Accurate tumor staging is essential for dogs and cats affected by neoplasia.1–9 Lymph node evaluation is an important initial step in the management of these patients, providing accurate prognostic information, and helps to guide treatment.1–9 Many studies3–9 have revealed that lymph node size as assessed by palpation or advanced diagnostic imaging correlates poorly with the presence or absence of tumor metastasis. Cytologic examination of cells aspirated from a lymph node is a readily available, inexpensive diagnostic procedure that has good sensitivity and specificity for detecting neoplastic cell infiltration. However, fine-needle aspiration of a palpable lymph node is easier, compared with aspiration of nonpalpable lymph nodes.10,11 Despite good sensitivity and specificity, cytologic examination may still yield inaccurate results mainly because of the small amount of aspirated material and the inability to evaluate tissue structure and organization.3–8,11–13 As such, neoplastic cell infiltration of lymph nodes may not be determinable. Noninvasive techniques for identifying neoplastic cells in lymph nodes, such as contrast-enhanced ultrasonography and CT, sonoelastography, and lymphoscintigraphy, have been investigated, but histologic examination of lymph node tissue remains the most accurate method, especially in cases of micrometastasis.7,14–17 Sentinel (the first lymph node to which tumor cells are most likely to spread from a primary tumor) or regional lymphadenectomy and histologic examination of the excised lymph nodes allow for identification of overt metastasis, small metastatic aggregates, and micrometastatic foci, especially when examination includes application of

**OBJECTIVE**

To evaluate ultrasound-guided placement of an anchor wire (AW) or injection of methylene blue (MB) to aid in the intraoperative localization of peripheral lymph nodes in dogs and cats.

**ANIMALS**

125 dogs and 10 cats with a total of 171 lymphadenectomies.

**PROCEDURES**

Medical records of dogs and cats that underwent peripheral lymphadenectomies with or without (N) the AW or MB localization technique were reviewed. Data retrieved included clinical, surgical, and histologic findings. The proportions of successful lymphadenectomies, lymph node characteristics, and complications among the 3 groups were analyzed.

**RESULTS**

143 (84%) lymph nodes were successfully excised. Lymphadenectomy success was significantly affected by the localization technique, with 94% for group AW, 87% for group MB, and 72% for group N. Lymph node size was smaller in groups AW and MB, compared with group N. Duration of lymphadenectomy was shorter in group AW, compared with groups MB and N, and in group MB, compared with group N. Intra- (7%) and postoperative (10%) complications and final diagnosis did not significantly differ among groups.

**CONCLUSIONS AND CLINICAL RELEVANCE**

Both lymph node localization techniques were highly successful and reduced surgery time, compared with unassisted lymphadenectomy. Specifically, these techniques were effective for localization of normal-sized and nonpalpable lymph nodes and were efficient and practical options for peripheral lymphadenectomies, particularly for those that were small or nonpalpable.
Materials and Methods

Study design and animal eligibility criteria

This retrospective study was approved by the University of Liverpool (protocol No. VREC933) and University of Pisa (protocol No. 48/2019) Research Ethics Committees. The medical records from the University of Liverpool Small Animal Teaching Hospital and Centro Veterinario Dott.ri Pisani-Carli-Chiodo were searched for dogs and cats that had undergone excision of ≥ 1 peripheral lymph nodes between January 2015 and March 2020. Data retrieved included signalment, body condition score (range, 1 to 9), primary tumor histotype, number of excised lymph nodes and their anatomical locations, presence of palpable peripheral lymphadenomegaly (determined through comparison with the contralateral or other lymph nodes), diagnostic imaging used for tumor staging at the time of the initial examination, lymph node diameter determined via diagnostic imaging, duration of preoperative ultrasound, lymph node localization technique, time required for lymph node localization and MB injection or AW placement, any concomitant surgical procedure, duration of lymphadenectomy, results of histologic examination of the excised lymph nodes, intra- and postoperative complications, and any further surgical intervention or medical treatment. Complications were classified as minor, defined as those that did not require additional surgical or medical treatment to resolve, or major, defined as those that required further surgical or medical treatment to resolve. Lymphadenectomy was performed for diagnostic or therapeutic purposes for all animals. The lymph node to be excised was determined on the basis of lymph node size at the time of physical examination, location of the primary tumor, nodal appearance in diagnostic radiographic or ultrasonographic images, previous results of lymph node cytopathology examination, results of (sentinel) lymph node mapping by use of lymphangiography, and site of anatomical drainage when lymphangiographic mapping was not performed. Successful lymphadenectomy was defined as accurate lymph node localization and excision as confirmed by histologic examination.

