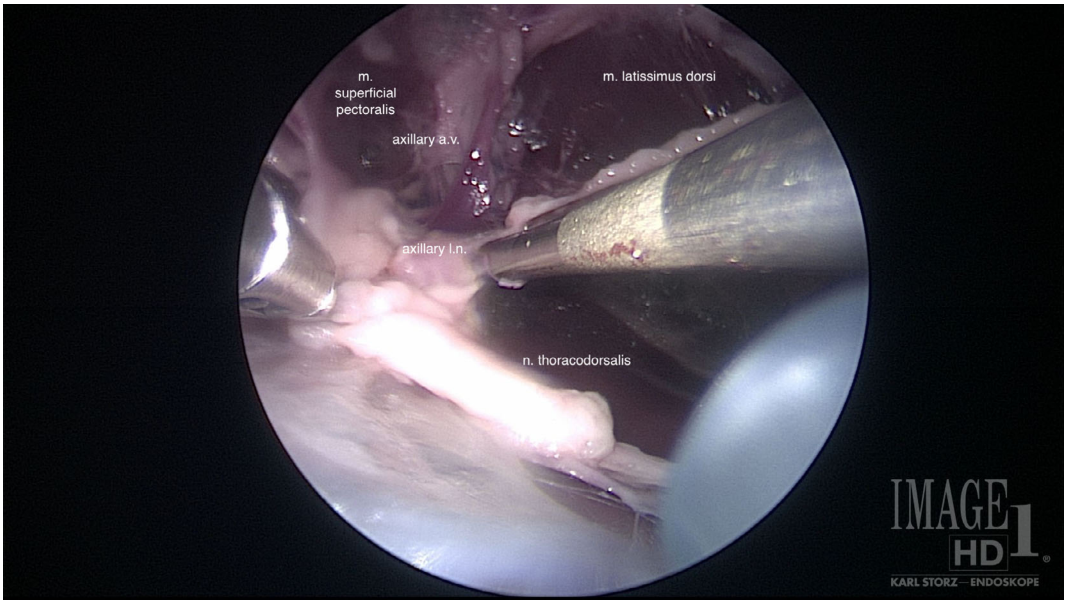

FIGURE 6 Endoscopic retrieval of axillary node showing the location relative to the axillary artery and vein and the thoracodorsalis n. bundle.

The patient was discharged the day following surgery. No lymphedema was identified. There was mild bruising with a small seroma at the lymphadenectomy surgical site which resolved together with the lameness by the 3-day postoperative recheck. Histopathology revealed hyperkeratosis and inflammation of the deep tissues of the digit, and lymph node hyperplasia. Eight months following surgery there had been no recurrence of the original lesion and no lameness or other issues were identified.

3.3 | Clinical case 2

A 9-year-old MN Labrador mix (44.3 kg) was presented for treatment of a 2 × 2 cm cutaneous mast cell tumor on the right antebrachium. Staging (thoracic radiographs, abdominal ultrasound, ultrasound guided aspirates of the spleen and axillary lymph node) was performed. Splenic aspirates were unremarkable. Lymph node aspirates were acellular. Curative intent excision of the primary tumor and excisional biopsy of the axillary and accessory axillary nodes were performed. The accessory axillary node was identified without difficulty. The axillary space was explored endoscopically, and the node location identified, however excision of the node required conversion to an open approach with muscle retraction due to difficulties grasping and elevating the node from the thoracic wall to allow completion of the dissection. The total surgical time was 58 minutes. Postoperatively, the dog received routine analgesic management (methadone 0.1 mg/kg IV every 4 h as needed). The dog showed normal limb use the following day, with minimal to no swelling/ bruising at the axillary incision and was discharged. No seromas or limb edema had developed by recheck 3 weeks later. Histopathology of the primary tumor and lymph nodes revealed incomplete excision of a grade II/III (Patnaik) intermediate (Kiupel) grade mast cell tumor, and the lymph nodes were both expanded by multifocal sheets of mast cells expanding the cortical and medullary sinuses (HN2 classification). No complications or recurrence of the primary tumor occurred over a 6 month follow-up period. Reoperation of the primary tumor was declined due to the lymph node status. Adjunctive radiation therapy and chemotherapy were also discussed and declined.

3.4 | Clinical case 3

A 6 year old FS Labrador Retriever (33.8 kg) was presented for treatment of a 2 × 3 cm diameter cutaneous mast cell tumor on the left ventral thorax. Staging (thoracic radiographs, abdominal ultrasound, ultrasound guided aspirates of superficial cervical and axillary lymph nodes) was performed and revealed occasional mast cells in the axillary node. The accessory axillary node could not be identified on ultrasound. Curative intent excision of the primary tumor and excisional biopsy of the axillary node was performed. The accessory axillary node could not be identified intraoperatively. The axillary space was explored endoscopically and the axillary node retrieved. The total surgical time was 35 minutes. Postoperatively,

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the dog received routine analgesic management (methadone 0.1 mg/kg IV every 4 h as needed). The dog showed normal limb use the following day, with minimal to no swelling/bruising at the axillary incision. Some discomfort associated with palpation around the surgical site for the primary tumor was noted, prompting hospitalization for pain management extending to 48 hours postoperatively. No abnormalities associated with the lymphadenectomy procedure were noted at a 3-week recheck. Histopathology of the primary tumor and lymph node revealed complete excision of a grade II/III (Patnaik) low grade (Kiupel) mast cell tumor with the lymph node showing early mast cell infiltration (classified as HN2). No other complications were encountered over a 3-month follow-up period.

