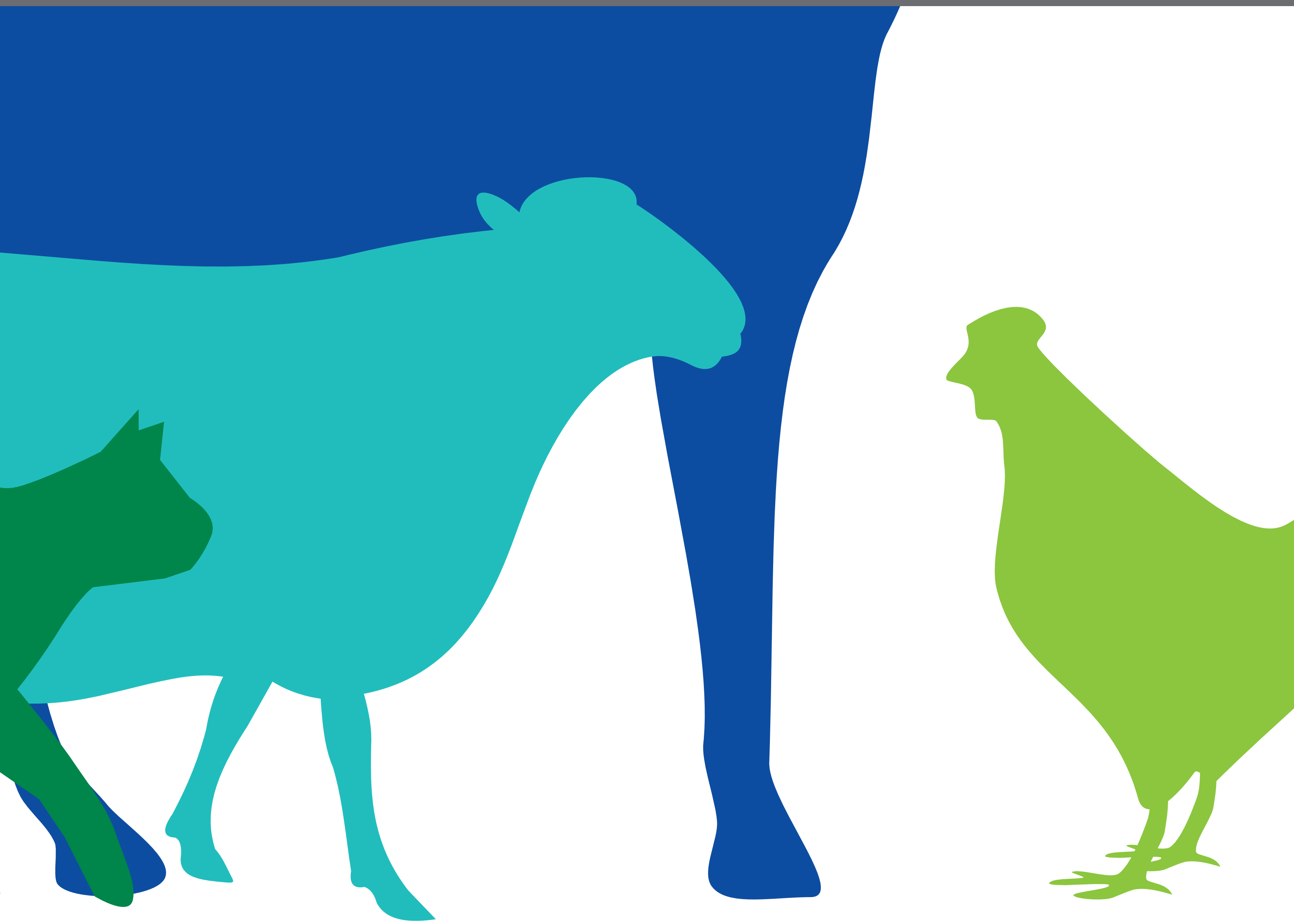
RECENT ADVANCES IN ORAL AND MAXILLOFACIAL SURGERY

EDITED BY: Boaz Arzi and Nadine Fiani
PUBLISHED IN: Frontiers in Veterinary Science



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ISSN 1664-8714
ISBN 978-2-88974-187-8
DOI 10.3389/978-2-88974-187-8

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RECENT ADVANCES IN ORAL AND MAXILLOFACIAL SURGERY

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Citation: Arzi, B., Fiani, N., eds. (2022). Recent Advances in Oral and Maxillofacial Surgery. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88974-187-8

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frontiers in Veterinary Science

ORIGINAL RESEARCH
published: 27 February 2020
doi: 10.3389/fvets.2020.00059

Biomechanical Testing of a Calcium Phosphate-Phosphoserine–Based Mineral-Organic Adhesive for Non-invasive Fracture Repair of Mandibular Fractures in Dogs

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OPEN ACCESS

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Specialty section:
This article was submitted to
Veterinary Dentistry and
Oromaxillofacial Surgery,
a section of the journal
Frontiers in Veterinary Science

Received: 29 November 2019
Accepted: 24 January 2020
Published: 27 February 2020

Citation:
Geddes AT, Thatcher GP, Hetzel S, McCabe RP, Vandereby R Jr and Snyder CJ (2020) Biomechanical Testing of a Calcium Phosphate-Phosphoserine–Based Mineral-Organic Adhesive for Non-invasive Fracture Repair of Mandibular Fractures in Dogs. Front. Vet. Sci. 7:59. doi: 10.3389/fvets.2020.00059

Mandibular fracture repair is complicated by limited availability of bone as well as the presence of the neurovascular bundle and an abundance of tooth roots. Fractures at the location of the mandibular first molar teeth are common and it can be particularly challenging to apply stable fixation. Non-invasive fracture repair techniques utilize intraoral placement of fixation devices typically involving polymerized composites and/or interdental wiring. A novel calcium phosphate-phosphoserine–based mineral–organic adhesive was tested *ex vivo* to determine its effects on augmenting strength of different non-invasive fracture fixation techniques. This study both tested the use of mineral–organic adhesive for the purpose of stabilizing currently used non-invasive fracture repair constructs (intraoral composite splinting ± interdental wiring) and evaluated adhesive alone or with subperiosteally placed plates on buccal cortical bone surface. Aside from controls, not receiving an osteotomy along the mesial root of the mandibular first molar tooth, six treatment groups were tested to evaluate ultimate strength, stiffness, angular displacement, bending moment, and application time. All forms of fixation were found to be significantly weaker than control ($p < 0.001$). Only the control ($p < 0.001$) and mineral–organic adhesive and composite ($P = 0.002$) groups were found to be significantly stronger than wire and composite. No difference was noted in stiffness between any groups with control or wire and composite. Application times varied from the mineral–organic adhesive group (mean = 206 s) to mineral–organic adhesive and composite (mean = 1,281 s). Twenty-three fixation devices exhibited adhesive failure, 20 demonstrated cohesive failure, and 5 failed by cohesive and adhesive failure. When evaluating the ultimate strength of the fixation device groups, mineral–organic adhesive, and composite was shown to be the strongest construct. The use of resorbable bone adhesive and composite may provide a stronger fixation construct over interdental wire and composite for mandibular fracture repair in dogs.

Keywords: non-invasive, fracture, repair, mandible, dogs, adhesive, strength, stiffness bone healing

INTRODUCTION

Mandibular fractures are the most commonly occurring maxillofacial fractures in small animal veterinary patients (1–4). Approximately 90% of maxillofacial injuries in canines are reported to be mandibular fractures (1, 2) with 47% sustained in the area of the mandibular first molar tooth (1). In a study characterizing mandibular first molar root volumes compared with mandibular volume, relative root volume increased as patient body weight decreased (5). This predisposition for fracture helps explain the propensity for fracture occurrence at this location in small breed dogs.

Mandibular fracture repair can be particularly challenging in the caudal mandible of dogs. Muscular attachments and neurovascular structures in the caudal mandible complicate surgical exposure compared to rostral fracture repair. Anatomic structures, such as tooth roots and the inferior alveolar neurovascular bundle, severely limit locations where pilot screw holes can be created for conventional plate fixation (6). Non-invasive fracture repair techniques minimize surgical exposure of the fracture site and minimize risk of damaging or disrupting anatomic structures such as tooth roots and neurovascular structures. These non-invasive techniques have gained in popularity, due to extensive experience with clinical application of dental composites in veterinary medicine (7–15). The splints created of human dental composites and used in dogs and cats have been shown to be strong (16, 17) and clinically effective at achieving fracture union (8–14). Using the tension band principle, placement of non-invasive fracture repair constructs along the oral surface of the mandible capitalizes on the creation of a natural compressive force along the ventral surface of the mandible, thus stabilizing mandibular body fractures (15).

A previous study compared interdental wiring techniques and acrylic/composite splints determining that interdental wire with composite splint was stronger than either technique used alone (16). The increased strength of the combined techniques was noted to be particularly important when the mandibular first molar tooth crown was absent for use in fixation of experimentally induced fractures occurring at this location (17). The Stout multiple loop wiring technique provides the benefit of anchoring the wire device to multiple teeth on either side of the fracture, thus distributing the force of the fixation apparatus (18). Disadvantages of interdental wiring include inciting periodontal disease (19), prolonged anesthetic periods (17), and for application and removal and inadvertent wire sticks (20). In humans, it appears long-term consequences of these disadvantages are minimal (19–21).

To date, the availability of bone cements has been limited to non-resorbable materials that are comprised of polymethylmethacrylate, used commonly for the cementation of implants such as total hip replacements (22, 23). Infection (24) and adhesive failure (25) are inherent risks with materials not removed or resorbed. A variety of bone graft materials exist for the purpose of promoting bone formation or bone healing but lack adhesive properties and include autografts, allografts, and synthetics (alloplasts) (26). Obstacles to using techniques to enhance bone healing in veterinary patients include a lack of chemical adhesion and/or mechanical structure, limited commercial availability (bone morphogenic protein), increased surgical time (autograft collection), and cost of allografts (27). Alloplasts function to serve primarily as a scaffold for osteoblasts depositing bone (28). Depending on the chemical makeup of the particular alloplast product, some materials take more than 1 year to resorb and remain incorporated into healed bone (29). Calcium phosphate–based materials serve as a scaffold and over time are broken down into calcium and phosphate and ultimately incorporate into bone (28).

The novel calcium phosphate-phosphoserine–based mineral–organic adhesive (Tetranite® Stabilization Material; LaunchPad Medical, Lowell, MA, USA) is a mixture of the powder forms of both tetracalcium phosphate (TTCP) and phosphoserine, which is mixed in an aqueous medium. Once mixed, this material is self-setting as it cures, and it precipitates primarily as an amorphous calcium phosphate-phosphoserine phase, which creates strong bonds to the surfaces of both bone and metallic implants (30). Within days, the solid evolves into a more crystalline phase of calcium phosphate and calcium phosphoserine. The combination of properties affords a unique potential to serve as a mechanism to enable fracture fixation stabilization while also being resorbed and incorporated into healed bone. The material has been demonstrated to be safe, biocompatible, and resorbed in studies using canines (31). Applying the adhesive to the end surfaces of fractures provides an opportunity for intraoperative bony alignment and may serve as a primary or adjunctive form of fixation to facilitate bone healing.

Evaluating fracture fixation strength in the caudal aspect of the mandible is a highly appropriate, clinically relevant research question due to limited anchorage locations for non-invasive repair techniques. Fractures involving the mandibular first molar tooth may have limited interdental wiring or composite splint anchorage points caudal to this location. Experimental benchtop investigation into strengthening non-invasive repair techniques warrants exploration and analysis with the objective of effectively implementing these techniques clinically. By generating more predictable patient outcomes through the safe implementation of non-invasive repair techniques, the intent is to maximally stabilize bone without creating additional complications such as that seen with the more invasive forms of fracture repair such as the application of plates and screws. This study aims to determine whether the use of resorbable bone adhesive by itself, or in combination with other non-invasive fracture repair techniques, provides biomechanical advantage over interdental wiring and composite splinting. Results of this study may begin to elucidate the benefit of stabilizing mandibular fractures using resorbable bone adhesive as either a replacement for or an adjunct to other non-invasive fracture repair techniques.

MATERIALS AND METHODS

Specimen Preparation

Right and left mandibles were collected from 28 medium-sized dogs (mean = 10.9 kg, range = 8.0–13.6 kg) over 1 year of age (mean = 16.7 months, range = 12–24 months). Ethical approval for this study was not required according to national legislation because the acquired specimens were humanely euthanized prior to, and for reasons unrelated to, this study. Cadaveric specimens rather than synthetic models were necessary because it replicates the clinical scenario and enamel and/or dentin is necessary for chemical adhesion and micromechanical retention of the *bis*-acryl composite to tooth structure (8, 9, 32). Only mandibles with complete dentition between the canine tooth and mandibular third molar tooth were selected. Dogs with periodontal disease greater than stage 1 (gingivitis only) were excluded, based on periodontal probing (33).

Eight mandibles were randomly assigned to each of seven treatment groups: (1) mineral–organic adhesive (adhesive) applied to the ends of the cut surfaces, (2) adhesive on the cut surface and *bis*-acryl composite interdental splint, (3) interdental wire and *bis*-acryl composite splint, (4) adhesive on the cut surface and non-resorbable titanium plate adhered with adhesive, (5) adhesive on the cut surface and resorbable plate made from cured mineral–organic adhesive adhered with adhesive, (6) *bis*-acryl composite only, and (7) control mandibles (**Figures 1A–E**).

Fresh specimens were harvested, and all attached soft tissues were removed with the exception of attached gingiva using combination of blunt and sharp dissection. Once harvested, specimens were stored at −20°C wrapped loosely in moist paper towels to maintain hydration until preparation for testing during a single freeze–thaw cycle.

For preparation, specimens were thawed and refrigerated until potting. Specimens were potted with the ramus in polyester resin (Feather-Rite Lightweight Filler; U.S. Chemical and Plastics, Massillon, OH, USA) within a preformed mold. The mandibular body extended perpendicular from the potted ramus with the height of the mandible oriented perpendicular to the base of the mold. Osteotomies were performed in all treatment groups excluding controls. The osteotomy was created along the mesial root of the mandibular first molar tooth, perpendicular to the long axis of the mandibular body and perpendicular to the buccal bone surface using an oscillating saw (MM40 Oscillating Tool; Dremel, Racine, WI, USA) and thin kerf (0.6 mm thick) blade [MM485B, 31.7 mm Blade (0.6 mm, thick); Dremel]. Following osteotomy, specimens were returned to plastic bags submerged in 100 mL of phosphate-buffered saline (PBS) (PBS tablets; Life Technologies Corp., Carlsbad CA, USA) and placed into a 37°C circulating warming bath for 24 h prior to fixation. All fixation application times were recorded.

The interdental wiring and *bis*-acryl composite group received Stout multiple loop wiring technique [24 gauge orthopedic wire (24 g orthopedic wire; Miltex, Plainsboro NJ, USA)] applied by a single investigator (C.J.S.) extending from the first premolar tooth through the third molar tooth as previously described (15–17). A *bis*-acryl composite (Maxi Temp HP 50 mL; Henry Schein Inc., Melville, NY, USA) splint was applied as described below.

All adhesive groups received the same preparation of the material prior to placement. The mineral–organic adhesive (Tetranite® Stabilization Material; LaunchPad Medical) was provided by the manufacturer in predosed vials. The material is composed of calcium phosphate (61.5%), primarily composed of TTCP phase, and phosphoserine (38.5%), which are mixed with water and applied to the fracture site. Water for injection was added to dry powder in increments of 540 μL, followed

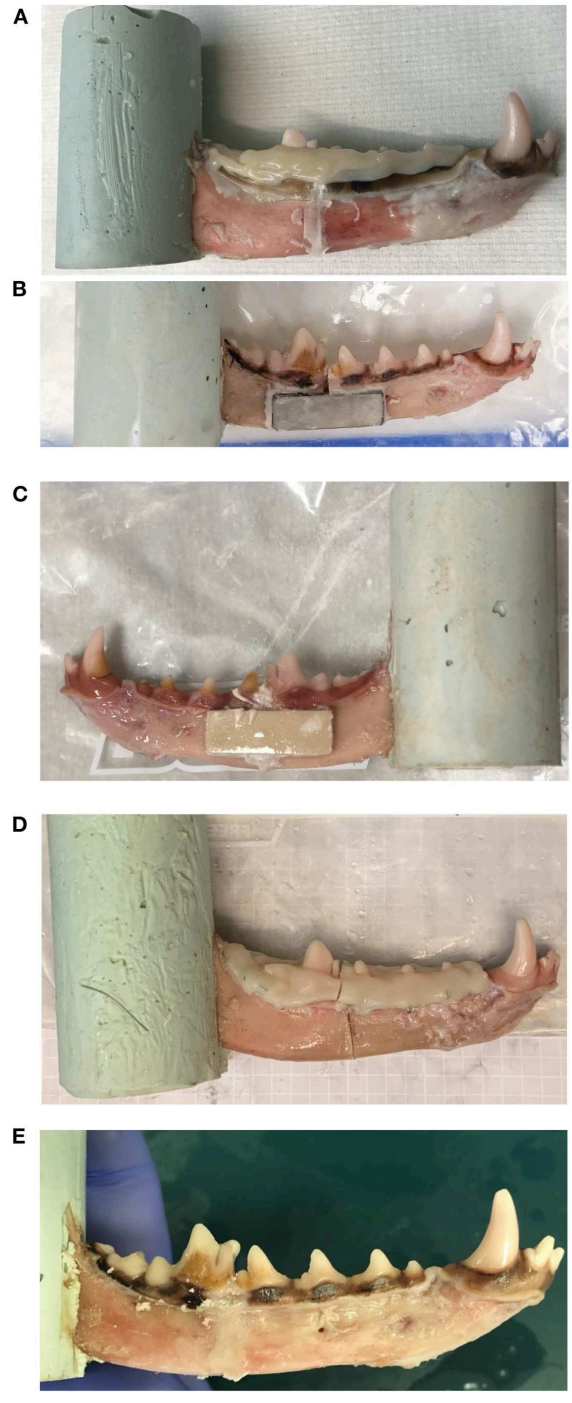

FIGURE 1 | (A–E) Specimens demonstrating multiple repair constructs. **(A)** Adhesive and composite, **(B)** adhesive and titanium plate, **(C)** adhesive and resorbable plate, **(D)** wire and composite, and **(E)** adhesive-only are shown. **(D)** The wire and composite construct demonstrates cohesive failure of the *bis*-acryl composite splint.

by immediate mixing of contents for 20 s in a silicone bowl with a dental spatula. Once mixing was complete, the adhesive material was transferred into a 3 mL syringe and injected onto both fracture surfaces within 90 s. Following the reconstitution of powder with water, the adhesive was applied to the bone end surfaces and manually apposed for 210 to 270 s.