For an animal to be included in this study, each lymph node had to be excised with a separate surgical approach from that for the excision of the primary tumor or the other lymph nodes and had to have a minimal follow-up period of 14 days. For animals that underwent many lymphadenectomies, each lymph node was included as 1 case only if it was from a different lymphoid center. A lymph node was also considered as a new case if lymphadenectomies were performed at other time points because of lymph node changes that were noted during tumor restaging or when another tumor was diagnosed. Animals were excluded if a lymphadenectomy was performed through the same surgical field as another lymph node (ie, lymph nodes were from the same lymphoid center or a ventral approach was used for bilateral cervical lymphadenectomies) or the primary tumor or a lymph node was included in the tissue excised with the primary tumor (eg, limb amputations and radical mastectomies). Popliteal lymphadenectomies were not included in this study because assisted localization was usually unnecessary in our experience.
Localization techniques

The patients were anesthetized, their hair was clipped, and their skin was aseptically prepared prior to the use of any localization technique; preoperative ultrasonography was performed aseptically approximately 15 minutes before surgery by a board-certified radiologist or an experienced ultrasonographer, with the patient positioned for optimal access to the desired lymph node. Patients were grouped on the basis of the localization technique used as follows.

Group N (unassisted localization)—Unassisted localization and surgeon-requested preoperative ultrasonography were used to determine the size and position of the regional lymph node.

Group MB (localization with MB)—On the basis of a lymph node’s location and its depth and on the patient’s size and body condition score, 0.1 to 0.5 mL of a 1% aqueous solution of MB was aseptically injected with a 23- to 27-gauge needle and a 1-mL syringe into the lymph node or perinodal tissue with ultrasound guidance. If the surgeon requested, an MB track from the lymph node to the skin was created by depositing approximately 0.01 mL of MB every few millimeters as the needle was removed from the lymph node to serve as a guide for surgical dissection. Animals that had peritumoral MB injection for sentinel lymph node identification were excluded.

Group AW (localization with AW placement)—The ultrasound probe was positioned above the lymph node. With an aseptic technique and ultrasound guidance, a 20-gauge, 70-mm localization needle (IM-IMX, 01M2007; Biomedical Srl) was passed immediately adjacent to or in a lymph node (Supplementary Figure S1). The point of introduction of the wire guide was agreed to with the surgeon on the basis of the planned surgical approach, or the needle was introduced perpendicular to the skin over the lymph node as close as possible to the intended site of the surgical incision. The beveled needle tip was positioned centrally in or adjacent to the lymph node. The wire component protruded beyond the base of the needle and was used to gently push the hook out of the needle when the needle tip position was satisfactory. The hook was deployed by fixing the needle firmly with one hand and gently advancing the wire with the other hand until a click was audible or palpable or the black mark on the wire was no longer visible, indicating that the AW had been deployed. At that point, the hook wire was anchored in the tissue and could not be retracted. The needle was removed over the wire until the thread extending from the tip of the hook wire was visible on the skin surface. The ultrasound probe was positioned again over the lymph node to verify the correct placement of the AW, and the wire was trimmed approximately 2 cm from the skin at the insertion site.

Surgical approach

After ultrasonography, each patient was transferred to the operating room and skin preparation with an alcohol-based solution was repeated over the surgical field. Lymphadenectomy was performed by an experienced surgeon or by a small animal surgery resident under direct supervision by an experienced surgeon and, where a localization technique was used, the procedure was performed before excision of the primary tumor in all patients in the AW group and in most patients in the MB group. The surgical procedure for each animal in each group was as follows.

Group N—The skin was incised over the lymph node, and a combination of blunt and sharp dissection and electrosurgery was used to identify and excise the lymph node.

Group MB—The skin was incised over the lymph node or where the MB track was visible on the skin. Careful blunt and sharp dissection was used to trace the MB track or to locate the MB stain within the lymph node or perinodal tissue.