4 | DISCUSSION

In this study, removal of the axillary lymph nodes by an MIS technique in the small number of cases reported was feasible and associated with minimal complications. The main axillary node is situated adjacent to the axillary artery and vein and brachial plexus in a relatively inaccessible location due to the overlying forelimb musculature. Subjectively, the endoscopic technique facilitated visualization of these hazards compared with an open approach. The technique might be further refined with the addition of an indocyanine green fluorescence technique to aid the intraoperative identification of the nodes within the surrounding fat[18,19] and thus accelerate surgical times. Indocyanine green fluroscence has been used in this manner both for sentinel node mapping[19] and also for optimizing node identification.[20]

The correct placement of the SILS port was important in creating a successful operating space. Due to adipose tissue present in the region, if the SILS port was placed too deep, the axillary lymph node and associated adipose layer relocated to the roof rather than the floor of the viewing window when the axilla was inflated with gas. Thus, if the landmarks are identified and the lymph node cannot be located, the clinician should probe the dorsal region caudal to the axillary artery and vein. In one of the clinical cases performed, it was necessary to convert to an open approach due to difficulties grasping the node and elevating it away from its position adjacent to the vascular bundle. This was likely due to an excess of caution on the part of the operator, although an articulating dissector might also have made this easier.

At our institution, ultrasound guided fine needle aspirates of locoregional nodes are routinely performed as part of our neoplastic staging protocol when the nodes cannot be readily palpated for palpation guided aspirates, or if palpation guided aspirates yield nondiagnostic samples. However, in select cases we have also started mapping the sentinel lymph node by indirect CT lymphangiography followed by surgical excision and histopathology due to data suggesting improved sensitivity for detection of metastatic disease.[21] This has been particularly helpful in guiding informed decision making when surgical excision/ reconstruction of the primary tumor would require a large surgical dose. However, an unanticipated outcome of this change in protocol is that we have found our mapping taking us to the axillary lymphocenter more frequently than previously. In our experience, this has been a deeper dissection requiring more retraction than other cutaneous lymphocenters. This has proved an incentive to identify a less invasive method of excision that provides good visualization of necessary landmarks. The success rate of unassisted surgical localization of lymph nodes by open techniques has been reported at 72%,[22] implying room for improvement. Combining an MIS technique with a localization technique may help achieve the optimal balance between patient recovery, minimal postoperative complications, and successful lymph node retrieval.

While lymphedema is a common complication after dissection of the axillary nodes in human patients, we did not observe this as a complication. This might be due to accessory lymphatic channels in the canine species, or the small number of clinical cases assessed. Suami et al.[23] found that lymphatic breaks created by lymph node dissection in a canine model repaired and restored via collateral pathways to second tier lymph nodes in the region. Similarly, to our knowledge, no case developed pneumothorax or pneumomediastinum as a complication of insufflation in this area. This has also not been reported as a complication following endoscopic axillary dissection in the human patient population,[14,24] presumably because the dissection does not need to extend into the thoracic wall.

While we were unable to identify previous reports of an endoscopic approach to noncavitary lymph node retrieval in the veterinary literature, a human endoscopic sentinel lymph node biopsy and endoscopic axillary lymphadenectomy study without liposuction demonstrated that this approach was feasible, had a low complication rate and samples obtained were comparable to those achieved with an open dissection.[14] Based on our experience in the clinical cases reported, some discomfort on peri-incisional palpation and mild lameness may be anticipated in the immediate postoperative period and should resolve over the next couple of days post procedure. Proper analgesia needs to be provided.

Limitations of this study include the small sample size and limited number of surgeons performing the procedure. All the clinical cases were large breed dogs and

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the technique may be more challenging in smaller animals.

In conclusion, this study demonstrated that a minimally invasive approach to the axilla and subsequent visualization of the axillary node is possible and can be utilized in those patients in which an excisional biopsy of the axillary lymph node is required. A controlled study with more patients is required to determine whether the hypothesized benefits of an MIS approach compared with an open technique can be verified.

AUTHOR CONTRIBUTIONS

Kuvaldina AB, DVM: Assisted with study design, data acquisition and interpretation; paper draft, revisions, and final approval; agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy and integrity of any part of the work are appropriately investigated and resolved. Buote N, DVM, DACVS: Assisted with study design, data acquisition and interpretation; paper revisions, and final approval; agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy and integrity of any part of the work are appropriately investigated and resolved. Luis Campoy L, LV, Cert VA, DipECVAA, MRCVS: Assisted with study material acquisition; paper final approval; agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy and integrity of any part of the work are appropriately investigated and resolved. Porter I, DVM, DACVR: Assisted with study design and study material acquisition; paper revisions, and final approval; agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy and integrity of any part of the work are appropriately investigated and resolved. Hayes GM, BVSc, PhD, DACVS, DACVECC: Created the study design, assisted with data acquisition and interpretation; paper draft, revisions, and final approval; agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy and integrity of any part of the work are appropriately investigated and resolved.

CONFLICT OF INTEREST

The authors declare no conflict of interest related to this report.

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