The adhesive and plate and adhesive and resorbable plate groups received additional application of respective plates to the subperiosteal bone surface. Details of the plates included use of a type 2 titanium with sand blast surface finish, with dimensions 1.0 × 3.0 × 0.163 cm, and a resorbable plate, with dimensions 1.0 × 3.0 × 0.250 cm centered over the fracture line. Following fracture reduction with adhesive, the mandible was transferred into a bag of PBS (37°C) for 300 s. At 600 s, the mandible was removed from PBS, and a second mixture of the mineral–organic adhesive was used to adhere the plate to the buccal cortical surface of the mandible. The plates were allowed to cure in an ambient environment for 120 s before being returned to PBS bag and returned to the warming bath.

The adhesive and composite group received placement of adhesive to the osteotomy end surfaces, followed by application of *bis*-acryl composite to the clinical crowns from the canine tooth to third molar tooth in the fashion described below. For the adhesive and plate, adhesive and resorbable plate, and adhesive and composite groups, a 10-min curing window at 37°C was provided between adhesive stabilization and application of secondary forms of fixation.

Treatment groups receiving *bis*-acryl composite splinting as part of two-treatment stabilization underwent placement following either interdental wiring or adhesive placement. Tooth crowns were ultrasonically scaled and polished with fine grit pumice and then etched with phosphoric acid gel (Max Etch 35% phosphoric acid etchant blue; Clinicians Choice Dental Products Inc., New Milford, CT, USA) for 20 s and rinsed with distilled water as per manufacturer's instructions. The tooth's surface was lightly air dried prior to *bis*-acryl composite application. An intraoral splint was fashioned by a single investigator (C.J.S.) using the supplied auto-mixing tips and placed on the tooth crowns extending from the first premolar tooth through third molar tooth, as previously described in a clinical fashion (16, 17). All groups involving *bis*-acryl composite were timed from the start of expressing the auto-mixed composite and were stopped when the *bis*-acryl composite was considered to be clinically cured (stable and firm to the touch).

Following adhesive application, all groups were returned into the PBS environment and maintained at 37°C for 24 ± 1 h prior to mechanical testing.

Mechanical Testing

Mechanical data were measured using a servohydraulic testing system (MTS Bionix 858; MTS Systems Corp., Eden Prairie, MN, USA) in a manner previously reported (17, 34). The load application was designed to mimic cantilevered bending forces acting on the mandible. Mandibular length was measured from the canine tooth cusp to the ramus as it meets the polyester resin mold. A cantilever bending test was subsequently performed on each specimen, with a force applied to the crown of the canine tooth (**Figure 2**). The hydraulic actuator applied loads at a constant rate of linear deformation (10 mm/min) until fixation device failure (fracture). Actuator deformation and compressive force were recorded throughout the test. These data provided one measure of structural behavior (force vs. displacement plots). In addition, bending moments and angular displacements were calculated from these data. The moment arm was measured from the canine tooth cusp to the osteotomy location in the treatment groups and measured to where the mandibular body fractured under maximal load in the controls. Bending moment–angular displacement were calculated using the distance from the MTS grip to the canine tooth (controls) and the osteotomy to the canine tooth (treatment groups). The bending moment vs. angular displacement curves provided another measure of structural behavior. Mode of failure (adhesive or cohesive failure) was noted.

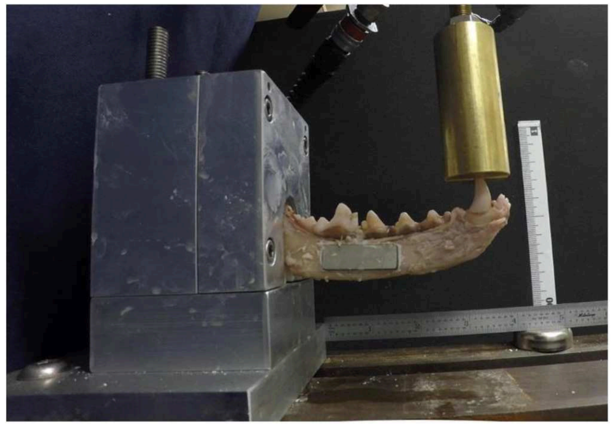

FIGURE 2 | A specimen treated with endosteal resorbable bone adhesive and titanium plate is loaded in a custom jig and undergoing point force cantilevered bending with a servohydraulic testing system.

Data Analysis

Failure was defined as the point at which either adhesive failure (gross separation of material from adhered structure) or cohesive failure (obvious fracture resulting in material breakage) occurred. This point represented the maximum force or moment. This point was identified on the force-vs.-displacement curves [load at failure (N) in **Table 1**] and on the bending moment–vs.–angular deformation curves [**Figure 3** and bending moment at failure (Nm) in **Table 3**]. Linear stiffness (N/mm) was calculated from the initial linear slope of each force-vs.-displacement curve. These data are summarized in **Table 2**. Angular displacement at failure (degrees) was identified on each moment-vs.-angular displacement curve as the angular change of the distal mandible from uptake of load to failure. Results are summarized in **Table 4**.

Statistical Analysis

The average and standard deviation (SD) were calculated for load at failure, stiffness, bending moment, and angular displacement for all treatment groups.

TABLE 1 | Summary of load at failure (applied to the canine tooth) with comparisons.

Group	Mean (SD) {N}	*P*-value*	*P*-value^
Wire and composite	111.6 (40.0)	Ref	<0.001#
Control	402.1 (82.0)	<0.001†	Ref
Adhesive	18.7 (4.1)	<0.001#	<0.001#
Adhesive and composite	191.6 (47.6)	0.002†	<0.001#
Adhesive and plate	40.8 (17.7)	0.009#	<0.001#
Adhesive and resorbable	19.3 (6.6)	<0.001#	<0.001#
Composite only	121.3 (40.9)	0.974	<0.001#

Data are presented as mean (SD) for all treatment groups. P-values are all groups compared to wire and composite and separately all groups compared to control. P-values have a Dunnett adjustment for comparison of multiple groups to a single group.
**P-value indicates comparison to wire and composite.*
^P-value indicates comparison to control.
†Group is significantly stronger to comparator.
#Group is significantly weaker to comparator.

To reduce the amount of statistical tests and hence reduce the probability of committing a type I error, we *a priori* selected to only compare the experimental groups (composite only, adhesive and resorbable, adhesive and plate, adhesive and composite, and adhesive only) to controls and then separately compare the experimental groups to wire and composite group. The control group and wire and composite group were chosen as reference groups because the control condition was an intact model of true strength potential, and wire and composite is the standard of care for repairing these types of fractures. Data were analyzed via two-sample *t*-tests with Dunnett adjustment for multiple comparisons of different groups to a single reference group. Analysis was performed to determine if a significant difference exists between groups in load to failure, stiffness, bending moment, and angular displacement. Analyses were all done using R for statistical computing, and all tests were conducted at a two-sided Dunnett adjusted 5% significance level.

RESULTS

Mean (±SD) load at failure for all groups and comparisons against wire and composite and controls are summarized in **Table 1**. All groups were significantly weaker ($P < 0.001$) than the control group (402.1 ± 82 N). The adhesive and composite group (191.6 ± 47.6 N) was the only group significantly stronger ($P = 0.002$) than the wire and composite group (111.6 ± 40.0 N). Adhesive (18.7 ± 4.1 N), adhesive and plate (40.8 ± 17.7 N), and adhesive and resorbable (19.3 ± 6.6 N) were all significantly weaker ($P < 0.001$, $P = 0.009$, $P < 0.001$, respectively) than the wire and composite group (111.6 ± 40.0 N).

Mean stiffness for all groups and comparisons against wire and composite and control groups are summarized in **Table 2**. No significant difference was noted between groups compared to wire and composite or control. Adhesive and composite (51.6 ± 16.0 N/mm) was noted to be stiffer at failure than wire and composite (28.3 ± 7.9 N/mm) but not considered to be significant ($P = 0.135$).

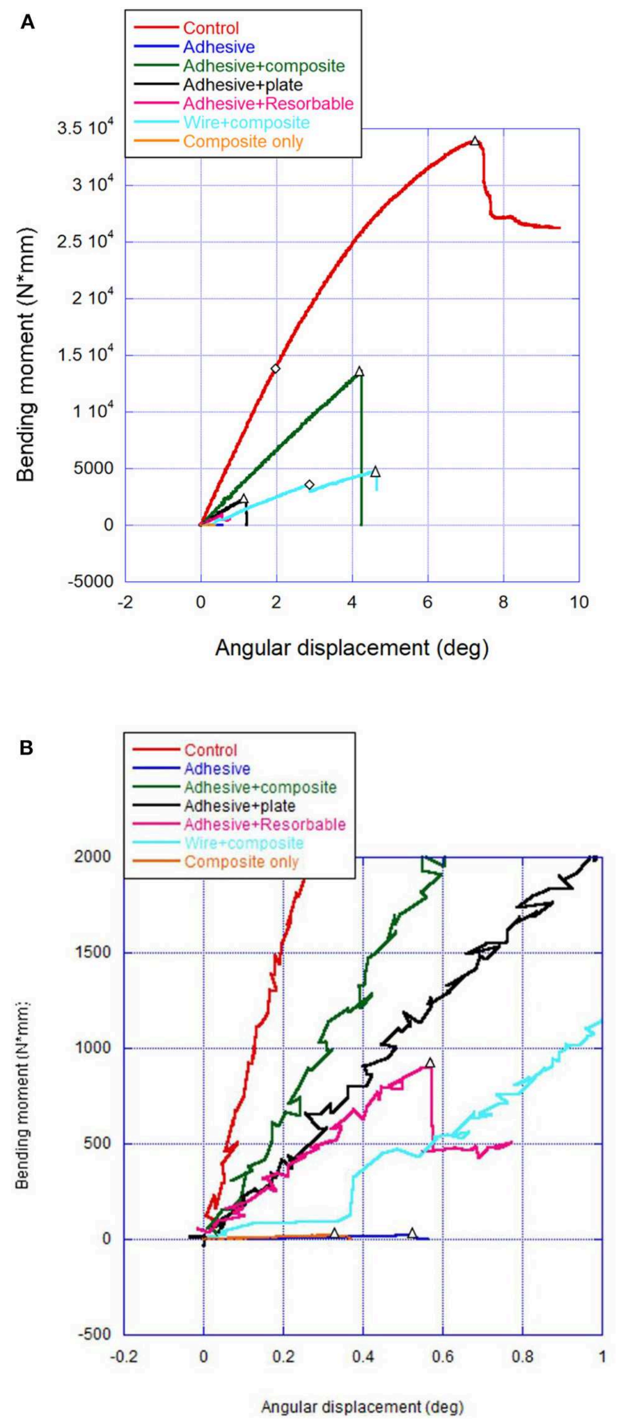

FIGURE 3 | Load displacement curve. **(A,B)** Representative bending moment–angular displacement curves (**A** = full graphs, **B** = magnified insert) that depict linear limit for stiffness (diamond) when different from peak moment (triangles).

Mean bending moment for all groups and comparisons with wire and composite are summarized in **Table 3**. The bending moment for all groups was no different than wire and composite (5.6 ± 2.2 Nm) except the control group. The bending moment

TABLE 2 | Summary of stiffness (from actuator force vs. displacement) with comparisons.

Group	Mean (SD) {N/mm}	*P*-value*	*P*-value^
Wire and composite	28.3 (7.9)	Ref	0.567
Control	42.3 (10.4)	0.567	Ref
Adhesive	39.6 (18.0)	0.731	0.994
Adhesive and composite	51.6 (16.0)	0.135	0.832
Adhesive and plate	48.0 (22.4)	0.261	0.956
Adhesive and resorbable	30.9 (17.4)	0.996	0.72
Composite only	42.7 (38.6)	0.546	1

Data are presented as mean (SD) for all treatment groups. P-values are all groups compared to wire and composite and separately all groups compared to control. P-values have a Dunnett adjustment for comparison of multiple groups to a single group.
**P-value indicates comparison to wire and composite.*
^P-value indicates comparison to control.

TABLE 3 | Summary of bending moment at failure with comparisons.

Condition	Mean (SD) {Nm}	*P*-value*
Wire and composite	5.6 (2.2)	Ref
Control	17.2 (13.6)	<0.001†
Adhesive	0.9 (0.2)	0.334
Adhesive and composite	9.4 (2.6)	0.515
Adhesive and plate	2.0 (0.9)	0.580
Adhesive and resorbable	1.0 (0.3)	0.345
Composite only	5.9 (2.0)	0.999

Data are presented as mean (SD) for all treatment groups. P-values are all groups compared to wire and composite. P-values have a Dunnett adjustment for comparison of multiple groups to a single group.
**P-value indicates comparison to wire and composite.*
†Groups with a bending moment significantly greater than wire and composite.

for the control group was 17.2 ± 13.6 Nm but was not tested because of the high loads causing the bone to bend and twist.

Mean angular displacement at failure for all groups was tested against wire and composite (**Table 4**). The adhesive (0.8° ± 0.7°), adhesive and plate (1.2° ± 0.4°), and adhesive and resorbable (0.9° ± 0.6°) groups all demonstrated significantly lower angular displacement ($P \leq 0.001$) compared to the wire and composite (4.7° ± 1.6°) group. The mean angular displacement for the control group was 6.9° (±1.1°) and was dissipated over the whole length of the jaw and very different in character than the treatment groups because of the high force applied and therefore was not compared.

The application times and modes of failures are summarized in **Table 5**. The adhesive-only group demonstrated the shortest application time (mean 206 ± 19.6 s), whereas the adhesive and composite group showed the longest (mean 1,281 ± 49.6 s) because of the 10-min curing period between adhesive application and *bis*-acryl composite application. There was no variation in application times for adhesive and plate group and adhesive and resorbable group because of following the manufacturer's recommended time for mixing adhesive and applying adhesive. All specimens in the adhesive (8/8) and adhesive and plate (8/8) groups demonstrated adhesive failure.

TABLE 4 | Summary of angular displacement at failure (angular change of distal mandible from uptake of load to failure) with comparisons.

Condition	Mean (SD) {°}	*P*-value*
Wire and composite	4.7 (1.6)	Ref
Control	6.9 (1.1)	0.082
Adhesive	0.8 (0.7)	<0.001#
Adhesive and composite	4.9 (2.5)	0.998
Adhesive and plate	1.2 (0.4)	0.001#
Adhesive and resorbable	0.9 (0.6)	<0.001#
Composite only	5.1 (3.3)	0.97

Data are presented as mean (SD) for all treatment groups. P-values are all groups compared to wire and composite. P-values have a Dunnett adjustment for comparison of multiple groups to a single group.
**P-value indicates comparison to wire and composite.*
#Groups with an angular displacement significantly less than wire and composite.

The wire and composite group only demonstrated cohesive (7/8) or cohesive and adhesive (1/8) failure. The composite-only group demonstrated a combination of either adhesive (4/8), cohesive (3/8), or adhesive and cohesive failure (1/8). The adhesive and resorbable plate demonstrated cohesive failure of the plate in seven of eight specimens, whereas one specimen demonstrated adhesive failure between the plate and bone. Seven of eight specimens in the adhesive and composite group demonstrated cohesive failure (four cohesive failure only, three adhesive, and cohesive failure), whereas one specimen demonstrated adhesive failure only.

DISCUSSION

This investigation provides the first experimental data for the application of a resorbable novel calcium phosphate-phosphoserine–based mineral–organic adhesive (Tetranite® Stabilization Material; LaunchPad Medical) for the purpose of augmenting various forms of non-invasive fracture repair. Historically, the use of interdental wiring (typically Stout's multiple loop technique) in conjunction with a *bis*-acryl intraoral composite has yielded greater strength (16) and stiffness compared to composite only (17). Considering the results of wire and composite in previous forms of testing, all treatment groups in this study were evaluated against both the control group and the wire and composite treatment group.

Load at failure is a clinically relevant measurement considering the mandible's role in prehension when assessing the qualities of fixation methods. The strength of a construct is important to clinically achieve successful bone healing. Load at failure is commonly used to compare fixation methods (6, 16, 17). All testing groups in this study demonstrated a lower load to failure as compared to the control, which is consistent with the previous literature. Considering the load to failure exceeded 100 N, on average, in all three test groups utilizing *bis*-acryl composite, the composite appears to be a large contributor to the strength of these fixation devices. The use of mineral–organic adhesive as a sole form of fixation or in combination with a titanium plate or plates made of adhesive material failed at a

TABLE 5 | Summary of fixation application times and modes of failure.

Treatment	Mean application time (s) (SD)	Method of failure: adhesive	Method of failure: cohesive	Method of failure: adhesive and cohesive
		(Frequency of failure within treatment group/overall)		
Adhesive	206 (19.6)	8 (100%/16.7%)	0 (0%/0%)	0 (0%/0%)
Adhesive and composite	1281 (49.6)	1 (12.5%/2.1%)	4 (50%/8.3%)	3 (37.5%/6.3%)
Adhesive and plate	990 (0)	8 (100%/16.7%)	0 (0%/0%)	0 (0%/0%)
Adhesive and resorbable	990 (0)	0 (0%/0%)	8 (100% /16.7%)	0 (0%/0%)
Wire and composite	951 (139.1)	0 (0%/0%)	7 (87.5%/14.6%)	1 (12.5%/2.1%)
Composite only	357 (25.2)	4 (50%/8.3%)	3 (37.5%/6.3%)	1 (12.5%/2.1%)

Mean application times for fracture fixation are reported for each group. Observational evaluation of the form of failure for each specimen is reported. Cohesive failure represents gross fragmentation of a fixation device, whereas adhesive failure represents gross delamination of the fixation device from bone or tooth. Frequency of failure occurrence is reported within the treatment group/overall.

lower load. Importantly, it was noted that a synergistic effect was achieved by combining adhesive and composite splinting. This suggests that the use of adhesive applied to the bone end surfaces in combination with composite splinting improves fixation strength when compared to interdental wiring and composite splinting.

Stiffness is an important consideration in fracture repair in order to achieve direct bone healing (35). A lack of stiffness may reduce relative stability and hence negatively impact the potential for direct bone healing. There was no difference noted between groups regarding stiffness. Despite the adhesive and composite group demonstrating the greatest stiffness, none of the constructs tested were significantly stiffer than others. The importance of stiffness may prove to be more clinically relevant in tests using cyclic loading and should be explored further.

The thickness of a material directly affects its structural stiffness (32). A single investigator (C.J.S.) applied all *bis*-acryl composite splints consistent with previous experiential clinical success. Each composite splint was fashioned in a similar manner. Stiffness is calculated from the slope of the load displacement curve (32). Materials undergoing less displacement to reach the same load demonstrate greater stiffness. For this study, the rate of displacement was fixed and directly related to time. Stiffer materials would be more appropriate for primary bone healing by stabilizing and protecting the fracture site and have been shown to ensure more advanced healing at the same time point (35). The relative flexibility of bone likely contributed to no differences between stiffnesses when comparing groups (36). Voluntary bite force in humans immediately following fracture repair with miniplating techniques demonstrates reduced forces following repair (37). Similar protective tendencies in veterinary jaw fracture patients may explain why non-invasive fracture repair techniques such as wire and composite with lower loads to failure and stiffness no different than bone may result in successful bone healing despite not demonstrating optimal biomechanical environments consistent with primary bone healing such as rigid fixation.

