Bilateral medial iliac lymph node excision by a ventral laparoscopic approach: technique description

Hyunjoo LIM1), Jina KIM1), Li LI1), Aeri LEE1), Junemoe JEONG1), Jonghyeok KO1), Sungin LEE1), Oh-Kyeong KWEON1) and Wan Hee KIM1)*

ABSTRACT. The aim of this study was to describe a ventral laparoscopic technique for bilateral medial iliac lymphadenectomy in dogs. Twelve intact male purpose-bred research dogs, weighing less than 15 kg, were positioned in dorsal recumbency, and a 3-portal technique was used. Bilateral dissection was performed with vessel-sealing devices while tilting the surgical table by up to 30° towards the contralateral side of the target medial iliac lymph node (MILN) without changing the surgeon's position. Using a ventral laparoscopic approach, bilateral MILNs were identified and excised in all dogs. The mean times for unilateral and bilateral MILN dissections were 9.7 ± 3.8 and 21.0 ± 6.0 min, respectively. The mean times for the right and left MILN dissections were 10.8 ± 4.3 and 9.8 ± 2.5 min, respectively. The mean total surgery time was 43.7 ± 7.7 min. In total, 26 MILNs were dissected. Several complications, including mild to moderate capillary hemorrhage from perinodal fat and vessels (controlled laparoscopically), mild spleen trauma caused by the first trocar insertion and capsular damage of MILNs, were observed. However, there were no other major complications. All MILN samples were evaluated and deemed suitable for histopathologic diagnosis. Laparoscopic excision of MILNs is a useful method of excisional biopsy for histopathologic diagnosis. Using this ventral laparoscopic approach with the 3-portal technique, bilateral MILN dissection suitable for obtaining histopathologic samples could be achieved in a short time in dogs weighing less than 15 kg.

KEY WORDS: biopsy, dog, laparoscopy, medial iliac lymph node, minimally invasive surgery

The medial iliac lymph node (MILN) belongs to the iliosacral lymph center. It is consistently observed in situ as a large lymph node located between the deep circumflex iliac and the external iliac arteries. Usually, a single MILN is present, but two MILNs on one or both sides may be observed. This lymph node receives efferent lymph vessels from the skin and muscles of the caudal abdomen and the pelvic limbs, as well as from organs located in the caudal abdomen and pelvis, including, the colon, rectum, anus, vagina, prostate gland, ureter, bladder and urethra. Considering this anatomical position of MILN, their assessment for metastatic disease is considered important in patients demonstrating neoplasia in these anatomic locations [14].

Evaluation of the sentinel lymph node, even when it appears unremarkable on palpation or shows normal appearance on imaging, is recommended to check for microscopic metastatic disease in several human cancers [8]. While the MILNs have not been demonstrated to be the true sentinel lymph nodes for all dogs with perineal neoplasia, such as anal sac adenocarcinomas, they are currently the most commonly assessed regional lymph nodes for evidence of metastasis based on the anatomic features of the MILN [1–3, 7, 13, 16, 25, 33]. It has been reported that more than 50% of dogs with anal sac adenocarcinoma display metastasis to regional lymph nodes at diagnosis [3, 13, 25, 33].

The methods to evaluate MILNs include diagnostic imaging, fine needle aspiration (FNA) or excisional biopsy. As abdominal ultrasonography is a useful diagnostic tool for evaluating the morphology and echogenicity of the MILN, if there is susceptible evidence of metastasis, including lymphadenomegaly or change of echogenicity, FNA or excisional biopsy of MILN can be recommended for exact diagnosis. Fine needle aspiration (FNA) is a comparatively less aggressive and useful diagnostic tool for tissue assessment, especially in cases of a cutaneous or subcutaneous mass which is easy to contact [12]. However, as the MILNs are located deep in the retroperitoneum and adjacent to large blood vessels, including aorta, caudal vena cava and external iliac arteries, the risk of hemorrhage while performing FNA of MILN with ultrasound guidance increases. In particular, cases of micrometastases of the lymph nodes can be misdiagnosed as non-metastatic tissue with other diagnostic tools, including...
ultrasonography and FNA [1, 6, 16].

In case of dogs with anal sac adenocarcinoma, extirpation of metastatic MILNs showed a positive effect on the prognosis in several studies [2, 13, 25]. In most of these cases, extirpation of lymph nodes was performed after lymphadenopathy or lymphadenomegaly was detected in the iliac region on ultrasound images [13, 25], and ultrasound-guided fine needle aspiration was performed for metastasis evaluation [25]. In patients with abnormal ultrasonographic features of the MILNs bilaterally, or in case of identification of both MILNs as true primary sentinel lymph nodes, excision of bilateral MILNs with primary tumor resection could be considered as the first-line treatment.