Group AW—The skin was incised at the exit site of the wire. A combination of blunt and sharp dissection was used to follow the wire until the lymph node was located.

Once the lymph node was located, the associated vasculature was coagulated or ligated, and without disrupting its capsule, the lymph node was excised. Closure of the surgical site was routine.

Statistical analysis

Statistical analyses were performed with commercially available software (Excel version 14.00 2011 [Microsoft Corp] and SPSS version 26.0 [IBM Corp]). Descriptive statistics were reported for all variables. The Shapiro-Wilk test confirmed that the data were not normally distributed (P < 0.001), so the data were reported as median and range. Assessed continuous explanatory variables were age, body weight, lymph node width, and duration of lymphadenectomy. Assessed categorical variables were species, sex, neuter status, body condition score, presence of palpable lymphadenomegaly, occurrence of intra- and postoperative complications, successful lymphadenectomy, and histologic diagnosis (metastatic vs nonmetastatic disease).

A Kruskal-Wallis H test was used to determine whether any significant association existed between the continuous variables and the localization techniques, and a χ² test was used to determine whether any significant association existed between the categorical variables and the localization techniques. When a significant association was confirmed, the Fisher exact test for categorical variables and the Mann-Whitney U test for continuous variables were used to characterize the results. Values of P < 0.05 were considered significant.

Results

A total of 171 peripheral lymphadenectomies from 125 dogs and 10 cats met the inclusion criteria. Mixed breed (n = 25 [20%]) was the most common...
type of dog and domestic shorthair (7 [70%]) was the most common type of cat. Patient characteristics, physical examination findings, lymph node characteristics, lymph node localization techniques, duration of lymphadenectomy, and perioperative complications are summarized in Table 1. Age, body weight, neuter status, sex, species, and body condition score did not significantly differ among the groups.

Lymphadenomegaly was more common in group N than in groups MB (P = 0.038) and AW (P = 0.012), whereas the frequency of lymphadenomegaly did not significantly differ between groups MB and AW (Supplementary Table S1). All animals but 1 underwent preoperative tumor staging; lymph nodes were evaluated with ultrasonography for 102 (60%) lymphadenectomies and CT for 68 (40%). On the basis of the findings of preoperative diagnostic imaging, target lymph nodes were significantly smaller in group AW, compared with those in groups N (P = 0.001) and MB (P = 0.002), and in group MB, compared with group N (P = 0.04).

Of the 135 animals, 30 (22%) had undergone only lymphadenectomy, whereas 105 (78%) had also undergone another surgery during the same anesthetic period, including cutaneous or subcutaneous mass excision (n = 81), pinnectomy (6), scar excision (5), mandibulectomy (3), thyroidectomy (3), medial iliac lymphadenectomy (3), splenectomy (3), nasal planum excisional biopsy (2), pelvic limb amputation (2), enucleation (1), digit amputation (1), radical mastectomy (1), orchietomy (1), and arytenoid lateralization (1). Several peripheral lymphadenectomies were performed during the same anesthetic period in 37 (27%) animals. The most common tumor was mast cell tumor (n = 89), followed by melanoma (12), mammary carcinoma (7), thyroid carcinoma (6), soft tissue sarcoma (6), lymphoma (4), adenocarcinoma (3), squamous cell carcinoma (3), plasmacytoma (2), systemic histiocytosis (2), fibrosarcoma (2), hemangiosarcoma (2), and myxosarcoma (1). In 1 dog, granulomatous inflammation was diagnosed. Histologic examination of 143 lymph nodes revealed the presence of neoplastic cells in 106 (74%), and 56 (53%) of these lymph nodes were of normal size or nonpalpable. Neoplastic cells were not seen in the remaining 37 (26%).