Bending moment describes the load placed on the construct causing angular deformation. It is a common physiological load placed upon bones, and its measurement can help determine strength of a construct (38, 39). Consistent with load to failure, the control group's bending moment was significantly greater compared to treatment groups at the moment of failure, reflecting bone's natural tendency to bend (36). Despite bending moments being significantly lower compared to controls, this may be less relevant in the clinical setting. All bending moments for treatment groups were calculated from the canine tooth cusp to fracture plane. The bending moment for controls was calculated from the canine tooth to the point where the fracture propagation extended to the alveolar crestal surface. The large load to failure for the control group likely contributed to the significant difference between bending moments of the control group compared with all treatment groups. The addition of mineral–organic adhesive to the construct contributed to the increase in bending moment, although not significant, and supports optimizing the stability of the construct. Bending moment applied to the canine tooth exerts a larger bending moment than if the load is across multiple teeth because of a larger moment arm (40). This is in contrast to *in vivo* biomechanical force application where loads are distributed across multiple teeth in a quadrant, including caudal to the fracture site. This reduces the moment arm and resultant load on the fracture site (40).

The invasiveness for application of different fracture repair techniques should be considered when selecting a fixation method. Interdental wiring is reported in the human literature to be associated with a low complication rate and minimal impact on periodontal health (19–21). Similar application techniques are used in veterinary interdental wiring. Veterinary patients create additional challenges due to the lack of bunodont dentition, which results in difficulty when placing interdental wire supragingivally. As a result, subgingival perforations are frequently necessary to successfully anchor the wiring (18). The consequences on the periodontal tissues of this technique in veterinary patients remain unreported in an objective fashion. However, clinical experience suggests that while interdental wiring is non-invasive relative to creating holes in bone that can impact tooth vitality, periodontal health is impacted. Unless interdental wire constructs can be improved to spare negative effects on periodontal health, the use of adhesives for stabilization of fractures becomes an attractive alternative, especially if it adds strength as shown in this model. The contribution of splints to inciting periodontal disease is expected, and quantification of the improvement on periodontal health through the avoidance of interdental wiring in those constructs requires investigation. Unlike the necessity to remove wire, resorbable mineral–organic adhesive has been shown to be osseointegrated and negates added time for removal.

Fracture fixation time required and ease of application of the fixation method become considerations if construct strength and stability are comparable. It is worth noting that the composite-only application time was less than half the time of the wire and composite group. The longer duration of application for the adhesive and composite group included a 10-min cure time between adhesive application to bone ends and composite placement. These 10 min were included to mimic time necessary for closure of the soft tissues between adhesive application and composite placement. Despite this technique being the slowest method to execute, the advantage of a greater load to failure over wire and composite is seemingly clinically relevant. The interdental wire and composite and adhesive and composite both require second anesthetic episodes for splint ± wire removal. The amount of time saved and resultant periodontal health without the wires have not been quantified; however, it would be expected to result in healthier periodontium and expedited splint removal in the adhesive and composite group.

Various forms of failure occurred between treatment groups. All specimens in the adhesive-only and adhesive and titanium plate groups exhibited adhesive failure. The assessment of adhesive failure in those respective groups was based on gross visualization. Adhered surfaces were assessed to have suffered adhesive failure by gross examination. Microscopic examination of the adhesive would be necessary to determine if cohesive failure existed within the cured material. All specimens in the adhesive-only group suffered adhesive failure, which is unsurprising considering the adhesive was not expected to withstand loads exceeding bone. All specimens in the adhesive and titanium plate group demonstrated adhesive failure, which is consistent with the adhesive's strong affinity to bind to metal (30). It is possible that the adhesive and titanium plate was weaker than adhesive and composite or wire and composite due to the location of fixation device, as well as a mismatched strength for adhesion between adhesive-titanium and adhesive-bone surfaces resulting in delamination of the apparatus. The tension band principle is leveraged in constructs involving use of composite material on the alveolar surface. Both the resorbable and titanium plates, placed subperiosteally, do not leverage this advantageous location and are subsequent to shear forces, which disrupted adhesion. The adhesive and resorbable plate, made of cured segments of the precast adhesive, exhibited cohesive failure in seven of eight specimens. While this was not found to be a superior form of stabilization, it does demonstrate the adhesive's ability to adhere bone to the cured material itself.

The wire and composite group demonstrated cohesive failure in seven of eight specimens, whereas one of eight specimens demonstrated adhesive failure between composite and tooth structure and cohesive failure. The composite-only group exhibited a mixture of failures including 4/8 adhesive, 3/8 cohesive, and 1/8 adhesive failure between composite and tooth structure and cohesive failure. The mean load to failure was not noted to be different ($P = 0.974$) between the wire and composite (mean = 111.6 N) and composite-only (mean = 121.3N) groups. The contribution of wire to ultimate strength appears to be insignificant in cantilevered bending tests and may have contributed to the type of failure noted in this group. Stabilization with orthopedic wire is not considered a rigid form of fixation, and additional investigation, such as the use of finite element analysis, would be necessary to determine if the distribution of forces through the wire contributes to the propensity for a large number of cohesive failures to occur. The predominately even distribution of failure types of failures in the composite-only group suggests that the adhesion between tooth and composite is stronger than the forces that the composite can withstand in tension. Despite efforts to standardize positioning of the mandible when potted, varying degrees of bending and twisting of the mandible were observed during point force loading, which may have impacted on the repair construct's ability to withstand point force loading. Bending and twisting of the mandible may be mitigated in clinical patients by contributions to stabilization of the rostral fragment across the symphysis. The adhesive and composite group had a similar number of cohesive failures compared to the composite-only group; however, the adhesive and composite group demonstrated only one adhesive failure.

When utilizing adhesives, surface area is a key consideration, and an increase in surface area would likely provide improved adhesion (41). The transverse fractures generated the smallest surface area for adhesion. This fracture confirmation may be a contributing factor to the relatively low strengths of the testing groups relying solely on the bone adhesive, or with other repair constructs that rely on the bone adhesive. Oblique fracture configurations may provide greater surface area for effective adhesion, but the shear forces acting upon unfavorable fractures may compromise this surface area advantage (42). Considering the high number of fractures through the mandibular first molar tooth (43), this testing model highlights possibly the most limited benefit of using bone adhesive for fracture fixation. The role that adhesive may play at significantly stabilizing oblique fractures, with additional surface area, could positively impact load to failure and other construct mechanical qualities.

Efforts were made to mimic the physiologic environment (hydration and temperature) that the adhesive would be applied in clinical patients. Maintaining specimen hydration in PBS and a 37°C water bath were necessary to test biomechanical properties of the mineral–organic adhesive, *bis*-acryl composite, and interdental wiring, as would be present in living patients. Time for application of fixation devices was impacted by the clinical experience of the author (C.J.S.) and may not be a representative time required to perform interdental wiring or applying composite splints for others. Furthermore, despite the fact that bite forces are reduced in human patients following mandibular fracture repair, the testing with cyclic loading may be more representative of actual functional use necessitating further studies to clearly define the biomechanical behavior of non-invasive fracture repair devices. Furthermore, the use of novel resorbable mineral–organic adhesive to further augment the strength of wire and composite fixation devices has not been defined.

Cantilevered testing mimics a force distribution to load the mandible and quantify mechanical properties of non-invasive fracture repair devices. When evaluating the mechanical properties of adhesive as a sole form of fixation or as an adjunct form of fixation, it was shown that adhesive and composite was significantly stronger than wire and composite ($P = 0.002$). None of the tested repair techniques were shown to be as strong as the control (unfractured) mandibular bone, and stiffness was noted to be no different between treatment groups. Calcium phosphate-phosphoserine–based mineral–organic adhesive tightly binds to titanium and demonstrates tight adhesion to cured TTCP–phosphoserine material. The primary form of failure for wire and composite constructs was cohesive failure of the construct, whereas mixed cohesive or adhesive failure was noted in the composite-only group. The use of mineral–organic adhesive as a method to augment the strength of composite splint fabrication for the treatment of mandibular repairs appears promising.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

Ethical approval for this study was not required according to national legislation because the acquired specimens were humanely euthanized prior to, and for reasons unrelated to, this study. Cadaveric specimens rather than synthetic models were necessary since it replicates the clinical scenario and enamel and/or dentin is necessary for chemical adhesion and micromechanical retention of the *bis*-acryl composite to tooth structure 8, 9, and 31.

AUTHOR CONTRIBUTIONS

CS and RV: project conception and design. SH: statistical analysis. AG, GT, and CS: initial draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

FUNDING

This project was made possible by a Companion Animal Grant, University of Wisconsin-Madison, School of Veterinary Medicine. The project described was supported by the Clinical and Translational Science Award (CTSA) program, through the NIH National Center for Advancing Translational Sciences (NCATS), grant UL1TR000427. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

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Conflict of Interest: The authors would like to acknowledge LaunchPad Medical, LLC for donating equipment and materials in support of this project.

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Veterinary Science

ORIGINAL RESEARCH
published: 28 April 2020
doi: 10.3389/fvets.2020.00241

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Craniomaxillofacial Trauma in Dogs—Part I: Fracture Location, Morphology and Etiology

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Treatment of craniomaxillofacial (CMF) trauma in dogs often requires a multidisciplinary approach and a thorough understanding of the CMF skeletal structures involved. The aim of this retrospective study was to use a large number of CT studies of dogs evaluated for CMF trauma and to describe fracture location and morphology in relation to demographic data and trauma etiology. The medical records and CT studies of 165 dogs over a 10-year period were evaluated. The skeletal location of CMF fractures as well as the severity of displacement and fragmentation of each fracture was recorded. Patient demographic data and trauma etiology were also recorded. Animal bites accounted for the majority of trauma (50%), followed by unknown trauma (15%), vehicular accidents (13%), and blunt force trauma (13%). Small dogs, < 10 kg, and juveniles accounted for the majority of patients (41.8 and 25.5%, respectively). The most likely bone or region to be fractured was the maxillary bone, followed by the premolar and molar regions of the mandible. Up to 37 bones or regions were fractured in any given patient, with an average of 8.2 fractured bones or regions per dog. The most commonly fractured location varied according to trauma etiology. Specifically, vehicular accidents tended to result in more locations with a higher probability of fracture than other trauma types. A major conclusion from this study is that every bone of the CMF region was fractured in at least one case and many cases had a large number of fractured regions. Thus, the need for comprehensive assessment of the entire CMF region, preferably using CT, is underscored.

Keywords: craniomaxillofacial, trauma, computed tomography, fracture, displacement, dog

OPEN ACCESS

Edited by:
Jason W. Soukup, University of Wisconsin-Madison, United States

Reviewed by:
Christopher John Snyder, University of Wisconsin-Madison, United States
Carlos Alberto Antunes Viegas, Universidade de Trás-os-Montes e Alto Douro, Portugal

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Specialty section:
This article was submitted to Veterinary Dentistry and Oromaxillofacial Surgery, a section of the journal Frontiers in Veterinary Science

Received: *26 February 2020*
Accepted: *08 April 2020*
Published: *28 April 2020*

Citation:
De Paolo MH, Arzi B, Pollard RE, Kass PH and Verstraete FJM (2020) Craniomaxillofacial Trauma in Dogs—Part I: Fracture Location, Morphology and Etiology. Front. Vet. Sci. 7:241. doi: 10.3389/fvets.2020.00241

INTRODUCTION

Craniomaxillofacial (CMF) trauma is a relatively common reason for which dogs are presented to veterinarians on an emergency basis. CMF trauma may occur in isolation but often accompanies injury to other parts of the body and therefore requires a multidisciplinary approach to optimize patient care (1–3). Fracture morphology and spatial location play an essential role in clinical decision-making (3, 4). In the human medical literature, several classification systems and injury severity scores for the CMF region have been made (2, 5–8). Perhaps the most well-known of these, the Le Fort fractures, are based on the repeatable lines of weakness of the midface demonstrated by Rene Le Fort in his classic cadaveric studies (9). A multitude of other classification systems and severity scores have been created for people with a recent effort by the professional association AOCMF (Arbeitsgemeinschaft fur Osteosynthesefragen-craniomaxillofacial) to create one standardized, accepted classification system for the entire CMF region (10). At present, there is not a standardized, accepted classification system for the CMF region in dogs.

The potential of such systems lies in their ability to aid in clear and standardized communication between clinicians as well as to provide therapeutic and prognostic information (10, 11). This is especially important in the CMF region as specialists with distinct but overlapping training can be involved in CMF trauma management. For example, in the veterinary field, treatment of CMF trauma patients could conceivably be performed by veterinary dentists, surgeons, neurosurgeons, ophthalmologists, criticalists, and general practitioners. In addition, classification systems may provide a logical explanation for approaches to management of the CMF trauma patient for those practitioners who have less experience in this region (11). The basis of any classification system first requires knowledge of common fracture location and morphology, ideally based upon a large number of cases.

Given the anatomically complex and overlapping nature of structures in the CMF region, it is not surprising that the diagnostic yield of CT in reference to identifying fractures is greater than that of skull radiographs (12, 13). When assessing people who have sustained CMF trauma, CT is considered the standard of care, and there is increasing recognition that three-dimensional and multiplanar reconstructions are extremely important for accurate diagnosis and optimal treatment planning (14). While utilizing the two dimensional aspects of CT is essential for the smaller and more internal CMF fractures, it is well-recognized that the two-dimensional and three-dimensional modalities are best utilized together (9, 15, 16). In some cases, not only is preoperative CT used for diagnostic and planning purposes, but intraoperative CT is also being utilized during surgery and has been shown to change clinical decision-making (17). Fortunately, there is increasing access to CT in veterinary practice, which may improve the accuracy of diagnosis in CMF trauma patients.

In addition to the spatial location of CMF fractures, the fracture morphology also has an influence on treatment planning and outcomes (10). In general, fragmentation and displacement are discussed when describing fracture morphology regardless of the type or location of the fractured bone (18). One aspect of fracture morphology that is different between long bones and bones of the CMF region is that the descriptive terms of linear, spiral, transverse, oblique, etc. are not always applicable in the bones of the CMF region (18). Nevertheless, collecting and describing the severity of displacement and fragmentation of fractures in the CMF region is likely to be useful.

Literature regarding common causes of CMF trauma in dogs is sparse and typically includes animal altercations, vehicular accidents, falls from a height, and unknown trauma as the most common etiologies (19–23). Similarly to trauma etiology, a small number of studies have reported the physical location of fractures secondary to CMF trauma in dogs (19, 21–25). Currently, the mandible is reported to be vastly more likely to be fractured than other parts of the skull (19, 22, 25). In addition, in the mandible, the premolar and molar teeth regions are the most commonly fractured (21), and this may be dependent on patient size (23). Documenting whether these findings are upheld when using computed tomography will provide important information to veterinarians.

To the authors' knowledge, current veterinary literature lacks comprehensive reports detailing CMF fracture location, morphology, etiology, and the relationship of each of these variables in a large number of dogs utilizing CT for diagnosis. This retrospective, descriptive study includes CT findings of 165 dogs that sustained CMF trauma. The primary objectives of the study were: (1) to describe the most common fracture etiology, location, and morphology and (2) to determine whether relevant demographic data (size, age, sex) were related to any of these variables. Although the objective of this study did not include creation of a classification system, the information gathered here can be used for the basis of classification systems in the future. We hypothesized that fracture location and morphology would be influenced by trauma etiology and that demographic variables would influence fracture location, morphology, and trauma etiology. In the accompanying article, entitled "Craniomaxillofacial trauma in dogs- part II," the associations between fracture location, morphology, and trauma etiology are analyzed further.

MATERIALS AND METHODS

Case Selection

The electronic medical record database of the UC Davis Veterinary Medical Teaching Hospital was searched for dogs that had been presented for evaluation and treatment following CMF trauma between the years 2008 and 2018. For inclusion, all dogs must have undergone CT (conventional CT and/or cone-beam CT [CBCT]) at the initial visit. Exclusion criteria were as follows: trauma that had occurred > 1 week prior to presentation, patients with CT slice thickness of > 1.3 mm, and those for whom either the medical record or CT study were incomplete (e.g., the caudal most portion of the skull had not been included in the images). Cases were excluded if the trauma occurred > 7 days prior to presentation due to concern that (a) early signs of fracture repair and boney remodeling may make fracture identification more difficult and (b) further displacement may have occurred since the trauma. Exclusion of cases if the slice thickness was > 1.3 mm was chosen as a compromise between maximizing the number of cases that were included in the study while simultaneously ensuring that slice thickness was not so large that small or incomplete fractures could be missed.

Image Acquisition and Evaluation

All dogs underwent conventional CT (HiSpeed FX/i or LightSpeed16, GE Healthcare, Waukesha, WI) and/or CBCT (NewTom 5G CBCT Scanner, NewTom, Verona, Italy) imaging at their initial visit. Although many dogs presenting for CMF trauma at our institution undergo CBCT, including conventional CT allowed the study to capture those patients in which superior

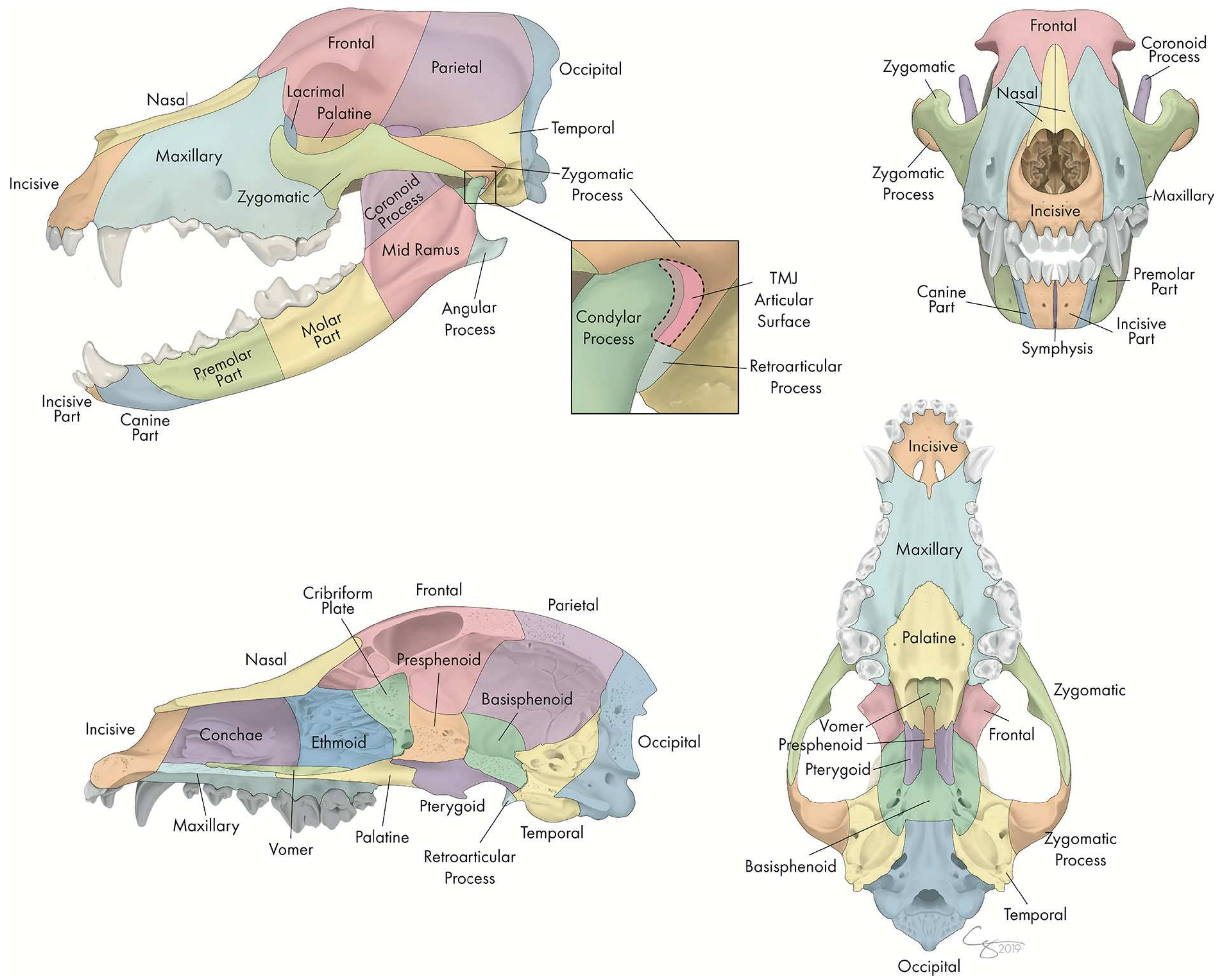

FIGURE 1 | Bones and regions of the CMF skeleton of the dog. This array of skulls was used when assessing which bone and regions of the CMF skeleton were fractured.

soft tissue imaging was medically necessary (e.g., those with concern for intracranial hemorrhage), those too large for the CBCT field of view, and those who received treatment prior to the availability of CBCT at this facility. All DICOM files from each study were viewed using a specialized software (*Invivo*5, Anatomage, San Jose, CA). Each case was viewed dynamically on medical flat-grade monitors (ASUS PB278Q 27-inch, ASUSTeK Computer Inc., Taipei, Taiwan), allowing the observers to utilize all viewing modes and tools to best assess all fractures. One observer (MD) viewed all studies and recorded all data after a period of calibration with one experienced board-certified veterinary radiologist (RP) and two board-certified veterinary dentists and Oral and Maxillofacial Surgery Fellows (FV, BA). When there was uncertainty regarding the presence or severity of a lesion, the study was reviewed with the board-certified radiologist (RP). Although soft tissue injuries were evaluated when the patient was in hospital, they were neither evaluated nor recorded for the purposes of this study.