Minimally invasive procedures for small animals have been described by many veterinary studies as feasible techniques, associated with less pain and rapid recovery [9, 10]. Providing that minimally invasive excision of lymph nodes is safe, efficient, and provides diagnostic biopsy samples, a minimally invasive approach may improve owner acceptance of MILN biopsy as a staging procedure.

While there have been many studies of laparoscopic excisional biopsy of lymph nodes in human medicine [4, 15, 21, 22, 26], only a few studies of minimally invasive lymph node extirpation have been reported in veterinary medicine [30, 31]. A recent study reported the technique of laparoscopic extirpation of the MILN in dogs with a lateral approach [30]. The medium- to large-breed dogs, body weight ranges between 15 and 25 kg, and access to bilateral MILNs is limited without changing the dog’s position and establishing additional laparoscopic cannulas.

The aims of this study were (1) to describe a ventral approach for the laparoscopic excision of bilateral MILNs in dogs weighing less than 15 kg, (2) to report the surgical time and complications of this technique and (3) to describe the quality obtained from the biopsy specimens.

MATERIALS AND METHODS

This study was approved by the Institutional Animal Care and Use Committees of Seoul National University (SNU-151230-2).

Animals

Twelve healthy intact male purpose-bred research dogs, weighing less than 15 kg, were included in this study. None of these
dogs had previously undergone abdominal surgery. Each dog underwent a complete physical examination and hematologic evaluation including a complete blood count and serum chemistry.

Surgical techniques

All surgical procedures were performed by the same surgeon. All dogs were premedicated with acepromazine (0.01 mg/kg, intravenously (IV), Sedaject®, Samu Medical Co., Ltd., Chungcheongnam-Do, Republic of Korea). Anesthesia was induced with alfaxalone (5 mg/kg, IV, Alfaxan®, Jurox Inc., Kansas, MO, U.S.A.) and maintained with inhalant isoflurane (Ifan®, Hana Pharm Co., Ltd., Naju, Republic of Korea) in oxygen. Tramadol (5 mg/kg, IV, once before surgery, and 5 mg/kg, orally, twice daily for 14 days after surgery) was administered for perioperative analgesia.

The dogs were positioned in dorsal recumbency. The hair was widely clipped from the xiphoid process to the pubic region. Urine in the urinary bladder was voided using an 8-Fr urinary catheter after anesthesia induction, prior to the procedure, to allow a better view of the caudal peritoneum. A Veress needle (Karl Storz, Veterinary Endoscopy, Goleta, CA, U.S.A.) was inserted through the right lateral abdominal wall, and CO₂ pneumoperitoneum (maximal pressure, 10–15 mmHg) was established and maintained using a pressure-regulating mechanical insufflator (Karl Storz, Veterinary Endoscopy).

In all dogs, a 3-portal technique was used; all dogs had two working portals and a single camera portal with 6-mm laparoscopic cannulas (Ternamian endotip cannula, 6.5 cm, Karl Storz, Veterinary Endoscopy) established after pneumoperitoneum was achieved. In the right upper abdominal region, 3 cm cranialateral to the umbilicus at the level of the last ribs, an approximately 1.5-cm skin incision was made with a No. 10 blade, and the first cannula (Ternamian endotip cannula with a multifunctional valve, 6 mm × 6.5 cm, camera portal, Karl Storz, Veterinary Endoscopy) was inserted into the abdomen at a 45° angle in the caudomedial direction. After exploring the entire abdomen with a 5 mm × 29 cm 0° laparoscope (Karl Storz, Veterinary Endoscopy) to evaluate the organs for iatrogenic damage, the second cannula (Ternamian endotip cannula, 6 mm × 6.5 cm instrumental portal, Fig. 1) was inserted at a point equidistant from the midline and the camera portal on the contralateral side of the animal under laparoscopic guidance, taking care to prevent damage to the abdominal organs. The surgical table was tilted by up to 15° towards the Trendelenburg position. The third portal (instrument) was established in the caudal abdomen at a location approximately one third of the distance between the pubic brim and the ipsilateral instrumental portal, using the method described for the second cannula (Fig. 1).

After all the portals had been established, a thorough inspection of the caudal abdomen was performed using a laparoscopic probe (Palpation probe, 5 mm × 36 cm, Karl Storz, Veterinary Endoscopy) and laparoscopic tissue grasping forceps (Kelly forceps or Babcock forceps, 5 mm × 36 cm, Karl Storz, Veterinary Endoscopy) through the second and third cannulas, respectively.