| Table 1 — Patient characteristics, physical examination findings, lymph node characteristics, duration of lymphadenectomy, and perioperative complications associated with 171 lymphadenectomies in 125 dogs and 10 cats classified by lymph node localization method, unassisted localization (N), methylene blue (MB) injection, or anchor wire (AW) placement. |
| Variable | Group N (n = 57) | Group MB (n = 67) | Group AW (n = 47) | P value |
| Species, No. (%) | Dog 46 (55) | 48 (62) | 31 (42) | 0.361 |
| Cat 2 (2) | 4 (5) | 4 (5) |
| Sex, No. (%) | Spayed females 24 (21) | 32 (28) | 17 (12) | 0.490 |
| Neutered males 24 (19) | 20 (15) | 18 (14) |
| Age (mo)* | 98 (45–168) | 108 (27–228) | 100 (37–178) | 0.167 |
| Body weight (kg)* | 22.4 (3.6–48.2) | 20.9 (2.6–51.5) | 23.3 (3.1–51) | 0.130 |
| Body condition score† | 5 (3–8) | 6 (3–9) | 6 (3–8) | 0.319 |
| Large lymph nodes, No. (%) | 19 (33) | 12 (18) | 5 (11) | 0.026 |
| Lymph node width (mm)* | 8 (3–40) | 6 (2.7–30) | 5 (2–30) | < 0.001 |
| Duration of lymphadenectomy (min)*‡ | 32 (15–95) | 25 (15–65) | 15 (4–35) | < 0.001 |
| Lymph node excision, No. (%) | 41 (72) | 38 (87) | 44 (94) | 0.009 |
| Mandibular | 18/19 (95) | 16/16 (100) | 6/6 (100) |
| Retropharyngeal | 4/6 (67) | 5/8 (63) | 2/2 (100) |
| Prescapular | 11/12 (92) | 15/16 (94) | 3/3 (100) |
| Axillary | 1/6 (17) | 4/6 (67) | 7/9 (78) |
| Inguinal | 7/14 (50) | 18/21 (86) | 26/27 (96) |
| Histologic diagnosis, No. (%) | Consistent with tumor metastasis | 28 (68) | 48 (83) | 30 (68) | 0.110 |
| Consistent with nonmetastatic change | 13 (32) | 10 (17) | 14 (32) |
| Complications, No. (%) | Intraoperative | 1 (1.8) | 6 (9) | 5 (9) | 0.153 |
| Postoperative | 6 (11) | 9 (13) | 1 (3) | 0.197 |

*Median (range). †Body condition was scored between 1 and 9. ‡Duration of lymphadenectomy recorded for only 123 (72%) lymphadenectomies.

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Duration of lymphadenectomy was significantly (P < 0.001) shorter for groups AW (median, 15 minutes) and MB (median, 25 minutes), compared with group N (median, 32 minutes), and for group MB, compared with group N (P = 0.026). Antimicrobial and analgesic treatments were prescribed postoperatively at the discretion of the surgeon, and all animals returned to the soft tissue surgery or oncology service by 14 days after surgery for reexamination or follow-up treatment.

Successful lymphadenectomy was achieved in 143 of the 171 (84%) cases, whereas the lymph node could not be located or excised in the remaining 28 (16%) cases; in 1 animal in group N, the mandibular salivary gland was excised instead of the mandibular lymph node. Successful lymphadenectomy was significantly (P = 0.009) affected by the use (vs non-use) of a localization technique; more lymph nodes in groups MB (58/67 [87%]; P = 0.044) and AW (44/47 [94%]; P = 0.005) were successfully excised than in group N (41/57 [72%]). The success of lymph node localization and lymphadenectomy did not significantly (P = 0.354) differ between groups MB and AW. Among the 3 groups, mandibular lymphadenectomy was the most successful procedure (40/41 [98%]), followed by prescapular (29/31 [94%]), inguinal (51/62 [82%]), and retropharyngeal (11/16 [69%]) lymphadenectomies. Axillary lymphadenectomy was the least successful procedure (12/21 [57%]). The proportions of mandibular and prescapular lymphadenectomies performed successfully were similar among the 3 groups, but proportionally, the AW technique was better for retropharyngeal lymphadenectomy, compared with the MB technique and N, whereas the use of a localization technique was better for axillary and inguinal lymphadenectomies.

Surgery was uncomplicated in 159 of 171 (93%) lymphadenectomies. Twelve intraoperative complications occurred in 11 dogs. One dog in group N had self-limiting hemorrhage during excision of an inguinal lymph node. Lymph node localization was impaired in 6 of 67 (9%) dogs because of leakage of MB into the subcutaneous tissue. In the AW group, 5 complications occurred intraoperatively: 2 AWs dislodged during surgery, but lymph node localization was not affected, and 3 AWs fragmented during excision of 2 axillary lymph nodes in 1 dog and 1 inguinal lymph node in 2 dogs. These 3 AWs could not be retrieved despite the use of radiography and fluoroscopy; the 2 axillary lymph nodes were not localized, whereas the inguinal lymph node was localized. During the follow-up period of 189 days for one of the dogs and 191 days for the other, no further complications were reported.