Fracture Evaluation

Each skull was divided into specific bones and regions as illustrated in **Figure 1**. For each bone or region, it was determined whether there was a fracture and, if so, fracture morphology was described in terms of displacement and fragmentation. The degrees of displacement and fragmentation were modeled after the AOCMF fracture classification system in humans (10). For both displacement and fragmentation, a score of 0 indicated that there was no fracture. When scoring displacement, a score of 1 indicated that there was no displacement, a score of 2 that there was minimal displacement with $<=50\%$ overlap remaining between fragments, and a score of 3 that there was severe displacement with $> 50\%$ overlap remaining. When scoring fragmentation, a score of 1 indicated an incomplete fracture, a score of 2 a complete fracture, and a score of 3 was consistent with a comminuted fracture. This process was repeated on both the right and left sides of the skull. Although use of the term "comminuted" is discouraged by the most recent recommendations in human CMF literature (26), the term and its associated meaning are still pervasive in veterinary medicine and was therefore utilized in this study. A comminuted fracture was defined as a fracture having 3 or more bone fragments, although “minute” fragments were ignored unless the entire bone or region had been reduced to microfragments (27).

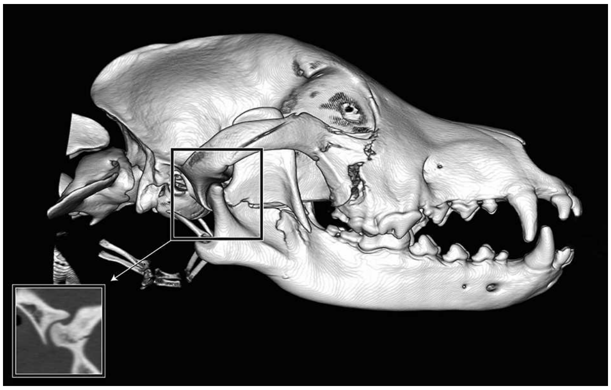

FIGURE 2 | Example of fragmentation and displacement of a single fracture line crossing multiple bones or regions. If the fracture affected individual bones or regions to a greater or lesser extent, the severity was recorded for each bone or region individually. In this example, the fracture line spanned multiple regions of the mandible. The mid-ramus was found to have fragmentation and displacement scores of 2, whereas the condylar process (not including the articular surface) had a fragmentation score of 1 (incomplete fracture) and a displacement score of 1 (no displacement). In addition, there are fractures of the right zygomatic bone.

FIGURE 3 | Example of symphyseal separation. By definition, all symphyseal separations were considered to be complete. However, in this example, the two sides of the symphysis were differentially displaced such that the left side of the symphysis remained essentially in place (displacement score of 1), whereas the right side of the symphysis was severely displaced (displacement score of 3).

Because the bones that form the temporomandibular joint (TMJ) may, in themselves, be fractured without there being a fracture that extends into the articular space, fractures of the TMJ were recorded as a unique category separate from the condylar process, the retroarticular process, and the temporal bone. It was expected that there would be frequent overlap between these fractures. However, recording the instances of a fracture involving the articular surface itself was considered important enough to be coded separately. Similarly, although the cribriform plate is technically considered part of the ethmoid bone (28), the possible prognostic implications of having breached the braincase were deemed important enough to record instances of cribriform fracture separately from other ethmoid fractures.

If a fracture occurred along a suture or at a border between two regions, the bone or region on both sides was considered fractured, and the morphology of the fracture was considered separately for each bone or region. By definition, all fractures along a suture were considered complete. However, the degree of displacement was recorded individually for the bone on either side of a suture (**Figure 2**).

For the intermandibular joint (symphysis), a fibrocartilaginous joint (synchondrosis), symphyseal separation was considered by definition to be bilateral. However, if the two sides were unequally displaced (**Figure 3**), the coding reflected this.

Fracture Etiology

For each case, one of seven different fracture etiologies were assigned, as depicted in **Table 1**.

Demographic Data

Patient sex (male and female, intact or neutered) and age (in years, or portion thereof) were recorded for each case. Although breed and skull shape may be related to fracture location and morphology, for the purposes of this study it was determined that patient weight in kilograms at time of presentation would be the only breed-related variable recorded. Patients were grouped into < 10 kg, 10–20 kg, 20–40 kg, and > 40 kg. Additionally, patients who were considered juvenile based on the presence of mixed or deciduous dentition were categorized separately as it was unlikely

TABLE 1 | Explanation of fracture etiology codes.

Fracture etiology	Code	Examples or clarification
Crush/slow velocity	1	Slow vehicular roll-over, stepped or sat on, shut in door, etc.
Vehicular injury	2	Any vehicular injury not specified in other categories.
Animal bite	3	Bite originating from any other animal.
Fall from height	4	Fall from building, fall from vehicle, etc.
Ballistic injury	5	Bullet, arrow, etc.
Blunt force trauma	6	Baseball bat, horse kick, running into an object (including a vehicle if vehicle stationary), etc.
Unknown/miscellaneous	7	Unknown injury or not otherwise characterized.

that their weight at the time of presentation would accurately reflect their final weight.

Statistical Methods

Exact binomial proportions and confidence intervals were calculated to evaluate the distribution of severe displacement and fragmentation by fracture location. These analyses were also used to assess the proportion of fractures present at each location, conditional on each of four trauma type etiologies for which at least 20 cases were represented in the data.

Fisher's exact test was used to compare trauma etiology across the four distinct groups defined by sex and gonadectomy status. The Kruskal-Wallis test was used to compare age distributions across the same four groups. Fisher's exact tests were also used to compare the association between the categorical variables (sex and size) with fracture location and morphology. Kruskal-Wallis tests were used to compare age between patients with fractures at specific locations and with specific morphologies. In addition, the chi-square test of homogeneity was used to compare the observed and expected counts in contingency tables defined by breed size (weight) categories and fracture etiologies. $P < 0.05$ were considered statistically significant.

RESULTS

Descriptive Statistics

Out of 165 dogs evaluated in this study, 45 dogs were spayed females, 39 were intact females, 38 were neutered males, and 43 were intact males (**Figure 4**). The ages ranged from 2 days to 16 years with an average age of 4.3 years. 25.5% of dogs were considered juvenile based on the dentition. The proportion of dogs in each size bracket was as follows: < 10 kg: 41.8%, 10–20 kg: 9.7%, 20–40 kg: 18.8%, and > 40 kg: 4.2% (**Figure 4**).

Incidence of trauma etiology, depicted in **Figure 5**, demonstrated that animal bites caused the majority (50.3%) of injuries. The average number of fractured regions or bones was 8.2 per dog, with up to 37 fractured regions, and only 7.2% of cases (12 dogs) having a solitary fractured region or bone. 41.2% of cases had bilateral fractures for at least one bone or region.

Most Commonly Fractured Locations

The most commonly fractured location was the maxillary bone (**Figures 6**, **7**), with 53.3% of dogs having sustained at least one fracture of this bone. The molar and premolar parts of the mandible were each affected in 41.2% of dogs. The least commonly affected locations were the occipital and parietal bones with each being fractured in 1.2 and 3% of cases, respectively. There was not a bone or region in the skull that was unaffected in all cases (i.e., no bone/region was fractured in 0% of cases). No attempt was made to determine significance based on possible overlapping of confidence intervals. However, a general trend of increasingly common fractures of the midface (maxilla, zygomatic, nasal, and incisive bones) as well as the premolar and molar parts of the mandible can be seen in **Figures 6**, **7**. The articular surface of the TMJ was fractured in 30.3% of cases.

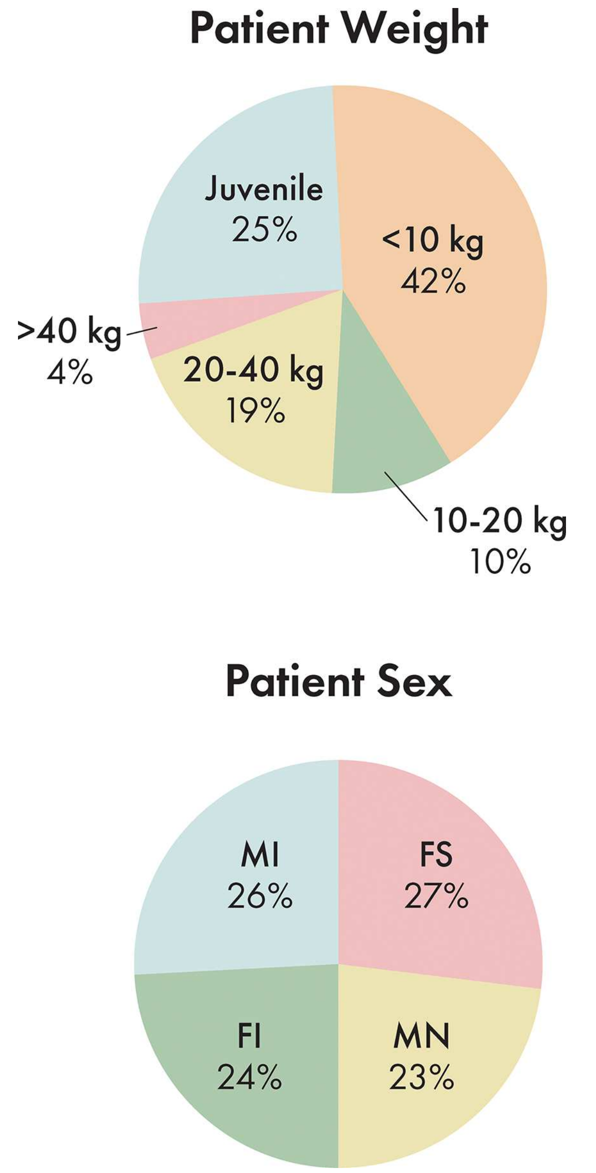

FIGURE 4 | Population distribution by patient size and by patient sex. MI, male intact; FI, female intact; FS, female spayed; MN, male neutered.

Fracture Morphology by Location

The proportions of severely displaced and fragmented fractures in each location are depicted in **Figures 8**, **9**. The maxillary bone had the highest proportion of severely displaced and fragmented fractures, with 28.5 and 33.9% of maxillary fractures being severely displaced or fragmented, respectively. The conchae were affected by severe displacement and fragmentation in ∼23% of cases. In general, regions of the mid-face and the body of the mandible were also more likely to be affected by severe fragmentation and displacement.

Fracture Etiology by Location

The proportion of fractures at each location varied according to etiology as seen in **Figure 10**. Etiologies that occurred in <

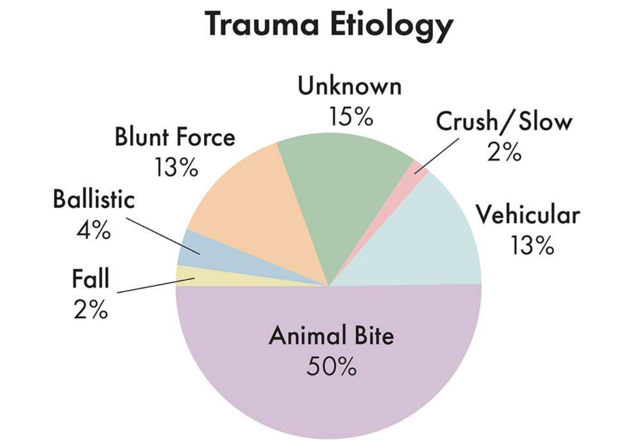

FIGURE 5 | Distribution of trauma etiology. Note that animal bites accounted for the largest proportion of trauma etiologies.

20 dogs in the study population included crush injuries, falls from height, and ballistic injuries. These were not included in this part of the assessment. For vehicular accidents, animal bites, and blunt force trauma, the maxillary bone was the most commonly fractured region, occurring in 81.8, 54.2, and 45.5% of cases, respectively. However, in cases of CMF trauma occurring secondary to an unknown etiology, the premolar part of the mandible was the most likely to be fractured, occurring in 56.0% of cases. In cases of vehicular trauma, the premolar and canine parts of the mandible were fractured in 50% of patients, whereas the molar part was more likely (47.0%) to be fractured in animal bites and the premolar part (41.9%) in blunt force traumas. Fractures of the TMJ articular surface also exhibited variation according to trauma etiology such that animal bites and vehicular accidents resulted in fractures in 34.9 and 40.9% of cases, whereas blunt force trauma and unknown trauma only resulted in articular surface fractures in 18.2 and 16.0% of patients, respectively. The frontal (36.4%) and temporal bones (13.6%) were most likely to be fractured in cases of vehicular trauma. The occipital and parietal bones were infrequently fractured in all trauma etiologies.

Demographic Data and Trauma Etiology

A Fisher's exact test revealed no significant association between trauma etiology and sex ($p = 0.29$). Similarly, a Kruskal-Wallis equality-of-populations rank test revealed no significant difference in patient age between trauma etiologies ($p = 0.34$). However, a Pearson chi-squared revealed that there were significant ($p < 0.001$) associations between patient size and trauma etiology as seen in **Table 2**. Specifically, patients < 10 kg were significantly less likely to be affected by vehicular trauma. Patients between 20 and 40 kg were significantly more likely to be affected by vehicular trauma and less likely to be affected by an animal bite. Patients < 40 kg were significantly more likely to have experienced blunt force trauma.

Demographic Data and Fracture Location

Sex: There was a significant difference in presence or absence of lacrimal bone ($p = 0.044$) and conchae ($p = 0.010$) fractures according to sex such that intact animals (groups 3 and 4) were more likely to have fractured the lacrimal bone (30–36%) than were neutered animals (13–15%) and more likely to have fractured the conchae (35–44%) than neutered animals (13–20%). In addition, intact females (group 3) were significantly ($p = 0.007$) more likely (54%) to have fractured the nasal bone than other sex groups (21–30%).

Age: When there was a significant association between age and fracture location, younger animals were consistently more likely to have sustained fractures in all bones/regions than were older animals ($p < 0.05$) with the exception of the premolar part of the mandible which showed that dogs with fractures in this region were significantly ($p = 0.029$) older (5.0 years) than those without fractures in this region (3.8 years).

Size: There were two significant associations between size and presence of fractures at particular locations. There was a significant difference in presence or absence of pterygoid fractures according to size ($p = 0.044$) such that dogs that were < 10 kg (group 1) were less likely to have a fracture in this region (8.7%) than were any of the other size groups (25–28.6%). In addition, there was a significant difference in presence or absence of lacrimal bone fractures according to size ($p = 0.044$) such that dogs that weighed 10–20 kg and 20–40 kg (groups 2 and 3) were more likely to have a fracture in this region (37.5 and 25.8%, respectively) than were either of the other size groups (8.7–14.3%).

Demographic Data and Fracture Morphology

Sex: When there were significant associations between sex and severe displacement of fractures of particular locations, intact animals were significantly ($p < 0.05$) more likely than neutered animals to have sustained severe displacement of fractures of the conchae, lacrimal, and palatine bones. Intact females were significantly ($p = 0.008$) more likely (21%) to have severe displacement of the premolar part of the mandibles than were other sex groups (0–11%), whereas neutered males were more likely (7.9%) to have severe displacement of the angular process than were other sex groups (0–2.5%; $p = 0.025$).

When there were significant associations between sex and severe fragmentation of fractures at particular locations, intact animals were significantly ($p < 0.05$) more likely than neutered animals to have sustained severe fragmentation of fractures of the conchae, lacrimal, and palatine bones. Intact females were more likely to have severe fragmentation of the condylar process (7.7%; $p = 0.037$) and articular surface of the TMJ (13%; $p = 0.032$) than were other sex groups (0–2.6% and 0–5.3%, respectively). Finally, there was a significant difference in presence or absence of severe fragmentation of the maxilla according to sex ($p = 0.24$) such that spayed females (group 1) were less likely to have severe fragmentation (27%) than were any of the other sex groups (38–51%).