For dissection of the right MILN, the surgical table was tilted to the left within 30° to allow the organs to displace towards the dependent aspects of the peritoneal cavity. After identification of the right external iliac artery, right deep circumflex iliac artery and the silhouette of the MILN behind the retroperitoneal tissue, a laparoscopic vessel-sealing device (LigaSure-Dolphin tip, 5 mm–37 cm, Covidien, Inc., Mansfield, MA, U.S.A.) was introduced through the second cannula. The retroperitoneum was incised at a point caudal to the right deep circumflex iliac artery and between the right external iliac artery and the testicular vessels. The tissue surrounding the retroperitoneum and the small vessels attached to the lymph nodes were dissected using blunt dissection and sealing/transaction by the vessel-sealing device, respectively. During the procedure, the lymph node was retracted away with laparoscopic Babcock forceps from the large vessels, including the right external iliac artery and the right deep circumflex artery. The dissection was performed in the cranial-to-caudal direction and toward the dorsomedial side of the MILN in order to completely separate the MILN from the surrounding tissues and vessels. The excised lymph nodes were withdrawn through the third port. For dissection of the left MILN, the surgical table was tilted to the right, and the procedure was performed on the opposite side in a similar manner.

The portal sites were sutured in a simple interrupted pattern using a monofilament absorbable suture (3–0, PDS II, Ethicon, Cincinnati, OH, U.S.A.) in the abdominal wall and the subcutaneous tissue, and monofilament nylon (4–0, Nylon, Ethicon) was used for the skin layer in simple interrupted pattern. All dogs successfully recovered from anesthesia.

The surgery time, anatomic landmarks, any changes of respiratory rate, heart rate, peripheral capillary oxygen saturation (SpO₂) or end-tidal carbon dioxide (EtCO₂) during table-tilting, peri-operative complications including abdominal hemorrhage from insertion of the laparoscopic cannula, mechanical trauma of the lymph nodes from direct manipulation of lymph nodes, any clinical symptoms for suspecting post-operative abdominal hemorrhage and any complications of the incision sites during the monitoring period of 14 days after surgery were recorded. The size, number and length of the MILNs were also recorded.

Histopathologic evaluation

Each excised MILN was immersed in 10% buffered formalin and bisected longitudinally. Hematoxylin and eosin staining was performed for histologic evaluation of the mechanical trauma caused during dissection. Both halves of the cut surface of the bisected MILNs were evaluated and scored using previously described criteria [30]. Histologic artifacts were characterized as central (pinch/pressure/crush artifacts affecting larger central areas of the lymph node) or peripheral (pinch/pressure/crush artifacts affecting the peri-nodal tissue and the capsular and superficial cortex/paracortex of the lymph node). The histologic artifacts were scored according to the following criteria: 0=no artifact, 1=minimal/marginal deformation, 2=mild crush, 3=moderate crush, 4=severe crush. When different areas within a central or peripheral region of a given lymph node received a range of scores, the worst score received was assigned to that lymph node as the overall score.
Statistical analysis

Statistical analyses were performed using SPSS, version 22.0 (SPSS Inc., Chicago, IL, U.S.A.). A Kolmogorov-Smirnov test was used to evaluate the normal distribution of body weight of dogs and dissection time for each MILN and for bilateral MILNs. Normally distributed data are reported as mean values with the variability expressed as standard deviation, and non-normally distributed data are reported as median values with ranges.

RESULTS

The median age of the dogs was 5 years (range, 4.5–5.5 years). The mean body weight was 10.4 ± 0.713 kg. The mean body condition score was 4.5 ± 0.45/9. All dogs were determined to be healthy based on the physical examination and hematologic evaluation.

Surgical findings

MILNs were successfully identified and excised by using the ventral approach in all dogs; no animal required conversion from laparoscopic procedure to open laparotomy. The 3-portal technique and table tilting up to 30° in the opposite direction to the target MILN were useful to identify bilateral MILNs without changing the surgeon’s position.

Right MILNs were located lateral to the right external iliac artery and right ureter, caudal to the right deep circumflex iliac artery, and medial to the right testicular vessels (Fig. 2). Left MILNs were located symmetrically with respect to the right MILNs (Fig. 3). In cases with an approach to the medial side of the ureter, the access to MILN and manipulation of the perinodal tissue...
were more difficult than when approaching the lateral side of the ureter, as the ureter disturbed the working space of the dissection procedure. The dogs were positioned in the Trendelenburg position and rotated in both directions within 30°. There were no abrupt changes in respiratory rate, heart rate, peripheral capillary oxygen saturation (SpO₂) or end-tidal carbon dioxide (EtCO₂) after tilting the table.

The mean number of identified MILNs was two (range, 2–3). There were additional MILNs in two dogs—both located on the left. These two additional MILNs were observed in the caudal position of left MILN and cranial to left external iliac artery. The mean dissection time was 9.7 ± 3.8 min for unilateral and 21.0 ± 6.0 min for bilateral MILN dissection. The mean dissection time was 10.7 ± 4.3 min for right and 9.8 ± 2.5 min for left MILN dissection. The mean total surgery time was 43.7 ± 7.7 min (Table 1).