In the postoperative period, 17 (10%) complications occurred. Sixteen were considered minor and included seroma (n = 9), wound breakdown (4), erythema (2), and cellulitis (1). One complication was considered major: 1 dog had a surgical site infection at the lymphadenectomy site that resolved after antimicrobial treatment. All complications occurred within 14 days after surgery. The median postoperative follow-up time was 115 days (range, 21 to 1,028 days), and follow-up time did not significantly differ among the groups.

Discussion

The results of the present study reveal that both AW and MB techniques were successful for facilitating peripheral lymphadenectomy, and proportionally, success did not significantly differ between these techniques. The American Society of Breast Surgeons suggested that any lymph node localization technique should be ≥ 85% successful.28 As demonstrated in the present study, both localization techniques were ≥ 85% successful (group MB, 87%; group AW, 94%) and were significantly more successful for regional lymph node localization, compared with unassisted localization (group N, 72%). In addition, use of these techniques significantly reduced the duration of lymphadenectomy, but lymphadenectomy following the AW technique took less time than lymphadenectomy following the MB technique.

The aim of regional lymph node evaluation is to determine the spatial extent of neoplastic disease, thereby helping to guide optimal treatment decisions and possibly influencing prognosis.17,29 The decision of whether to perform a lymphadenectomy is based on the results of preoperative diagnostic imaging, cytologic examination of cells aspirated from a lymph node, and primary tumor type. Lymphadenectomy can be associated with increased surgery time and patient morbidity and a lack of documented clinical benefit for some tumors.9

Fifty percent of normal-sized lymph nodes determined via palpation or nonpalpable lymph nodes that were excised for tumor staging in dogs with mast cell tumors had neoplastic mast cells that were confirmed with histologic examination.5 Possibly, tumor staging without regional lymphadenectomy and subsequent histologic evaluation would have led to different decisions regarding patient monitoring.9

In our experience, excision of nonpalpable and normal-sized lymph nodes (determined through palpation) is a surgical challenge. Excision of these lymph nodes may increase surgery time and morbidity because additional incisions or an extensive approach and more tissue dissection are needed; moreover, lymph node localization may be unsuccessful. Therefore at our institutions, the lymph node localization techniques MB and AW were instituted to overcome this surgical challenge.

MB has been used in various diagnostic and therapeutic procedures because it is generally considered safe, readily available, and inexpensive. However, in people, it may induce a hypersensitivity reaction that ranges from skin rashes to life-threatening anaphylaxis, an intense tissue reaction that may result in skin necrosis when it is injected intradermally,
a blue discoloration of the operating field, a spurious decrease in intraoperative oxygen saturation of hemoglobin, and permanent skin staining.5,10,28,30–34

Reported complications for dogs and cats following IV administration of MB include Heinz body anemia, pseudocyanosis, increased serum alkaline phosphatase activity, and kidney failure.20,35–35

In a recent study35 of the detection and excision of sentinel lymph nodes following peritumoral injection of a radionuclide and MB, no short- or long-term adverse effects were reported; however, MB injection was peritumoral, and the peritumoral tissues were excised with the tumor. Despite the difference in MB techniques between the present and previous studies, no perioperative adverse reactions, including skin necrosis, were noted in either study. Other adverse effects, such as anemia and kidney failure, which often occur within days following MB injection, or granuloma formation, which may occur weeks following MB injection, were also not seen in the present study. Moreover, no differences in postoperative complications were reported between the techniques.

One problem that was encountered with the MB technique in the present study was occasional leakage of MB into the subcutaneous tissue, such that the leakage interfered with the surgeon’s ability to identify the lymph node. In these situations, all the stained tissue was excised en bloc. Given the close anatomic relationship of the peripheral lymph nodes with major regional vessels, en bloc resection could result in vascular injury and hemorrhage; therefore, caution is recommended when performing the MB technique. However, vascular injury and hemorrhage were not noted in the affected dogs in the present study.