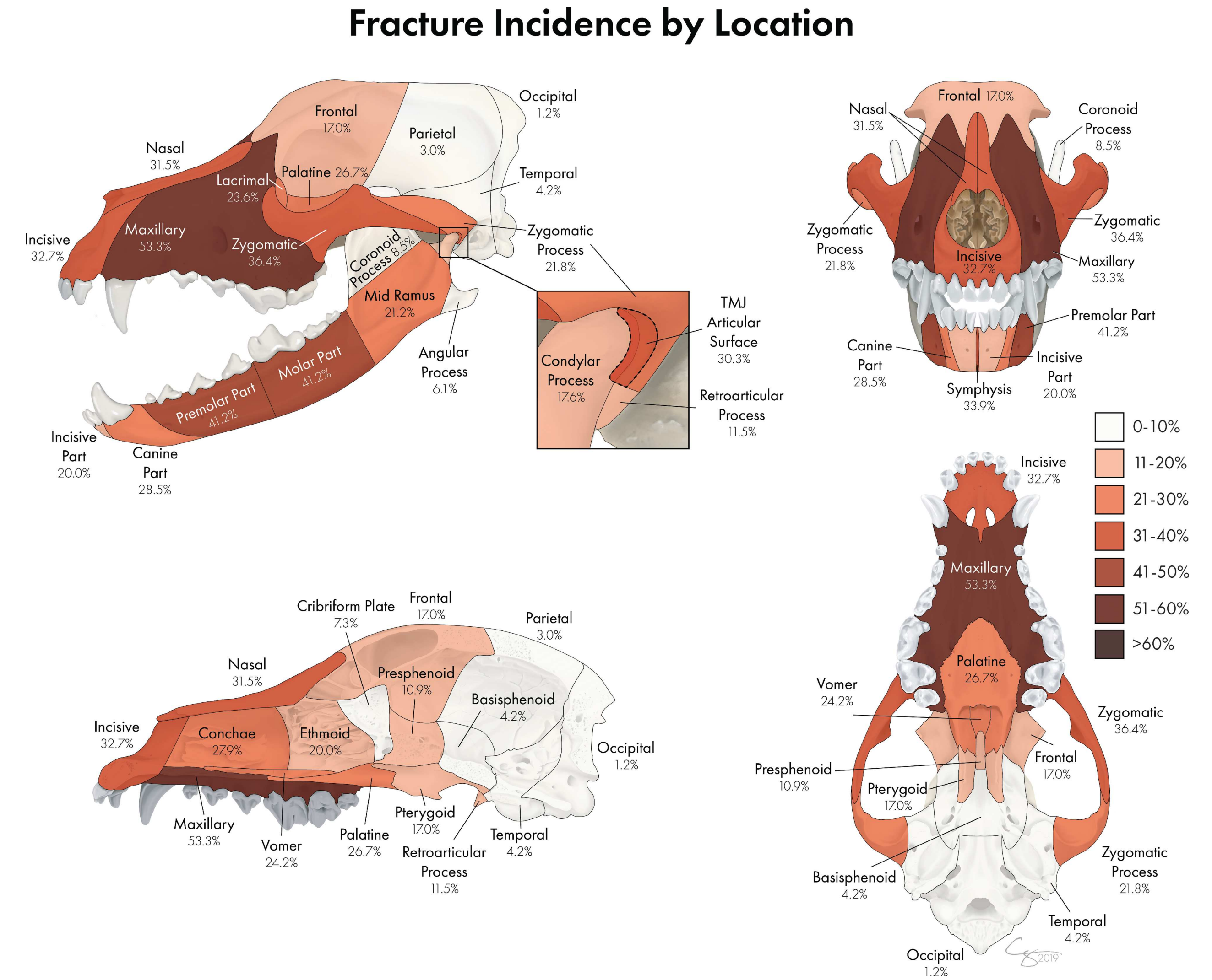

FIGURE 6 | A percentage heat map demonstrating the proportion of CMF fracture locations. Percentages represent the percentage of dogs sustaining a fracture in each location. For example, 53.3% of dogs in this study sustained fractures of the maxillary bone, whereas 1.2% of dogs in this study sustained fractures of the occipital bone.

Age: When there was a significant association between age and severe displacement, younger animals were more likely to have sustained severely displaced fractures in particular locations than were older animals ($p < 0.05$) without exception.

When there was a significant association between age and severe fragmentation, younger animals were more likely to have sustained severely fragmented fractures of particular locations than were older animals ($p < 0.05$). The exception was the zygomatic process for which dogs with severely fragmented fractures of this region were significantly ($p = 0.047$) older (4.3 years) than those without severely fragmented fractures (2.4 years).

Size: There were two significant associations between size and severe fragmentation of fractures. There was a significant difference in presence or absence of severely fragmented ramus fractures according to size ($p = 0.033$) such that dogs that were < 10 kg or > 40 kg were less likely to have a severely fragmented fracture in this region (0–2.9%) than were any of the other size groups (16–19%). In addition, there was a significant difference in presence or absence of severely fragmented pterygoid fractures according to size ($p = 0.036$) such that dogs that weighed 10–20 kg (group 2) were more likely to have a severely fragmented fracture in this region (19%) than were any of the other size groups (0–6.5%).

There were two significant associations between size and severe displacement of fractures of particular locations. There was a significant difference in presence or absence of severely displaced pterygoid fractures according to size ($p = 0.026$) such that dogs that weighed < 10 kg (group 1) were less likely to have a severely displaced fracture in this region (1.4%) than

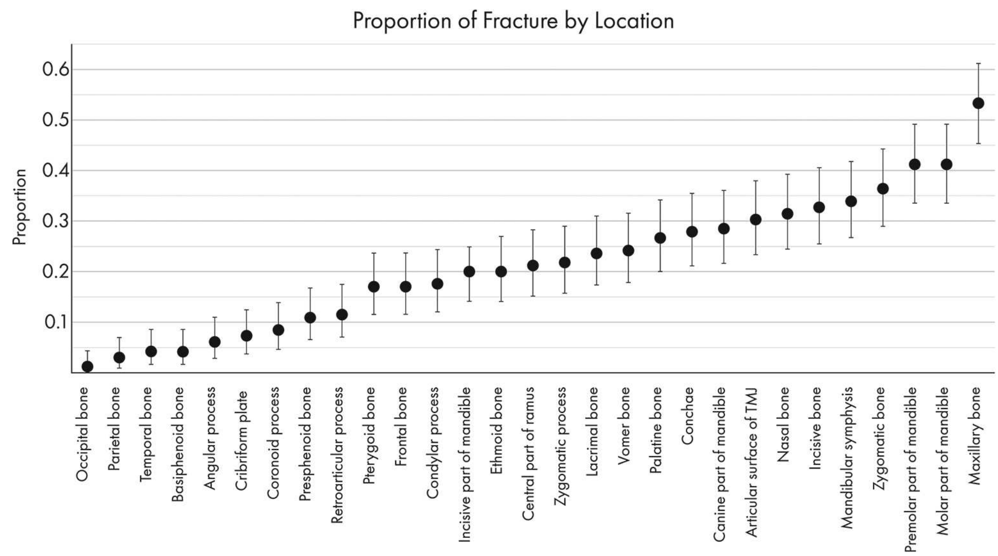

FIGURE 7 | Graphical projection of the proportion of CMF fractures by location. Note that the proportions of fractures spanned the entire CMF region but was the highest at the caudal mandibles and the maxillary bones.

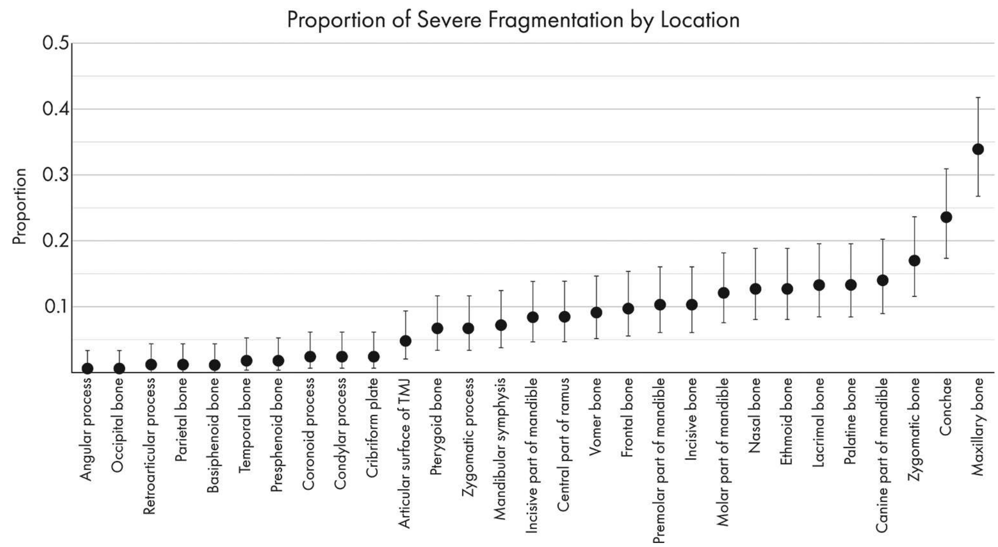

FIGURE 8 | Graphical projection of the proportion of severely fragmented fractures at each location. Note that the proportion of fractures with severe fragmentation (comminution) was highest in the maxillary bone, zygomatic bone, and conchae.

were any of the other size groups (12–14%). In addition, there was a significant difference in presence or absence of severely displaced ethmoid fractures according to size ($p = 0.033$) such that dogs that weighed < 10 kg (group 1) or > 40 kg (group 4) were less likely to have a severely displaced fracture in this region (3 and 0%, respectively) than were either of the other size groups (16–19%).

DISCUSSION

This comprehensive study documents CMF trauma in dogs using CT as a diagnostic tool and provides a detailed description and mapping of fracture location, morphology, and etiology. We report several key findings. First, dog age and sex were not associated with trauma etiology. However, dog size was associated with trauma etiology. Second, although causes of CMF trauma vary, the most common trauma etiology was animal bite. Third, the maxillary bone and the premolar and molar teeth regions of the mandible were the most commonly fractured. In addition, the more exposed the anatomical region, the higher the probability of severe fracture fragmentation and displacement. In addition, we demonstrated that the most commonly fractured regions of the skull vary according to the etiology of the causative trauma. Finally, younger dogs exhibited

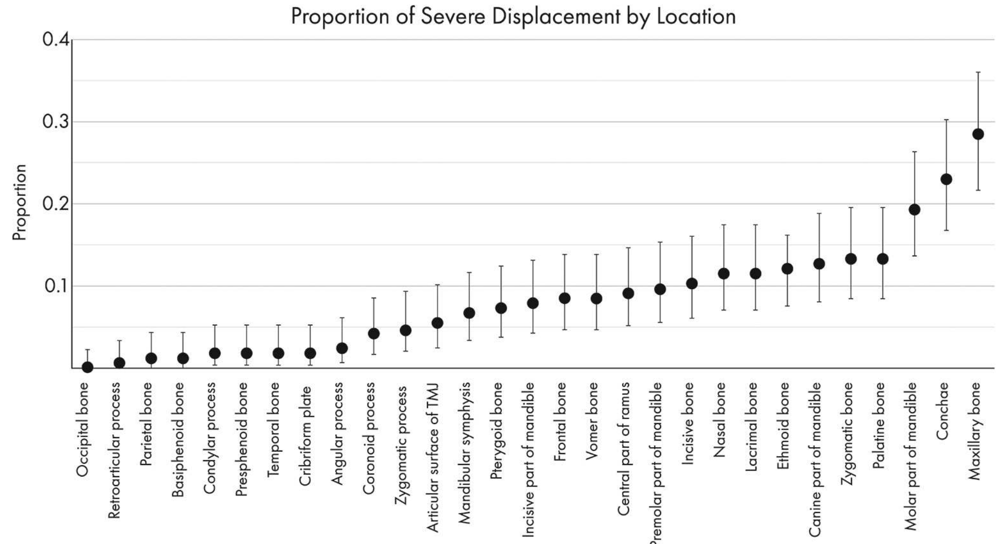

FIGURE 9 | Graphical projection of the proportion of severe displacement at each fracture location. Note that the most severely displaced fractures (<50% overlap between fracture segments) occurred at the maxillary bones, the conchae, and the molar part of the mandible.

more severe fragmentation in particular locations as compared to older dogs.

The present study demonstrates that small breed dogs are less likely to suffer CMF trauma as a result of a vehicular accident. This may be explained by several possibilities. First, it is likely that small breed dogs are less likely to be off leash near vehicles and hence, less likely to be involved in a vehicular accident. Another possibility is that dogs with a small head would die immediately following vehicular trauma rather than presenting to our hospital. The reason that medium-large breed dogs are more likely to sustain CMF trauma from vehicular accidents than from animal bites may reflect on their size (i.e., the larger the dog the less likely it is to be on the receiving end of a bite). In addition, blunt force trauma affected a number of giant breed (> 40 kg) dogs. This could be reflective of the fact that when they do collide with an object, there is often greater momentum involved.

Overall, the most common trauma etiology in our study involved an animal bite, whereas the previous literature has not consistently demonstrated a predominant etiology. For example, several studies (20, 21) found that vehicular accidents accounted for the majority (53–100%) of mandibular fractures, whereas others have found that an animal bite was the most common etiology (19, 22–24). It has also been found that dogs are less likely to sustain CMF trauma after a vehicular accident than they are to sustain trauma to other body parts, possibly due to an instinct to turn the head away from an oncoming object (24). In our study, unknown trauma and vehicular trauma were less common than animal bites. Although this discrepancy between studies may be partially due to regional or temporal variations, as has been pointed out in other publications (24), it is also possible that inconsistent reporting, lack of historical access to CT, and small sample sizes have previously prevented recognition of the most common causes of CMF trauma.

The most commonly fractured region was the maxillary bone followed by the premolar and molar parts of the mandibles. Our findings are in agreement with previous reports on fractured regions of the mandibles. However, our findings differ with regards to incidence of fracture in other regions. Previous reports, which did not all utilize CT for diagnosis, demonstrated that the mandible is vastly more likely to be fractured than other parts of the skull (19, 22, 25). Specifically, the premolar-molar part of the mandibles have been reported to be the most commonly fractured (21) and this may be dependent on patient size (23). In contrast, our study found that the maxillary bone is slightly more likely than the premolar-molar teeth region of the mandibles to be fractured. Previous reports on the physical location of CMF fractures due to trauma are sparse and typically focused on the mandibles. Historically, access to CT was much more limited, so concentrating on mandibular fractures likely reflects the relative ease of interpreting skull radiographic images of the mandible as compared to the difficulty of interpreting the superimposed structures of the maxilla, skull base, and cranial vault (29). This is important given that standard skull radiographs have been documented to significantly underdiagnose the number of fractures in a CMF trauma patient as compared to computed tomography (12). It is not surprising that the maxillary bone and premolar/molar parts of the mandibles are the most commonly fractured regions. As has been described elsewhere (1, 3), the maxilla of the dog is, in many breeds, a prominent and exposed structure and is therefore more susceptible to traumatic insults than other craniomaxillofacial structures. The premolar/molar part of the mandible is similarly exposed to traumatic insults, especially those occurring from the side as opposed to frontally.

Fragmentation and displacement of fractures tended to be more severe at the most exposed regions of the skull which

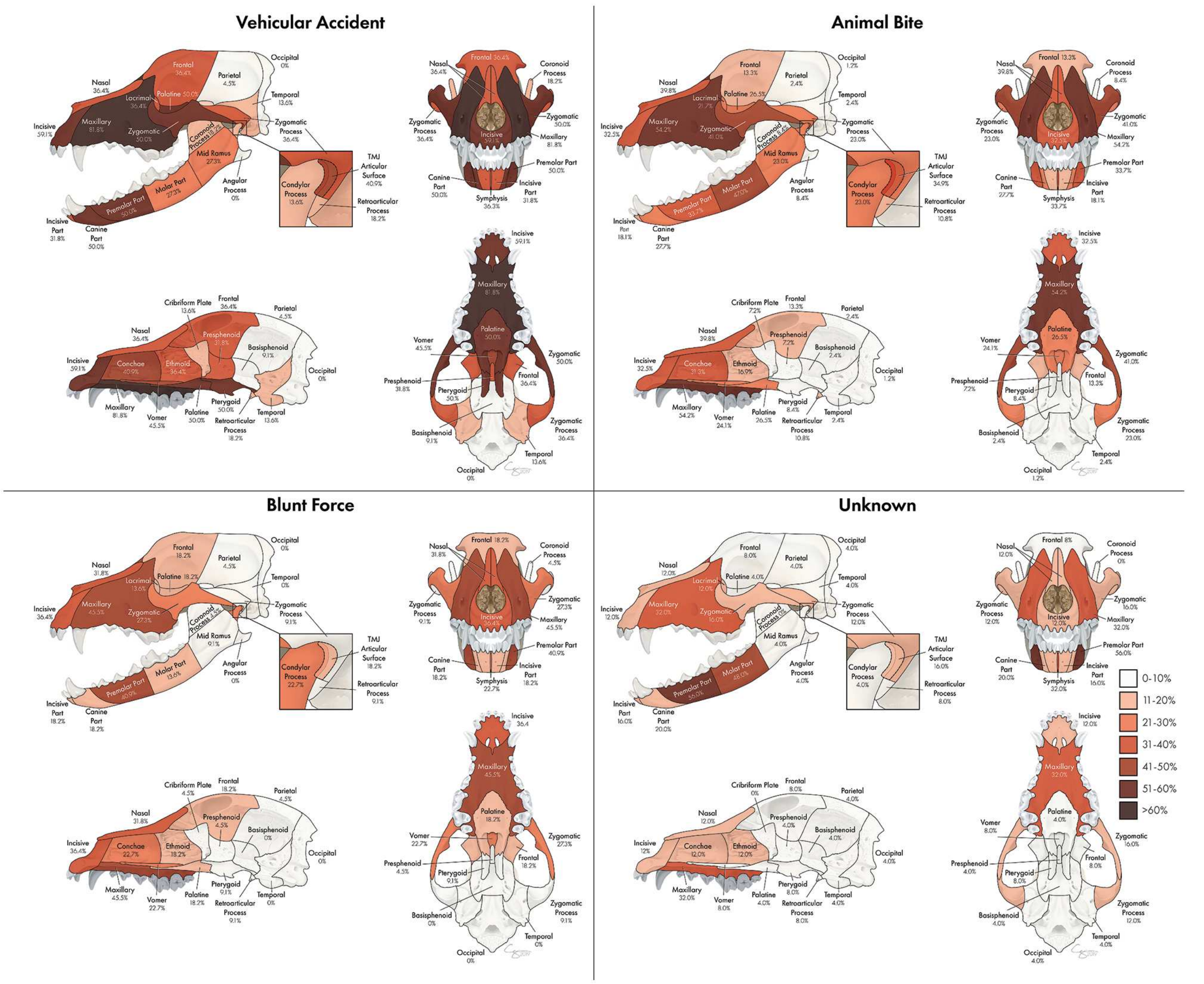

FIGURE 10 | Array of heatmaps according to trauma etiology. Trauma etiologies which accounted for fewer than 20 cases (fall from height, crush injury, ballistic injury) were not included in this portion of the analysis.

follows the rationale described above. Furthermore, younger dogs exhibited more severe fragmentation as compared to older dogs. The maxillary region, in general, is composed of thin, lightweight bones interposed with nasal and paranasal passages (1, 3). As may be expected in such an area, when fractured, these regions experience more severe impact and therefore exhibit more displacement and fragmentation than other, more protected structures such as the skull base. It is important to note, however, that the low overall incidence of skull base and cranial vault fractures in our study may also reflect the likelihood that such injuries are more often rapidly fatal and, therefore, these patients may not have lived long enough to enter our patient population. With regards to age and fragmentation, it is plausible that the younger the dog, the more fragile are the CMF bones and, in addition to the presence of cranial sutures, predispose to excessive fragmentation following CMF trauma.