The median measured length of the excised MILNs was 16.2 mm (range, 5–28 mm), and the median width was 6.8 mm (range, 3–14 mm). The median measured length of the excised right MILNs was 17.9 mm (range, 12–28 mm), and the median width was 7 mm (range, 4–10 mm). The median measured length of the excised left MILNs was 14.5 mm (range, 5–23 mm), and the median width was 6.6 mm (range, 3–14 mm) (Tables 2 and 3).

Postoperative management

The vital signs of all dogs remained within the normal range during the entire perioperative period, and they all recovered from anesthesia uneventfully. All dogs could stand and walk within 2–3 hr after the procedure and showed good appetite and activity during two weeks after surgery. No clinical symptoms were observed, indicating postoperative complications, including abdominal hemorrhage or inflammation, or infection of the incision site during the 2-week monitoring period.

Table 1. Summary data of the dogs and the surgery time

| Dog | BW (kg) | BCS | Dissection time of MILN (min) | Total surgical time (min) |
|-----|---------|-----|------------------------------|--------------------------|
|     |         |     | Right | Left | Additional | Bilateral |              |
| 1   | 9.5     | 4   | 15   | 15   | 30         | 52        |
| 2   | 10.2    | 4   | 18   | 11   | 29         | 54        |
| 3   | 10.5    | 5   | 18   | 11   | 29         | 49        |
| 4   | 9.8     | 4.5 | 12   | 12   | 24         | 51        |
| 5   | 9.5     | 4   | 7    | 10   | 4          | 21        | 48        |
| 6   | 11.2    | 4.5 | 6    | 8    | 3          | 17        | 38        |
| 7   | 10.3    | 4   | 9    | 7    |            | 16        | 35        |
| 8   | 9.6     | 4   | 6    | 6    | 12         | 32        |
| 9   | 10.9    | 5   | 11   | 10   | 21         | 40        |
| 10  | 10.3    | 4.5 | 9    | 9    | 18         | 36        |
| 11  | 11.3    | 5   | 10   | 11   | 21         | 42        |
| 12  | 11.5    | 5   | 7    | 7    | 14         | 35        |

*BW=Body Weight, BCS=Body Condition Score.

Table 2. Summary data for laparoscopically excised right MILN

| Dog | L × W × H (mm) | Central artifacts score (0–4) | Peripheral artifacts score (0–4) | Total surface area (%) of evaluated MILN affected by artifacts |
|-----|----------------|-----------------------------|---------------------------------|-------------------------------------------------------------|
| 1   | 14 × 9 × 5     | 0                           | 1                              | 5                                                          |
| 2   | 22 × 8 × 6     | 0                           | 1                              | 5                                                          |
| 3   | 18 × 8 × 6     | 0                           | 2                              | 10                                                         |
| 4   | 15 × 7 × 8     | 1                           | 2                              | 10                                                         |
| 5   | 15 × 8 × 4     | 0                           | 1                              | 5                                                          |
| 6   | 20 × 3 × 3     | 0                           | 1                              | 5                                                          |
| 7   | 28 × 9 × 3     | 0                           | 2                              | 10                                                         |
| 8   | 20 × 10 × 3    | 0                           | 1                              | 5                                                          |
| 9   | 12 × 4 × 4     | 2                           | 3                              | 20                                                         |
| 10  | 14 × 5 × 3     | 0                           | 1                              | 5                                                          |
| 11  | 18 × 6 × 3     | 1                           | 2                              | 15                                                         |
| 12  | 19 × 7 × 2     | 0                           | 1                              | 5                                                          |

L=Length, W=Width, H=Height.

Table 3. Summary data for laparoscopically excised left MILN

| Dog | L × W × H (mm) | Central artifacts score (0–4) | Peripheral artifacts score (0–4) | Total surface area (%) of evaluated MILN affected by artifacts |
|-----|----------------|-----------------------------|---------------------------------|-------------------------------------------------------------|
| 1   | 18 × 11 × 7    | 0                           | 1                              | 5                                                          |
| 2   | 15 × 5 × 5     | 0                           | 1                              | 5                                                          |
| 3   | 14 × 3 × 3     | 0                           | 1                              | 5                                                          |
| 4   | 15 × 9 × 7     | 0                           | 1                              | 5                                                          |
| 5   | 5 × 4 × 4      | 1                           | 2                              | 15                                                         |
| 6   | 9 × 3 × 3      | 1                           | 1                              | 10                                                         |
| 7   | 18 × 9 × 4     | 0                           | 1                              | 5                                                          |
| 8   | 18 × 14 × 4    | 2                           | 3                              | 20                                                         |
| 9   | 12 × 4 × 3     | 1                           | 2                              | 10                                                         |
| 10  | 12 × 6 × 4     | 0                           | 1                              | 5                                                          |
| 11  | 23 × 6 × 3     | 0                           | 1                              | 5                                                          |
| 12  | 15 × 5 × 3     | 2                           | 4                              | 30                                                         |