MB is usually easily visible in the subcutaneous tissue because of its color, so we recommend using a small quantity of MB when injecting it into the perinodal tissue. For deep (vs superficial) lymph nodes, a tracel of MB from the lymph node to the skin proved helpful in maintaining orientation during surgery and guiding surgical dissection.

The AW technique is an established, minimally invasive technique adopted in people for the surgical approach of nonpalpable mammary nodules and sentinel axillary lymph nodes; the AW technique has also been used in revision surgeries; foreign body removal; and excision of benign masses, pulmonary nodules, and head and neck lesions (eg, cervical lymph nodes and thyroid tumors).2,24,25,44–49

This technique was designed to improve the accuracy of resection of a lesion, reduce surgery time, and limit damage to nearby structures;24,25,46,47; the technique has similar advantages when used for the excision of inguinal lymph nodes in dogs.27

The AWs may be deployed within or near to the lesion, depending on the local anatomy and pathologic condition. Therefore, given our experience, the surgeon should be present at the time of ultrasonography to be aware of how the wire is deployed and its position, allowing for improved dissection along the wire tract and adequate lesion resection.24

When the AW technique was first used, the aim was to place the AW in the lymph node; however, in many animals, the AWs were found in the perinodal tissue. This may have occurred because of the small size of many of the lymph nodes, AW dislodgement during surgical preparation of the skin after preoperative ultrasonography, or the diagnostic imaging team’s lack of experience with placement of AWs. The exact location of the AW was not clinically relevant in the present study because lymph node localization and excision were highly successful. However, feedback from the surgeon following surgery is important to determine whether AW placement in a lymph node is optimal or required.

Wire transection and migration during surgery and damage to vital structures near lesions are well-known complications reported for the AW technique.49 Intraoperatively, the thin wire may be inadvertently transected such that the removal of the target lesion may be compromised and the wire fragment may be retained in the body, which happened in 1 dog during bilateral axillary lymphadenectomy and in another dog during inguinal lymphadenectomy. The wires had fragmented during dissection, and they could not be retrieved. No complications were reported during the follow-up period for these dogs, but close postoperative monitoring is advised when wire fragmentation occurs.

In people, failure to locate peripheral lymph nodes with the AW technique is associated with age; body mass index; tumor size, location, and grade; type of previous biopsy; and the surgeon’s experience.5 Prospective studies with large sample sizes of dogs and cats are needed to minimize selection bias and to evaluate the potential risk factors for failing to locate lymph nodes with the MB or AW techniques.

The present study was focused on the ability of these techniques to help locate various peripheral lymph nodes, including those that were not sentinel lymph nodes. Sentinel lymph nodes can be the regional lymph nodes but can also be lymph nodes at distant anatomic locations.14 Various mapping techniques have been developed to localize the sentinel lymph nodes.13,14,35 The localization techniques evaluated in the present study may be combined with those mapping techniques to guide excision of the sentinel lymph node for accurate tumor staging.

No differences in the proportions of successful lymphadenectomies or complications were noted between the MB and AW groups, but the target lymph nodes were significantly smaller in the AW group, and the duration of lymphadenectomy was significantly less, compared with the MB group. The use of the AW technique significantly reduced surgery time and facilitated the localization of small and nonpalpable lymph nodes.

The main limitation of the present study was its retrospective nature, such that patient management
varied. Although the patients were followed for a minimum of 14 days after surgery, long-term complications may have been missed. The size of the lymph nodes may have led to a selection bias because the attending clinician may have not elected to use a localization technique for a lymph node that was easily palpable. The techniques were used to localize only 1 lymph node from each lymphoid center, but they may be beneficial for the localization of multiple lymph nodes from the same lymphoid center. Additional studies are needed to assess these techniques’ ability to help in identifying multiple lymph nodes from the same lymphoid centers.

In conclusion, ultrasound-guided injection of MB and placement of an AW were safe and accurate techniques to locate various peripheral lymph nodes. The AW technique may be particularly beneficial techniques to locate various peripheral lymph nodes. The AW technique has the potential to reduce surgery time and minimize invasiveness. The AW technique may be particularly beneficial to help in identifying multiple lymph nodes from the same lymphoid center. Additional studies are needed to assess these techniques’ ability to help in identifying multiple lymph nodes from the same lymphoid centers.

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Supplementary Materials
Supplementary materials are posted online at the journal website: avmajournals.avma.org