The most commonly fractured location varied according to trauma etiology. Specifically, vehicular accidents tended to result in more regions with a higher probability of being fractured than other trauma types, likely due to the velocity and impact with which vehicles strike animals. Notably, the pterygoid bones were fractured in vehicular accidents more often than in other types of trauma. This is essential for clinicians to understand as these injuries can be easy to miss and can greatly affect patient discomfort and ability to swallow. Animal bites are also likely to result in multiple areas with a relatively high probability of fracture, but tend to be centered mostly on the maxillary bone, zygomatic bone, and molar part of the mandibles. One explanation is that if a dog is bitten with the upper teeth grasping the muzzle and the lower teeth grasping either the inside of the oral cavity or below the mandible, these areas of fracture are

TABLE 2 | Chi-squared analysis of patient size and trauma etiology.

		Patient size					
		<10 kg	10–20 kg	20–40 kg	>40 kg	Juvenile	Total
Crush/slow							
	Observed	0	0	2	0	1	3
	Expected	1.3	0.3	0.6	0.1	0.8	3
	Chi-square	1.3	0.3	3.7	0.1	0.1	5.4
Vehicular							
	Observed	2	5	9	2	4	22
	Expected	9.2	2.1	4.1	0.9	5.6	22
	Chi-square	5.6*	3.9	5.7*	1.2	0.5	16.9
Animal bite							
	Observed	42	3	3	2	33	82
	Expected	34.7	8	15.6	3.5	21.1	83
	Chi-square	1.5	3.2	10.2*	0.7	6.7	22.2
Fall from height							
	Observed	2	1	1	0	0	4
	Expected	1.7	0.4	0.8	0.2	1	4
	Chi-square	0.1	1	0.1	0.2	1	2.3
Ballistic							
	Observed	1	0	5	0	0	6
	Expected	2.5	0.6	1.1	0.3	1.5	6
	Chi-square	0.9	0.6	13.3	0.3	0.15	16.6
Blunt force							
	Observed	6	4	7	3	2	22
	Expected	9.2	2.1	4.1	0.9	5.6	22
	Chi-square	1.1	1.6	2	4.6*	2.3	11.6
Other/unknown							
	Observed	16	3	4	0	2	25
	Expected	10.5	2.4	4.7	1.1	6.4	25
	Chi-square	2.9	0.1	0.1	1.1	3	7.2
	Total	69	16	31	7	42	165

Asterisks (*) indicate significant differences between expected and observed incidence of cases.

logical. The palatine and frontal bones were similarly affected in cases of blunt force trauma and animal bites, but were more common in vehicular accidents and rare in unknown trauma types.

The pattern of TMJ fractures is important to note as articular surface fractures were most commonly confined to either the condylar process or the zygomatic process for blunt force and unknown trauma, respectively, but more commonly occurred on both surfaces in vehicular accidents and animal bites. Thorough evaluation of the TMJ following CMF trauma is essential as fractures associated with the articular surface may have long term adverse consequences such as joint pain, reduced mandibular opening, degenerative joint disease, masticatory dysfunction or ankylosis (30, 31). In addition, if the fracture of the TMJ occurred at an early age, it is likely to affect the growth and development of the mandibles (32).

The limitation of this study is inherent to its retrospective design. In addition, the patients included in this study were assessed at a tertiary referral institution, which could have affected the types of CMF trauma included in the study. For example, very mild cases may not have been referred to our institution if the primary veterinarian felt capable of treating the patient. Likewise, very severe cases may have died or been euthanized prior to referral. Because several of the trauma etiologies (crush injuries, fall from height, and ballistic traumas) occurred infrequently, the sample size for those etiologies was too small to draw any conclusions from the associated data. Finally, the inclusion of unknown trauma etiology category can be viewed as a limitation as it does not reveal precise information.

In conclusion, by assessing CT images of the entire CMF region in a large population of patients, this study has highlighted the most commonly fractured regions of the skull as well as the most common causative traumatic insults. In addition, we provided basic information regarding trauma etiology and the regions of the skull that are most likely to be fractured. In turn, this allows veterinarians to focus their physical exams and diagnostic imaging in the appropriate regions.

Importantly, a major takeaway from this study is that every bone of the CMF region was fractured in at least one case and many cases had a large number of fractured regions. Therefore, the need for careful assessment of the entire CMF region using CT has been underscored. In part 2, we report on the specific fracture locations and their tendency to co-fracture with other locations, as well as further elucidating the relationships between trauma etiology, fracture morphology, and fracture location.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

Ethical review and approval was not required for the animal study because the study is retrospective in nature and included clinical cases, hence, it is exempt from IACUC requirements. Written informed consent for participation was not obtained from the owners because the study is retrospective in nature and, hence, it is exempt from written informed consent.

AUTHOR CONTRIBUTIONS

MD: Study concept and design, image analysis, data acquisition, analysis and interpretation, drafting of the manuscript, final approval of the version to be published. RP: Study concept and design, image analysis, drafting of the manuscript, final approval of the version to be published. BA and FV: Study concept and design, data interpretation, drafting of the manuscript, final approval of the version to be published. PK: Data analysis and interpretation, drafting of the manuscript, final approval of the version to be published.

ACKNOWLEDGMENTS

We would like to thank Chrisoula Toupadakis Skouritakis for providing the illustrations and images used in this study. We would also like to thank Daria Farkondeh for her assistance with coding demographic data from the medical records.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Veterinary Science

ORIGINAL RESEARCH
published: 15 May 2020
doi: 10.3389/fvets.2020.00242

Craniomaxillofacial Trauma in Dogs—Part II: Association Between Fracture Location, Morphology and Etiology

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Edited by:
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Specialty section:
This article was submitted to
Veterinary Dentistry and
Oromaxillofacial Surgery,
a section of the journal
Frontiers in Veterinary Science

Received: 26 February 2020
Accepted: 08 April 2020
Published: 15 May 2020

Citation:
De Paolo MH, Arzi B, Pollard RE,
Kass PH and Verstraete FJM (2020)
Craniomaxillofacial Trauma in
Dogs—Part II: Association Between
Fracture Location, Morphology and
Etiology. Front. Vet. Sci. 7:242.
doi: 10.3389/fvets.2020.00242

Treatment of craniomaxillofacial (CMF) trauma in dogs requires a thorough understanding of the CMF skeletal structures involved. The human medical literature has several examples of CMF trauma and fracture classification, including the classically described Le Fort fractures. The recent classification schemes require large studies using computed tomography (CT). In the veterinary medical literature, such studies are lacking. The aims of part II of this retrospective study were to use a large number of CT studies of dogs evaluated for CMF trauma to determine whether specific fracture locations in the CMF region occur concurrently, and whether trauma etiology influences fracture morphology. This information may then be used to form a fracture classification scheme in the future. The medical records and CT studies of 165 dogs over a 10-year period were evaluated. The skeletal location of CMF fractures as well as the severity of displacement and fragmentation of each fracture was recorded. Dogs' demographic data and trauma etiology were also recorded. Fractured portions of the mandible tended to occur with fractures of adjacent bones, with the major exception of symphyseal separation, which occurred simultaneously with fractures of the cribriform plate. Fractures of the maxillary bone were accompanied by many concurrent fractures affecting the majority of the midface, skull base, and cranial vault. When the zygomatic bone was fractured, the other bones comprising the orbit also tended to fracture. Fractures of the relatively superficially located frontal and nasal bones were often accompanied by fractures of the skull base. Fracture etiology influenced fracture morphology such that vehicular trauma resulted in a relatively higher number of severely displaced and comminuted fractures than did other trauma etiologies. This study provides examples of fractures that, when found, should prompt veterinarians to look for additional injuries in specific locations. In addition, it further highlights the need for thorough CT evaluation of the entire CMF region, even when clinically apparent fractures appear relatively superficial.

Keywords: craniomaxillofacial, trauma, computed tomography, fracture, displacement, dogs

INTRODUCTION

Over a century has passed since the studies performed by Rene Le Fort in 1901, who demonstrated that fracture morphology and location are often closely related to trauma etiology (1). As in the human craniomaxillofacial (CMF) skeleton (2), the bones and anatomy of the canine CMF skeleton have many complex structures and interdigitations. Given this complexity, it is likely that neighboring bones will be fractured simultaneously as a result of trauma. What remains elusive is whether fractures of specific bones, such as the rostral mandible, are likely to occur simultaneously with more distant structures, for example the temporomandibular joint (TMJ). In people, there are specific fractures that, when found, should prompt clinicians to evaluate for further injuries (3). For example, fracture of the pterygoid bones is a feature of all Le Fort fractures and, when noted, should immediately prompt the clinician to look for evidence of additional fractures. Currently no such indicators exist for dogs that have sustained CMF trauma, and the potential usefulness of such indicators is evident.

Le Fort's studies were some of the earliest attempts at understanding how fracture etiology can affect the resultant fracture distribution and morphology (1). Since then, others have built upon his work and further refined it such that there are now different fracture patterns expected between and even within trauma etiology. For example, within ballistic injuries, different patterns are expected dependent upon weapon type and bullet caliber (4). Recently, it has been recognized that patterns of CMF trauma in people are likely to shift over time given that the nature of the trauma etiology (due to increased access to motor vehicles and weapons) itself is also shifting (2, 3, 5). In dogs, it is also intuitive that different trauma etiologies might result in different fracture patterns and severity. However, to the authors' knowledge this has not been documented. As we demonstrated in Part I, fracture location does tend to change based on etiology, but the resultant fracture morphology has yet to be reported.

An adequate understanding of these variables may provide a foundation for a fracture classification system in which trauma etiology; fracture location and morphology; and patient demographic factors are taken into account to inform prognosis and best practices. As described by Audigé et al. in the most recent AOCMF (Arbeitsgemeinschaft fur Osteosynthesefragen—craniomaxillofacial) trauma classification for humans (6), which has recently been validated (7), this process takes many iterations and requires collaboration between multiple specialists, and the first step requires documentation of the existing fractures and patterns.

At present, there is no evidence-based classification system for CMF fractures in the dog. An effective classification system for traumatic dentoalveolar injuries (TDI) in humans has recently been applied to TDI in dogs and cats with success (8). However, given the marked differences in CMF structure between humans and companion animals, no such classification system exists in the human literature that can be applied to CMF fractures in dogs. Therefore, an iterative process similar to that currently being undertaken by AOCMF will likely be needed in the future to produce a classification system that allows for appropriate communication across surgical specialties and, therefore, appropriate treatment of the dog as a whole.

In Part I, we demonstrated that trauma etiology is associated with fracture location (9). Similarly, fracture morphology can also vary based on the location of the fracture. In Part II, we elucidate whether certain bones or regions tend to fracture concurrently and whether there is a relationship between fracture etiology and fracture morphology. We hypothesized that specific bones or regions would fracture concurrently with others, and that fracture etiology would impact the resultant fracture morphology for each location differently.

METHODS

All methods relating to case selection, image acquisition, fracture evaluation, and categorization of demographic and trauma-related data were previously described in Part I of this study and are repeated below. All figures referenced in this Methods section appear in Part I of the accompanying paper (9).

Case Selection

The electronic medical record database was queried for dogs that had been presented to the UC Davis Veterinary Medical Teaching Hospital for evaluation and treatment following CMF trauma between the years 2008–2018. All dogs had undergone computed tomography (conventional and/or cone-beam CT [CBCT]) at the initial visit. Exclusion criteria were as follows: trauma that occurred >1 week prior to presentation, dogs with CT scan slice thickness of >1.3 mm, and dogs for whom either the medical record or CT study were incomplete (e.g., the caudal-most portion of the skull had been left out of the study). Cases were excluded if the trauma occurred >7 days prior to presentation due to concern that: (a) early signs of fracture repair and boney remodeling may make fracture identification more difficult, and (b) further displacement may have occurred since the trauma. Exclusion of cases if the slice thickness was >1.3 mm was chosen as a compromise between maximizing the number of cases that were included in the study while simultaneously ensuring that slice thickness was not so large that small or incomplete fractures could be missed.

Image Acquisition and Evaluation

All dogs received conventional (HiSpeed FX/i or LightSpeed16, GE Healthcare, Waukesha, WI) and/or cone beam CT (NewTom 5G CBCT Scanner, NewTom, Verona, Italy) scans at their initial visit. Although many dogs presenting for CMF trauma at our institution undergo CBCT, including conventional CT allowed the study to capture those cases in which superior soft tissue imaging was medically necessary (i.e., those with concern for intracranial hemorrhage, those too large for the CBCT field of view, and those who received treatment prior to the advent of CBCT at this facility). All DICOM files from each study were viewed using specialized software (Invivo5, Anatomage, San Jose, CA). Each case was viewed dynamically on medical flat-grade monitors (ASUS PB278Q 27-inch, ASUSTeK Computer Inc., Taipei, Taiwan), allowing the observers to utilize all viewing modes and tools to best assess all fractures. One observer (MD) viewed all studies and recorded all data after a period of calibration with one experienced board-certified radiologist (RP) and 2 board-certified diplomates and AVDC-OMFS Fellows (FJV, BA). When there was uncertainty, the particular study was reviewed with the board-certified radiologist (RP).

Fracture Evaluation

Each skull was divided into specific bones and regions as illustrated in Part I, Figure 1. For each bone and region, it was determined whether each bone or region was fractured. If so, fracture morphology was described in terms of displacement and fragmentation. The degrees of displacement and fragmentation were modeled after the AOCMF fracture classification system (6). For both displacement and fragmentation, a score of 0 indicated no fracture. When scoring displacement, a score of 1 indicated no displacement, a score of 2 minimal displacement with ≥50% overlap remaining between fragments, and a score of 3 severe displacement with <50% overlap remaining. When scoring fragmentation, a score of 1 indicated an incomplete fracture, a score of 2 a complete fracture, and a score of 3 a comminuted fracture. This process was repeated on both the right and left sides of the skull. Although use of the term "comminuted" is discouraged by the most recent recommendations in human CMF literature (10), the term (and its associated meaning) are still pervasive in veterinary medicine and was therefore utilized in this study. A comminuted fracture was defined as a fracture having 3 or more bone fragments, although "minute" fragments were ignored unless the entire bone or region had been reduced to microfragments (11).

Because the bones that form the TMJ may be fractured without a fracture extending into the articular space, fractures of the temporomandibular joint were recorded separately from the condylar process, the retroarticular process, and the temporal bone. It was expected that there would be frequent overlap between these fractures. However, recording the instances of a fracture involving the articular surface itself was considered important enough to be coded separately. Similarly, although the cribriform plate is technically considered part of the ethmoid bone (12), the possible prognostic implications of having breached the braincase were deemed important enough to record instances of cribriform fracture separately from other ethmoid fractures.

If a fracture occurred along a suture or at a border between two regions, the bone or region on both sides was considered fractured, and the morphology of the fracture was considered separately for each bone or region. By definition, all fractures along a suture were considered complete. However, the degree of displacement was recorded individually for the bone on either side of a suture (Part I, Figure 2).

For the mandibular symphysis, a fibrocartilaginous joint (synchondrosis) symphyseal separation was considered by definition to be bilateral. However, if the 2 sides were unequally displaced such as depicted in Part I, Figure 3, the coding reflected this.

Fracture Etiology

For each case, 1 of 7 different fracture etiologies were assigned, as depicted in Part I, Table 1.

Statistical Methods

For each fracture location (the "region of interest"), exact binomial proportions and 95% confidence intervals were calculated to evaluate the frequency of related fractures occurring at other CMF locations. Locations with proportions >0.5 were reported only if the same association was detected on both sides of the jaw. For example, when evaluating maxillary fractures, the association was not reported if the left maxilla fractured concurrently with the right frontal bone but the right maxilla did not fracture concurrently with the left frontal bone. This was done to minimize the chance of reporting outlier associations. In addition, associations in which either the region of interest or the concurrently fractured location had fewer than 10 occurrences were not reported. When all of these criteria were met, the locations which fractured concurrently with the region of interest were recorded. These locations are henceforth referred to as "significant locations."

Box-and-whisker plots were used to display the distribution of fragmentation and displacement severity scores at each potential fracture location. These analyses were conditional on each of 4 trauma type etiologies for which at least 20 cases were represented in the data.

RESULTS

Twenty bones or regions met inclusion criteria described earlier when assessing associations between fractured locations. Each of these associations is depicted in **Figure 1** through **Figure 12**. The number of significant associations varied according to the primary fracture location being examined (the "region of interest," which is depicted in dark blue), with some fracture locations only being associated with a single additional fracture location while others were associated with several additional locations (concurrently fractured locations highlighted in light blue). The bones or regions that met the criteria described above are grouped into 3 larger regions (the mandible, midface, and skull base/cranial vault) for further reporting of association.

Fracture Location Co-occurrence: Mandible

Number of Concurrently Fractured Locations

In the mandible (**Figure 1**), the number of significant locations that fractured concurrently with the bone or region of interest ranged from one to five. When examining the molar part of the mandible, the only significant location that fractured concurrently was the mid-ramus region of the mandible. In contrast, when examining symphyseal separation, 5 regions

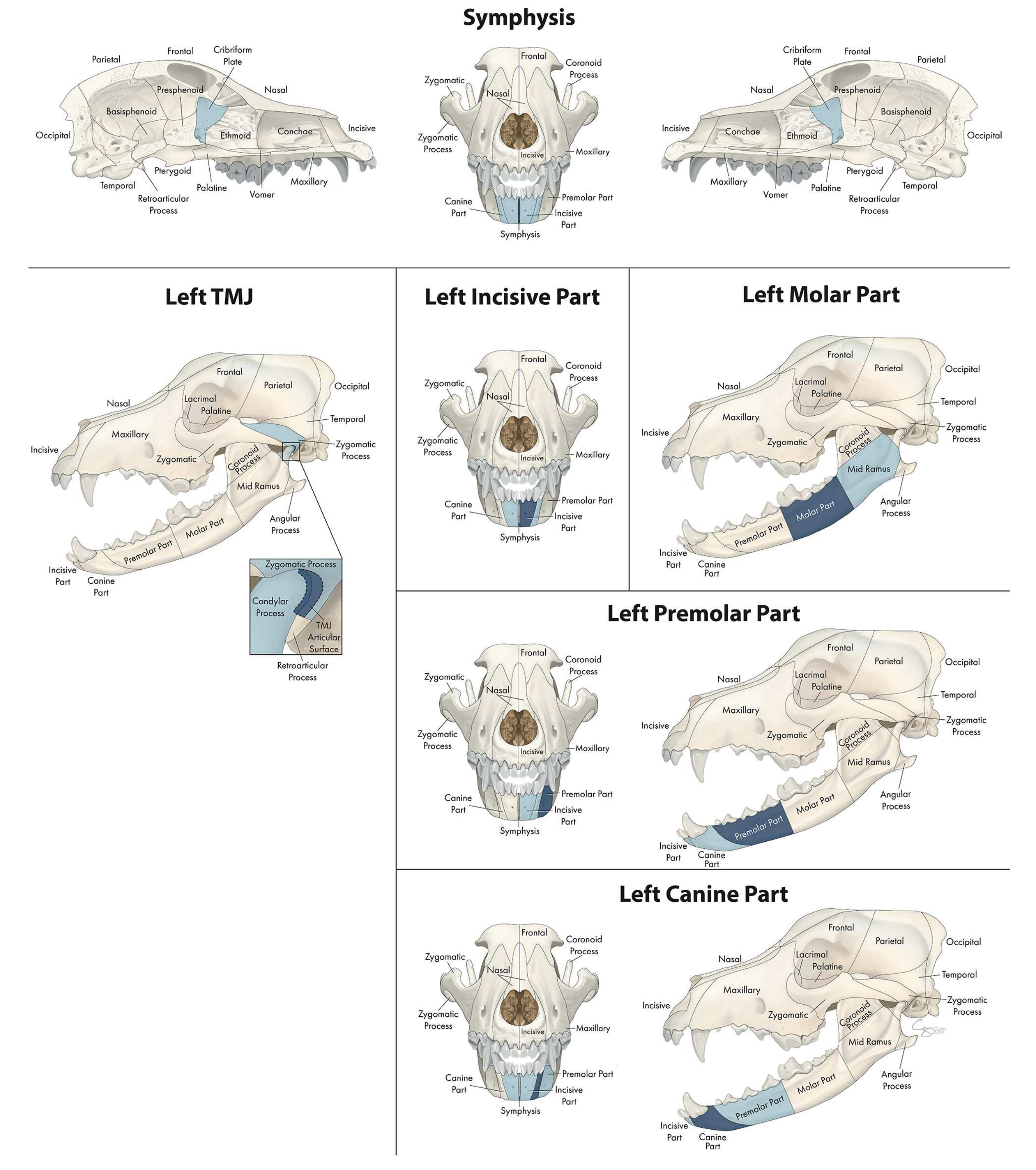

FIGURE 1 | Significant bones or regions fractured concurrently with the various regions of the mandible. Although the left mandible is shown, when the right mandible was the region of interest, a mirror image of concurrently fractured locations applied. Only views of the skull that demonstrate a fractured region are shown.

fractured (or separated) concurrently including the contralateral and ipsilateral incisive and canine regions in addition to the cribriform plate.