L=Length, W=Width, H=Height, a) Additionally identified MILN.
Histologic evaluation of samples

The collected samples were histologically confirmed as lymphoid tissue in all 12 dogs and categorized as MILNs based on the anatomic location. All samples were found to be histologically suitable for diagnosis. There were several peripheral artifacts caused by mechanical trauma in all samples, while artifacts in the central portions were relatively fewer. The median central artifact score for all MILNs was zero (range, 0–2), while the median peripheral artifact score was one (range, 1–4). For all MILNs, the median percentage of the surface area of the dissected MILNs affected by histologic artifacts was 5% (range, 5–30%) (Tables 2 and 3).

DISCUSSION

Laparoscopic lymphadenectomy at similar anatomical locations with this procedure, performed for staging and therapy of gynecological cancers, has been reported in a previous human medical study [26]. Using a 4-portal technique with a ventral approach, bilateral surgical excision of several lymph nodes was successfully achieved in this study.

In our study, we were able to access bilateral MILNs with laparoscopy using a ventral approach in a manner similar to that used in the above-mentioned study [26]. The need to change the position of the dog or the surgeon to explore both sides of the peritoneum was obviated by tilting the table to access both sides. This reduced the operation time and minimized the effort required from the surgeons. None of the dogs showed a remarkable change in physiological status during tilting of the table during the operation, and no anesthetic complications were observed.

As Steffey et al. [30] previously reported, the laparoscopic extirpation of MILN is a feasible technique in dogs with normal-sized MILNs for disease staging. The median dissection time for all 9 MILNs in that study was 13 min (range, 5–22 min), which included 5 left MILNs with a median dissection time of 14.5 min (range, 9–22 min) and 4 right MILNs with a median dissection time of 13 min (range, 5–19 min). In another previous study [30], the 3-portal technique was used with the lateral approach on the ipsilateral side of the MILN. To approach the side contralateral to the MILN, additional laparoscopic cannulas placement would be inevitable.

In our study, only three transperitoneal cannulas were needed for the entire procedure of bilateral MILN dissection, and the surgical time for each side excision was similar to that of the previous study [30]. In addition, as recorded in Table 1, the later dissection times were slightly reduced compared to the initial dissection time, which can be considered as a learning curve. The learning curves in laparoscopic surgery models were also reported in similar studies in human and veterinary medicine [28, 32]. Following these studies, surgical time was shown to decrease significantly with operative experience of surgeons. In cases requiring bilateral MILN dissection, this technique can be expected to reduce the total surgical time with less effort and time for repositioning patient or replacing the ports. Moreover, considering generally short time required for the procedure, it may be helpful for reducing the total surgical time when performed in conjunction with primary tumor resections.

While exploring the caudal abdomen, efforts were made to identify other lymph nodes of the iliosacral lymph center, including the internal iliac lymph nodes and sacral lymph nodes. However, the visualization and exploration of these lymph nodes were limited due to different reasons, including inappropriate position of portals for exploration and limitation of performing angle for laparoscopic probe and camera, or presence of abdominal fat and small size of the lymph nodes. Considering their anatomic intimacy with the MILN and their efferent lymphatic flow to the MILN, other lymph nodes of iliosacral lymphatic center should be also evaluated in cases of suspected metastasis from a primary mass in the perineal or pelvic region. The evaluation of the lymph nodes can be best realized by advanced preoperative imaging modalities, including computed tomography or magnetic resonance imaging [1, 20, 24], preoperative methods of sentinel lymph node mapping, which may include scintigraphy [34] or computed tomographic lymphography [18]. The inability to visualize other lymph nodes of iliosacral lymphatic center is a limitation of our minimally invasive approach and surgical technique and is consistent with the findings of laparoscopic exploration in dorsal recumbency reported in abstract form by Steffey et al. [29]. This abstract did document laparoscopic identification of internal iliac and sacral lymph nodes with positioning in lateral or sternal recumbency and lateralized port placements, although dissection of these lymph nodes was not attempted [29].

The observed MILNs were located lateral to the external iliac arteries, caudal to the deep circumflex iliac arteries, and medial to the testicular vessels. The mobility of the ureter made the appropriate positioning for incision of the retroperitoneum difficult. As mentioned above, the approach to the medial side of the ureter occurred in three dogs, and the access and manipulation of the MILN perinodal tissue were more difficult than when approaching the lateral side of the ureter. Direct manipulation of ureter was inevitable during the dissection procedure when approaching from the medial side of the ureter in these dogs, which could increase the risk of damaging the ureter.

Perioperative complications were controllable without conversion to laparotomy in all the patients; however, considering the anatomic intimacy of the MILN and large blood vessels, care should always be taken not to tear or damage the vessels. Capillary hemorrhages of the peritoneal fat and vessels were successfully controlled using the vessel-sealing device. As the capsule was easily torn by even minimal direct manipulation with laparoscopic Babcock forceps, retraction and dissection should be performed with manipulation of the perinodal tissues.

In our study, splenic hemorrhage occurred in two dogs, although the first cannula (camera portal) was established in the right upper abdomen to prevent trauma to the spleen. However, the tail of the spleen extended to the right upper abdomen in these dogs. It is thought that splenic congestion could be induced by injection of pre-anesthetic drugs, including acepromazine, during the preanesthetic period [19]. There was no splenic hemorrhage in other dogs without splenic congestion. The injury of abdominal organs caused by insertion of a Veress needle or laparoscopic cannula is not a rare complication in laparoscopy, and has been...
reported in a few studies in veterinary medicine [5, 23] and human medicine [17, 27]. Therefore, blind insertion of the Veress needle or the first cannula should be performed with extreme care considering the possibility of abdominal organs injury, including of the spleen. Other insertion methods, including the open method, Hasson’s technique or right intercostal Veress needle insertion [11], can be considered for prevention of this complication.

As the third cannula (the caudal instrumental port) was placed adjacent to the location of the inguinal ring, care should be taken not to tear the inguinal ring. The inguinal ring is a passage of vaginal tunic and the spermatic cord in males, the vaginal process and round ligament of the uterus in females, and the external pudendal vessels and the genitofemoral nerve in both sexes. [14] The risk of traumatizing these tissues while establishing or manipulating the third cannula should be considered. Inguinal hernia can also be an expected complication in case of an inguinal ring torn.

Histologic examination of samples revealed several artifacts, including a small portion of disrupted capsule and ruptured lymphatic follicles, especially in peripheral parts of the lymph nodes. The surface area of the lymph nodes was also affected, but the proportion was generally small. All samples were found to be suitable for histopathologic diagnosis; however, there might be some risks for compromising exact diagnosis by these artifacts in clinical patients, especially in cases with only small lesions of the lymph nodes. Therefore, as mentioned in Steffey et al. study [30], the effort should be taken to minimize direct manipulation of the lymph nodes.

There were a few limitations to our study. The sample size and the breed of the dogs were small. Therefore, we could not determine, if there is any variation of lymph node location among the breeds. The dogs were all young, medically fit, and healthy. There was no variation in body size and no excessive fat in the caudal abdomen. In cases of old, obese patients with tumors, the identification and dissection of MILNs may be more challenging, because of the excessive retroperitoneal fat presence, additional vascularization and a more fragile lymph node capsule. Furthermore, lymphadenomegaly of MILN can be another risk factor for lymph nodes dissection. Although the identification of the lymph node could be accomplished with less effort, the increased size of the lymph node could obstruct visualization of the large blood vessels adjacent to the lymph node and increase the difficulty of stable manipulation of the lymph node.

The position of the three transperitoneal portals was planned to establish a triangular position for approaching bilateral MILNs without changing the patient’s position. As a result, the portals were inserted through the abdominal wall, not through the linea alba. If the Veress needle was inserted through the linea alba and pneumoperitoneum was established, it could reduce the injury of the abdominal wall. Identification of other sublumbar lymph nodes could not be achieved, because of the organs and fat within the caudal abdomen and limitation of the performing angle of the laparoscopic devices due to the position of the dog and the position of the portals. Further investigation with relocation of the portal sites may allow identification of other lymph nodes of the iliosacral lymphatic center. In our study, all the MILN samples were normal in size and small enough to be retrieved via the 6-mm laparoscopic cannula. However, if an MILN is enlarged because of metastasis or inflammation, a commercial laparoscopic specimen retrieval bag, a wound retractor, or the finger of a sterile surgical glove can be utilized for removal.

Some authors have anecdotally attempted this procedure in intact female dogs in preliminary experiments, and the presence of broad ligament was found to inhibit the identification of important anatomic landmarks for MILN localization, as well as the dissection of the retroperitoneum and MILNs bilaterally. Trendelenburg position with head down tilt made the broad ligament move cranially; therefore, the anatomic landmarks were difficult to visualize. In procedures in intact females, laparoscopic ovariotomy or additional techniques, such as placement of stay suture in the ovaries to improve visualization should be considered to solve these problems.

We successfully identified and dissected MILNs bilaterally in a small cohort of normal dogs weighing less than 15 kg, with laparoscopic excision using a ventral approach. We were able to obtain biopsies with minimal artifact and of suitable quality for diagnostic purposes using this ventral 3-portal technique. This procedure may aid diagnostic efforts in cases with suspected metastatic disease or when FNA, ultrasound, core biopsies or other diagnostic modalities are inconclusive. Minor bleeding from perinodal tissue was an expected and controllable consequence of the procedure and did not affect outcomes in these 12 dogs.