Confinement to the Same Jaw

When the region or bone of interest was located in the mandible, the significant locations that were fractured concurrently were

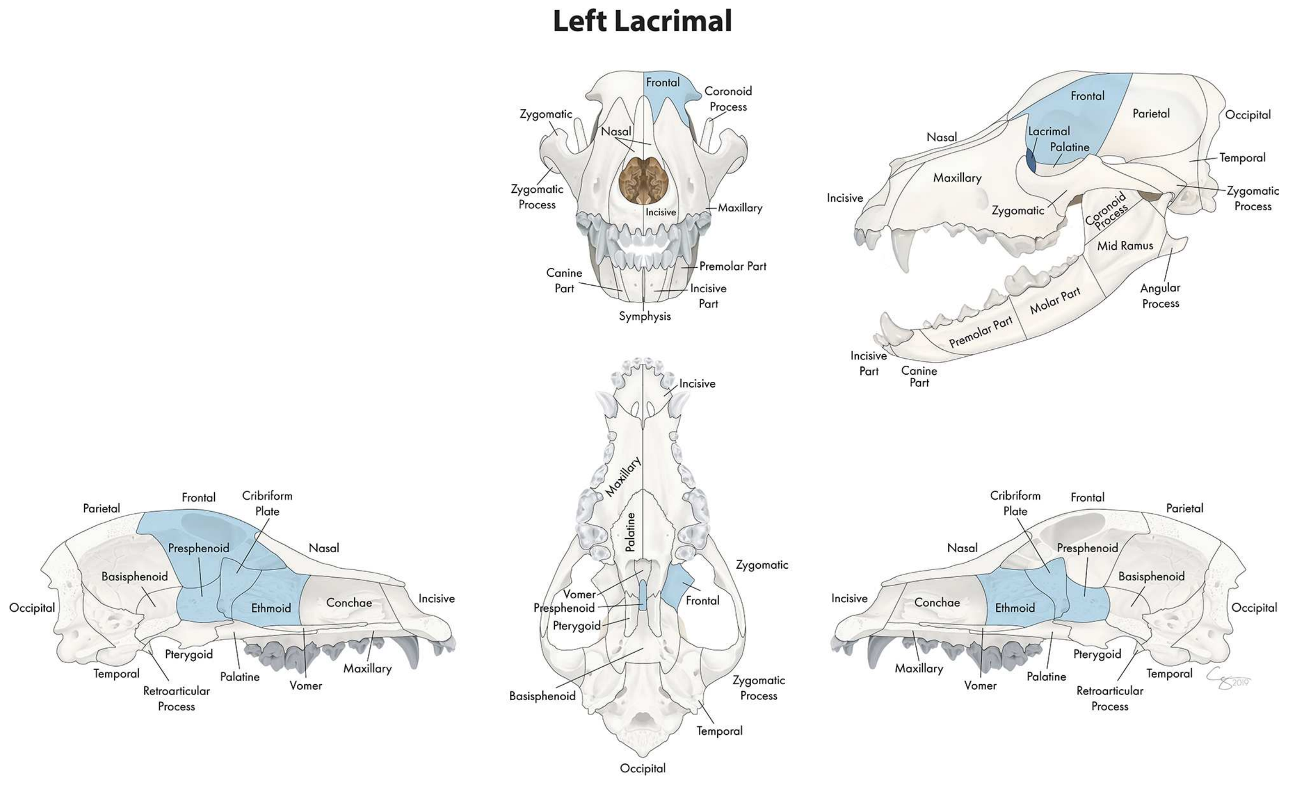

FIGURE 2 | Significant bones or regions fractured concurrently with the lacrimal bone. When the lacrimal bone was the region of interest, the cribriform plate, ethmoid, presphenoid, and ipsilateral frontal bones fractured concurrently. Although the left lacrimal bone is shown, when the right lacrimal bone was the region of interest, a mirror image of concurrently fractured locations applied. Only views of the skull that demonstrate a fractured region are shown.

also located in the mandible, with two exceptions. The first exception was the mandibular symphysis, which was separated concurrently with fracture of the cribriform plate (along with fractures of both incisive and canine regions of the mandible). The second exception was the articular surface of the TMJ, which fractured concurrently with both the zygomatic process of the temporal bone as well as the condylar process of the mandible.

Distance of Concurrently Fractured Locations From Region of Interest

When examining different regions of the mandible as the primary location of interest, locations that fractured simultaneously tended to be adjacent to the region of interest and on the ipsilateral side. For example, the molar region tended to fracture simultaneously with the ipsilateral mid-ramus, and the premolar region fractured with the ipsilateral canine and molar regions. The instances in which the contralateral side of the mandible was fractured occurred only when the primary location of interest was in the rostral mandible (i.e., symphysis, canine, or incisive parts).

Fracture Location Co-occurrence: Midface

Number of Concurrently Fractured Locations

In the midface, the number of locations that fractured concurrently with the bone or region of interest ranged from 4 to 23. When examining the lacrimal bone (**Figure 2**) or the zygomatic bone (**Figure 3**), for example, the number of significant locations that fractured concurrently was limited to 4 and 5, respectively. When the conchae (**Figure 4**), vomer (**Figure 5**), and maxillary bone (**Figure 6**) were isolated as the primary regions of interest, the number of significant locations that fractured concurrently was 17, 19, and 23, respectively. The number of locations that fractured with the incisive (**Figure 7**), palatine (**Figure 8**), and nasal bones (**Figure 9**) was intermediate, with 8, 11, and 15 concurrently fractured locations, respectively.

Confinement to the Same Jaw

When the region or bone of interest was located in the upper jaw, the significant locations that were fractured concurrently were also located in the upper jaw or the skull base and cranial vault.

Distance of Concurrently Fractured Locations From Region of Interest

When examining different bones and regions of the midface as the primary location of interest, locations that fractured simultaneously included those adjacent to the region of interest and on the ipsilateral side. However, a large number of fractures also occurred on the contralateral side and in regions not necessarily adjacent to the primary region of interest. For example, the conchae fractured with not only the surrounding

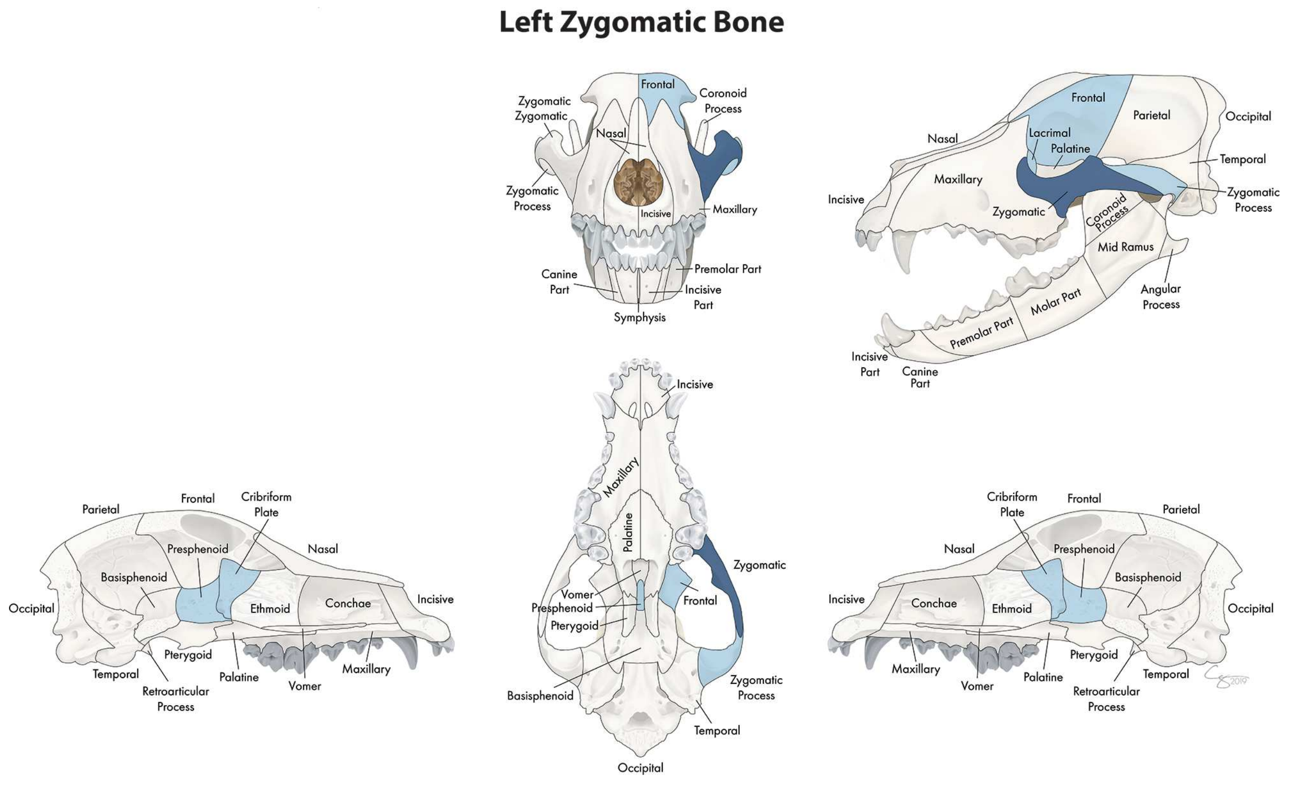

FIGURE 3 | Significant bones or regions fractured concurrently with the zygomatic bone. When the zygomatic bone was the region of interest, the cribriform plate, presphenoid, and ipsilateral zygomatic process, lacrimal, and frontal bones fractured concurrently. Although the left zygomatic bone is shown, when the right zygomatic bone was the region of interest, a mirror image of concurrently fractured locations applied. Only views of the skull that demonstrate a fractured region are shown.

regions but also many bones of the skull base and cranial vault. Similarly, the nasal bone fractured with multiple bones on the contralateral side as well as those of the skull base and cranial vault. The concurrently fractured bones and regions that occurred with fracture of the maxillary bone were the most broadly distributed, including the majority of the bones of the midface on the ipsilateral and contralateral sides of the skull. However, the zygomatic bone, conchae, incisive bone, lacrimal bone and vomer fractured concurrently with other bones and regions that were nearby.

Fracture Location Co-occurrence: Skull Base and Cranial Vault

Number of Concurrently Fractured Locations

In the skull base and cranial vault, the number of locations that fractured concurrently with the bone or region of interest ranged from 1 to 12. When examining fractures of the ethmoid bone (**Figure 10**) as a whole, 12 bones or regions fractured concurrently including frontal, palatine, pterygoid, and lacrimal bones bilaterally as well as the cribriform plate, presphenoid bone and conchae. The frontal bone (**Figure 11**) fractured concurrently with 5 other bones, and the presphenoid bone (**Figure 12**) with 4. When examining the pterygoid bones (**Figure 12**), they were found to fracture concurrently with the contralateral pterygoid bone as well as the cribriform plate and presphenoid bone. Whereas, when examining the cribriform plate (**Figure 12**), the only significant location that fractured concurrently was the presphenoid bone.

Distance of Concurrently Fractured Locations From Region of Interest

When examining different regions of the skull base and cranial vault as the primary location of interest, locations that fractured simultaneously tended to be adjacent to the region of interest. The exception to this finding was the ethmoid bone, which fractured concurrently with bones not just of the skull base and cranial vault but also with multiple bones of the midface such as the lacrimal and palatine bones.

Fracture Etiology, Location, and Morphology

Box and whisker plots demonstrate the severity of fragmentation and displacement of fractures occurring at each location (1-29) based on trauma etiology (**Figure 13**). As in Part I, only those trauma etiologies that occurred in more than 20 cases were included for analysis. Whiskers represent the fragmentation and displacement severity of the majority of the fractures recorded at that location, while the colored dots indicate outliers in the data. Therefore, locations with

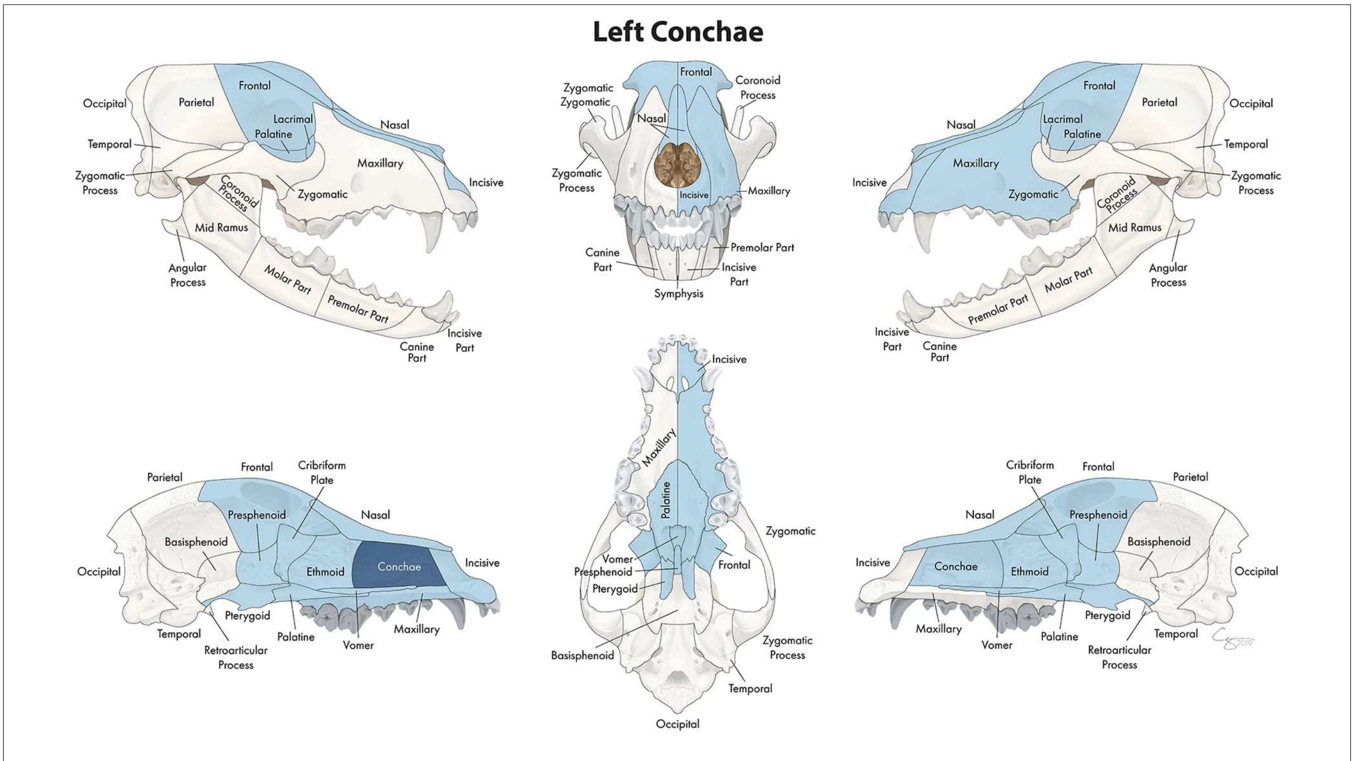

FIGURE 4 | Significant bones or regions fractured concurrently with the conchae. When the conchae were the region of interest, many other bones of the skull base/cranial vault and midface, both on the ipsilateral and contralateral side, fractured concurrently. Although the left conchae are shown, when the right conchae were the region of interest, a mirror image of concurrently fractured locations applied.