ACKNOWLEDGMENT. This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Ministry of Science, ICT & Future Planning (20163A9B6026771).

REFERENCES

1. Anderson, C. L., MacKay, C. S., Roberts, G. D. and Fidel, J. 2015. Comparison of abdominal ultrasound and magnetic resonance imaging for detection of abdominal lymphadenopathy in dogs with metastatic apocrine gland adenocarcinoma of the anal sac. Vet. Comp. Oncol. 13: 98–105. [Medline] [CrossRef]
2. Barnes, D. C. and Demetriou, J. L. 2017. Surgical management of primary, metastatic and recurrent anal sac adenocarcinoma in the dog: 52 cases. J. Small Anim. Pract. 58: 263–268. [Medline] [CrossRef]
3. Bennett, P. F., DeNicola, D. B., Bonney, P., Glickman, N. W. and Knapp, D. W. 2002. Canine anal sac adenocarcinomas: clinical presentation and response to therapy. J. Vet. Intern. Med. 16: 100–104. [Medline] [CrossRef]
4. Buist, M. R., Pijpers, R. J., van Lingen, A., van Diest, P. J., Dijkstra, J., Kenemans, P. and Verheijen, R. H. 2003. Laparoscopic detection of sentinel lymph nodes followed by lymph node dissection in patients with early stage cervical cancer. Gynecol. Oncol. 90: 290–296. [Medline] [CrossRef]
5. Case, J. B., Marvel, S. J., Boscan, P. and Monnet, E. L. 2011. Surgical time and severity of postoperative pain in dogs undergoing laparoscopic ovarioectomy with one, two, or three instrument cannulas. J. Am. Vet. Med. Assoc. 239: 203–208. [Medline] [CrossRef]
6. Casey, K. M., Steffey, M. A. and Afferter, V. K. 2016. Identification of occult micrometastases and isolated tumour cells within regional lymph...
nodes of previously diagnosed non-metastatic (stage 0) canine carcinomas. *Vet. Comp. Oncol.* **15**: 785–792. [Medline] [CrossRef]

7. Chaffin, K. and Thrall, D. E. 2002. Results of radiation therapy in 19 dogs with cutaneous mast cell tumor and regional lymph node metastasis. *Vet. Radiol. Ultrasound* **43**: 392–395. [Medline] [CrossRef]

8. Cochran, A. J., Ohise, S. J. and Binder, S. W. 2008. Pathobiology of the sentinel node. *Curr. Opin. Oncol.* **20**: 190–195. [Medline] [CrossRef]

9. Culp, W. T., Mayhew, P. D. and Brown, D. C. 2009. The effect of laparoscopic versus open ovariohysterectomy on postsurgical activity in small dogs. *Vet. Surg.* **38**: 811–817. [Medline] [CrossRef]

10. Devitt, C. M., Cox, R. E. and Hailey, J. J. 2005. Duration, complications, stress, and pain of open ovariohysterectomy versus a simple method of laparoscopic-assisted ovariohysterectomy in dogs. *J. Am. Vet. Med. Assoc.* **227**: 921–927. [Medline] [CrossRef]

11. Fiorbianco, V., Skalsky, M., Doerner, J., Findik, M. and Dupré, G. 2012. Right intercostal insertion of a Veress needle for laparoscopy in dogs. *Vet. Surg.* **41**: 367–373. [Medline]

12. Ghisleni, G., Roccabianca, P., Ceruti, R., Stefanello, D., Bertazzolo, W., Bonfanti, U. and Caniatti, M. 2006. Correlation between fine-needle aspiration cytology and histopathology in the evaluation of cutaneous and subcutaneous masses from dogs and cats. *Vet. Clin. Pathol.* **35**: 24–30. [Medline] [CrossRef]

13. Hobson, H. P., Brown, M. R. and Rogers, K. S. 2006. Surgery of metastatic anal sac adenocarcinoma in five dogs. *Vet. Surg.* **35**: 267–270. [Medline] [CrossRef]

14. Howard, E. Evans, A. d. L., 2013. Miller’s Anatomy of the Dog. Saunders, St. Louis.

15. Kamprath, S., Possover, M., Kühne-Heid, R. and Schneider, A. 1997. Value of laparoscopic pelvic lymph node excision in inguinal vulvar carcinoma metastasis. *Zentralbl. Gynakol.* **119**: 331–333. [Medline]

16. Llabrés-Díaz, F. J. 2004. Ultrasonography of the medial iliac lymph nodes in the dog. *Vet. Radiol. Ultrasound* **45**: 156–165. [Medline] [CrossRef]

17. McGinnis, D. E., Strup, S. E. and Gomella, L. G. 2000. Management of hemorrhage during laparoscopy. *Vet. Radiol. Ultrasound* **41**: 225–227. [Medline] [CrossRef]

18. Majeski, S. A., Steffey, M. A., Fuller, M., Hunt, G. B., Mayhew, P. D. and Pollard, R. E. 2017. Indirect computed tomographic lymphography for iliosacral lymphatic mapping in a cohort of dogs with anal sac gland adenocarcinoma: technique description. *Vet. Radiol. Ultrasound* **58**: 295–303. [Medline] [CrossRef]

19. Palladino, S., Keyerleber, M. A., King, R. G. and Burgess, K. E. 2016. Utility of Computed Tomography versus Abdominal Ultrasound Examination to Identify Iliosacral Lymphadenomegaly in Dogs with Apocrine Gland Adenocarcinoma of the Anal Sac. *J. Vet. Intern. Med.* **30**: 1858–1863. [Medline] [CrossRef]

20. Papadia, A., Remorgida, V., Salom, E. M. and Ragni, N. 2004. Laparoscopic pelvic and paraaortic lymphadenectomy in gynecologic oncology. *J. Am. Assoc. Gynecol. Laparosc.* **11**: 297–306. [Medline] [CrossRef]

21. Pollard, R. E., Fuller, M. C. and Steffey, M. A. 2017. Ultrasound and computed tomography of the iliosacral lymphatic centre in dogs with anal sac gland carcinoma. *Vet. Comp. Oncol.* **15**: 299–306. [Medline] [CrossRef]

22. Polton, G. A. and Brearley, M. J. 2007. Clinical stage, therapy, and prognosis in canine anal sac adenocarcinoma. *Vet. Surg.* **36**: 274–280. [Medline] [CrossRef]

23. Pope, J. F. and Knowles, T. G. 2014. Retrospective analysis of the learning curve associated with laparoscopic ovariohysterectomy in dogs and associated perioperative complication rates. *Vet. Surg.* **43**: 668–677. [Medline] [CrossRef]

24. Possover, M., Krause, N., Plaul, K., Kühne-Heid, R. and Schneider, A. 1998. Laparoscopic para-aortic and pelvic lymphadenectomy: experience with 150 patients and review of the literature. *Gynecol. Oncol.* **71**: 19–28. [Medline] [CrossRef]

25. Prieto, A. V., Kruger, P. A., Hståd, J., van Diest, P. J., Teule, G. J., Kenemans, P. and Verheijen, R. H. 2004. The sentinel node in cervical cancer: scintigraphy and laparoscopic gamma probe-guided biopsy. *Eur. J. Nucl. Med. Mol. Imaging* **31**: 1479–1486. [Medline] [CrossRef]

26. Rungé, J. J., Boston, R. C., Ross, S. B. and Brown, D. C. 2014. Evaluation of the learning curve for a board-certified veterinary surgeon performing laparoendoscopic single-site ovarioectomy in dogs. *J. Am. Vet. Med. Assoc.* **245**: 828–835. [Medline] [CrossRef]

27. Steffey, M. A., Radlinsky, M., Padgett, K. and Rawlings, C. 2014. Operative Assessment of Pelvic Lymphatic Drainage and Laparoscopic Surgical Access to the Iliosacral Lymphatic Center of the Dog: Preclinical Studies. Proceedings of the 2014 Meeting of the Veterinary Endoscopy Society, 54.

28. Steffey, M. A., Daniel, L., Mayhew, P. D., Afolter, V. K., Soares, J. H. and Fuller, M. C. 2015. Laparoscopic Extirpation of the Medial Iliac Lymph Nodes in Normal Dogs. *Vet. Surg.* **44** Suppl 1: 59–65. [Medline] [CrossRef]

29. Tekkis, P. P., Senagore, A. J., Delaney, C. P. and Fazio, V. W. 2005. Evaluation of the learning curve in laparoscopic colorectal surgery: comparison of right-sided and left-sided resections. *Ann. Surg.* **242**: 83–91. [Medline] [CrossRef]

30. Williams, L. E., Gliatto, J. M., Dodge, R. K., Johnson, J. L., Gamblin, R. M., Thamm, D. H., Lana, S. E., Szymkowski, M., Moore A. S., Veterinary Cooperative Oncology Group. 2003. Carcinoma of the apocrine glands of the anal sac in dogs: 113 cases (1985–1995). *J. Am. Vet. Med. Assoc.* **223**: 825–831. [Medline] [CrossRef]

31. Worley, D. R. 2014. Incorporation of sentinel lymph node mapping in dogs with mast cell tumours: 20 consecutive procedures. *Vet. Comp. Oncol.* **12**: 215–226. [Medline] [CrossRef]