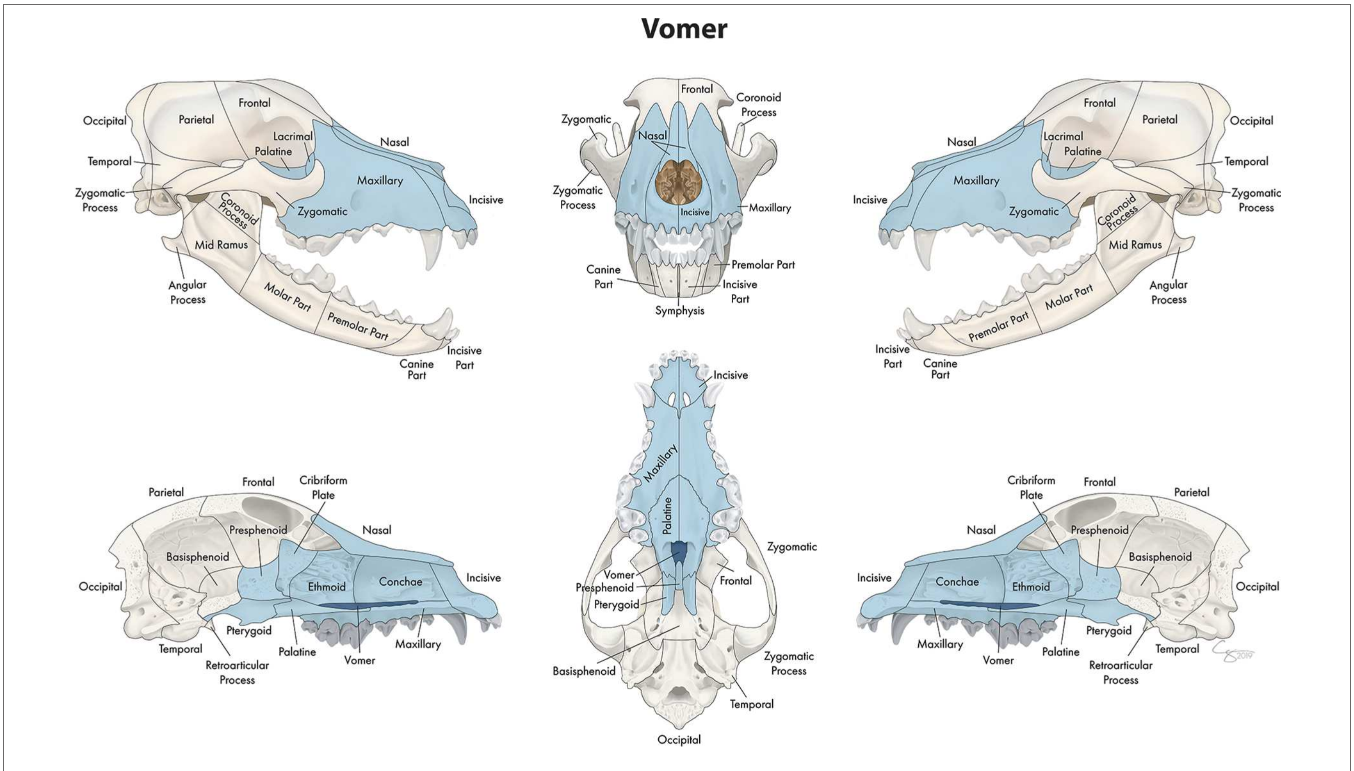

FIGURE 5 | Significant bones or regions fractured concurrently with the vomer. When the vomer was the region of interest, the conchae, cribriform plate, ethmoid, presphenoid, and bilateral incisive, maxillary, nasal, lacrimal, palatine, and pterygoid bones fractured concurrently.

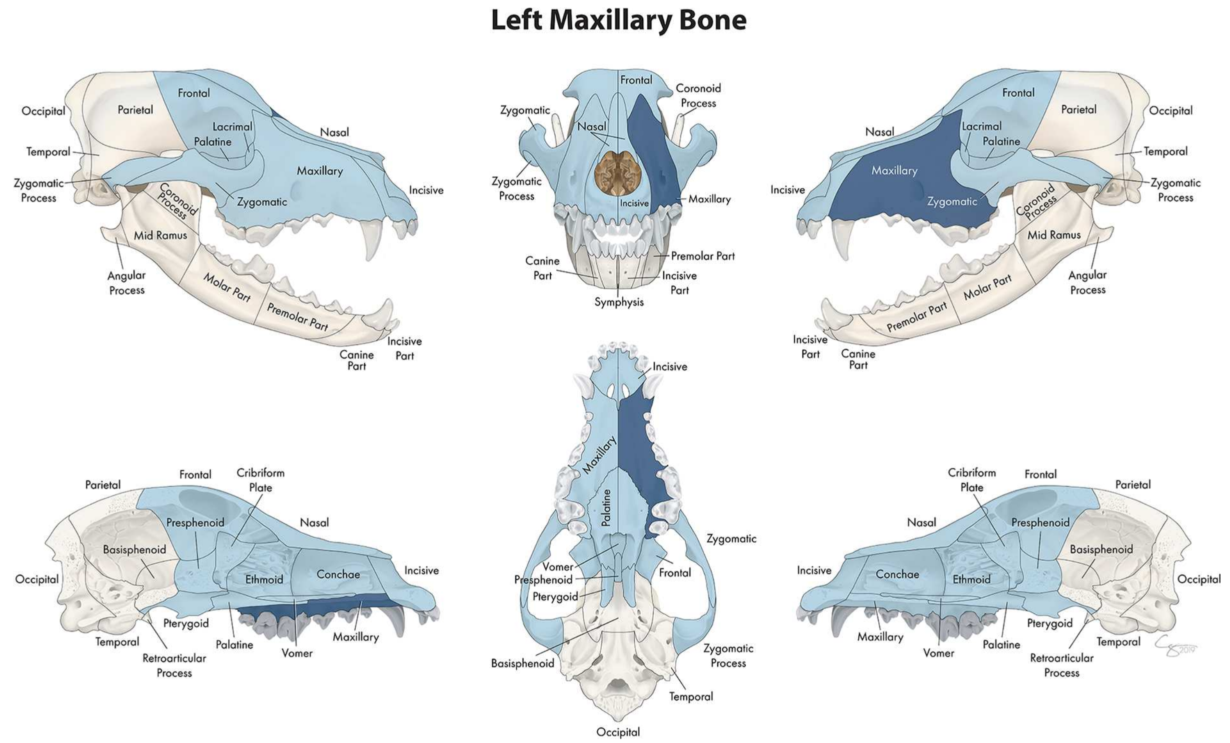

FIGURE 6 | Significant bones or regions fractured concurrently with the maxillary bone. When the maxillary bone was the region of interest, many other bones of the skull base/cranial vault and midface, both on the ipsilateral and contralateral side, fractured concurrently. Although the left maxillary bone is shown, when the right maxillary bone was the region of interest, a mirror image of concurrently fractured locations applied.

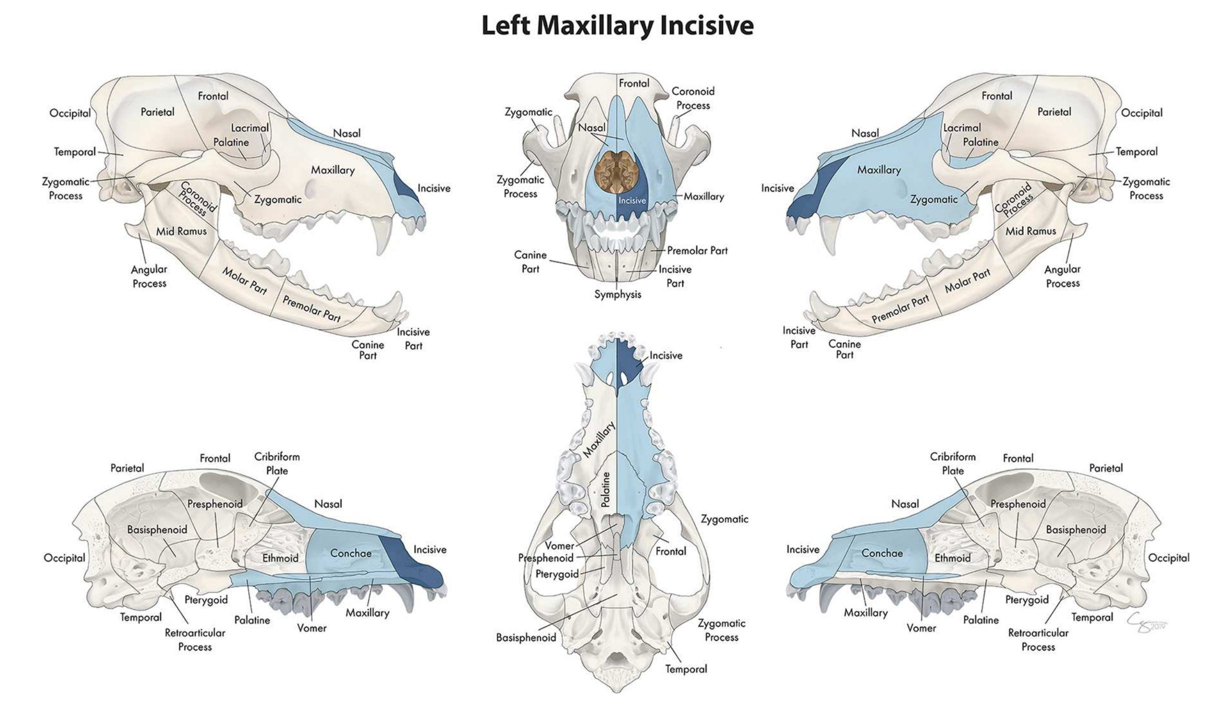

FIGURE 7 | Significant bones or regions fractured concurrently with the incisive bone. When the incisive bone was the region of interest, the vomer, ipsilateral maxillary and palatine bones, contralateral incisive bone, and bilateral conchae and nasal bones fractured concurrently. Although the left incisive bone is shown, when the right incisive bone was the region of interest, a mirror image of concurrently fractured locations applied.

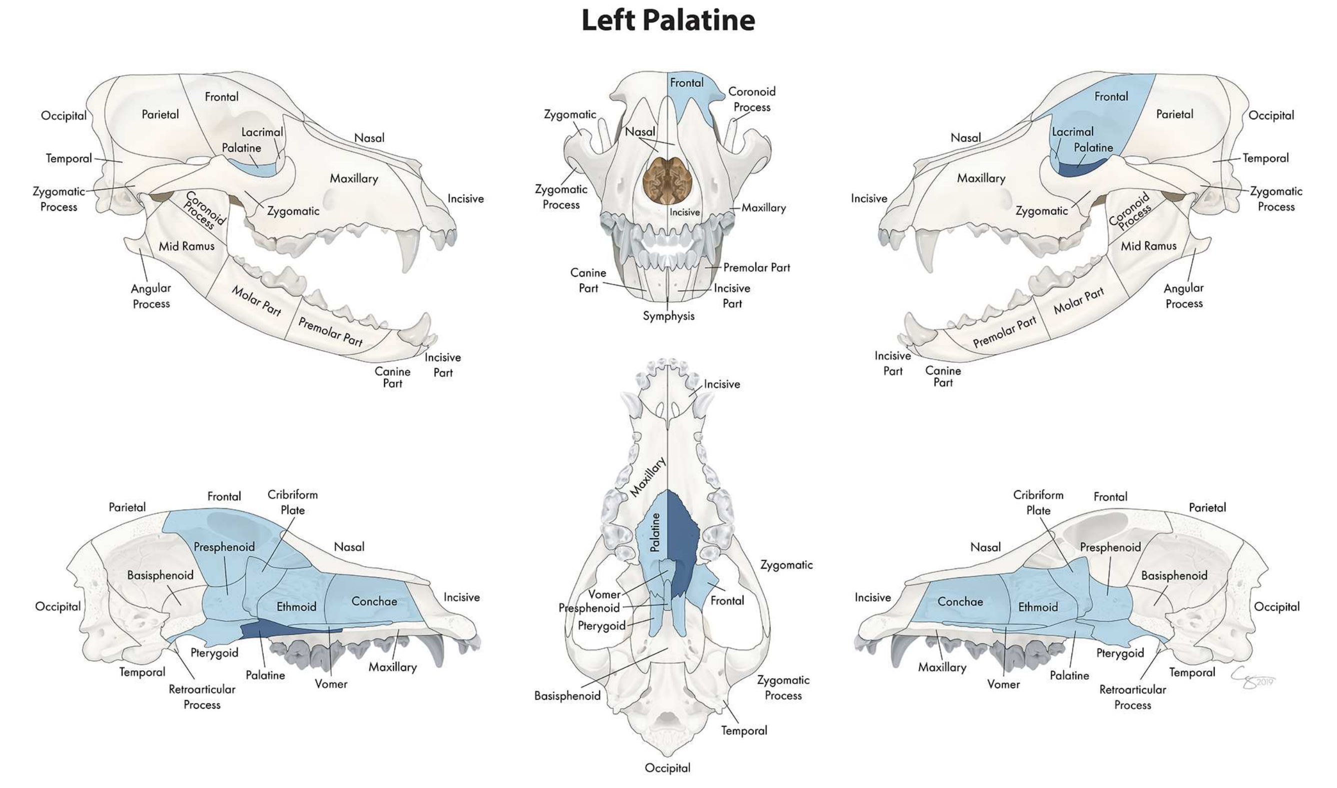

FIGURE 8 | Significant bones or regions fractured concurrently with the palatine bone. When the palatine bone was the region of interest, the cribriform plate, vomer, conchae, ethmoid bone, presphenoid bone, contralateral palatine bone, bilateral pterygoid bones, and ipsilateral lacrimal and frontal bones fractured concurrently. Although the left palatine bone is shown, when the right palatine bone was the region of interest, a mirror image of concurrently fractured locations applied.

only colored dots visible means that the fragmentation and severity score was 0 (the locations were not fractured) the majority of the time with several outliers. No attempt was made to determine significance in patterns across data. A general finding across all trauma etiologies was that those fracture locations with higher fragmentation scores also had higher displacement scores.

Vehicular Accidents

The majority of fractured locations occurring secondary to vehicular accidents had low, but >0, fragmentation and displacement scores, although the whiskers on the box plot did span the entire range of (0–3) for several locations. The notable exception was the maxillary bone, in which the majority of cases had fragmentation and displacement scores closer to 2 (complete fracture and minimally displaced fracture, respectively). The majority of vehicular accident cases had fractures of the vomer, ethmoid, and presphenoid bones with whiskers spanning the entire severity score range, whereas no other trauma etiology displayed this pattern.

Animal Bites

Eleven of the 29 possible fracture locations affected by animal bites had fragmentation and displacement scores >0 in the majority of cases, whereas the remaining 18 locations were unaffected in the majority of cases. Again, the most exposed regions of the CMF skeleton (the mid to rostral mandibles and the midface) had higher severity scores in both fragmentation and displacement than did other less exposed regions.

Blunt Force Trauma

Only 5 of the 29 possible fracture locations affected by blunt force trauma had fragmentation and displacement scores >0 in the majority of cases, whereas the remaining 24 locations were unaffected in the majority of cases. Unlike for the other 3 trauma etiologies, the symphysis and the molar region of the mandible had fragmentation and displacement scores of 0 (they were unaffected) in the majority of cases.

Unknown Trauma

Only 4 of the 29 possible fracture locations affected by unknown trauma had fragmentation and displacement scores >0 in the majority of cases, whereas the remaining 25 locations were unaffected in the majority of cases. Unlike the other three trauma etiologies, the maxillary, incisive, nasal, and zygomatic bones had fragmentation and displacement scores close to 0 (they were unaffected) in the majority of cases.

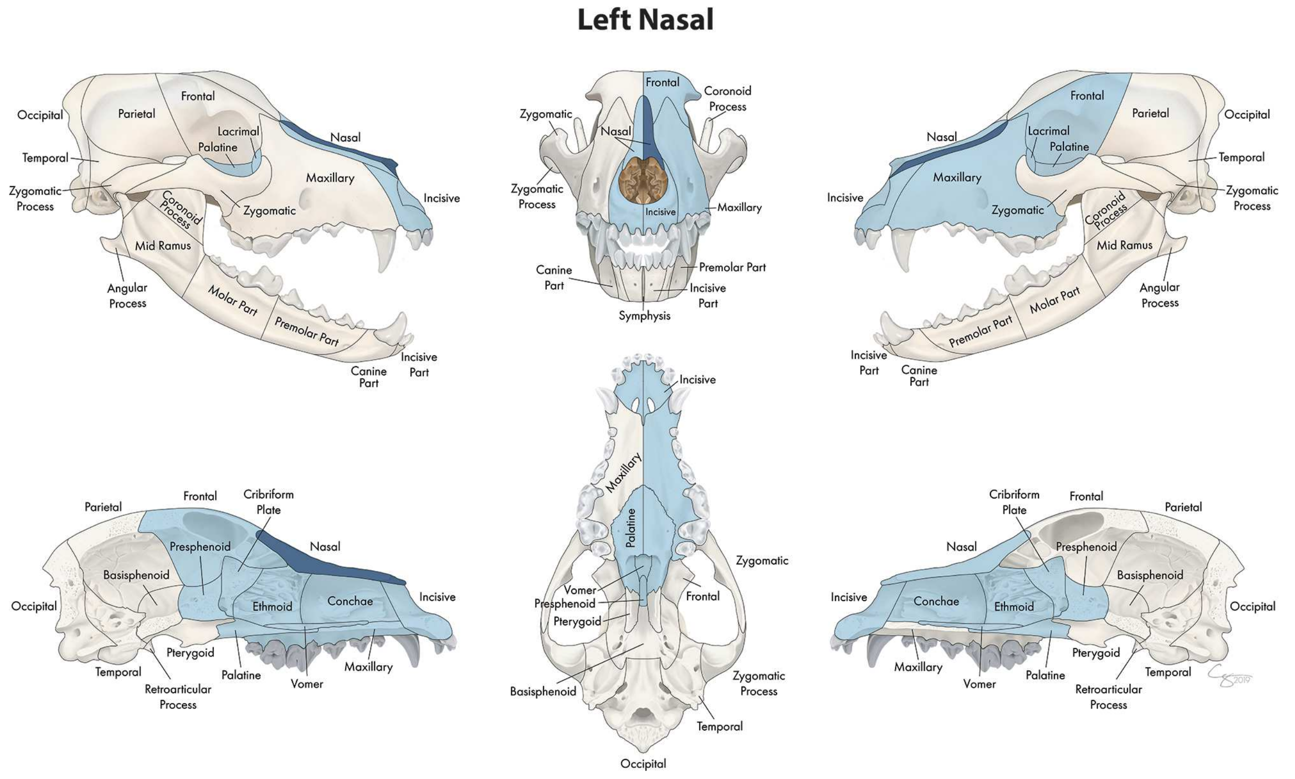

FIGURE 9 | Significant bones or regions fractured concurrently with the nasal bone. When the nasal bone was the region of interest, many other bones of the skull base/cranial vault and midface, both on the ipsilateral and contralateral side, fractured concurrently. Although the left nasal bone is shown, when the right nasal bone was the region of interest, a mirror image of concurrently fractured locations applied.

DISCUSSION

This study examines CMF trauma in dogs using CT as a diagnostic tool and further details the relationships between fracture location, morphology, and etiology. We report several key findings. First, the use of CT was instrumental in determining that spatially distant bones or regions fractured concurrently. Second, the bone fractures that occurred concurrently with fractured regions of the mandible, the orbit, the nasal cavity, and the maxilla are likely to be of clinical and prognostic significance. Finally, trauma etiology is associated with fracture morphology. As a result, the hypothesis that specific bones or regions would fracture concurrently with others, and that fracture etiology would impact the resultant fracture morphology for each location differently is accepted.

We demonstrated that in dogs affected by CMF trauma, there are often multiple bone fractures regardless of the etiology. This further signifies the importance of CT for complete and accurate diagnosis. As has been reported previously (13), skull radiographs typically underdiagnose the presence of fractures in maxillofacial trauma. In turn, this may have a significant impact on treatment plan and prognosis. For example, symphyseal separation is readily apparent on physical examination, and a clinician may make the erroneous assumption that this is the only injury in the absence of a CT. Importantly, our study showed that in cases of symphyseal separation, the cribriform plate is also fractured, which is highly relevant for treatment recommendations and prognosis. Although many human CMF trauma patients with CSF leaks heal without additional surgical intervention (14, 15), intensive monitoring for meningitis and other sequelae should be considered. It is reasonable to assume that similar recommendations are warranted in dogs.

In the mandible, there were fracture locations that co-occur, which are likely to be clinically relevant for treatment planning. Specifically, the molar part of the mandible fractures with the ramus of the mandible, whereas the premolar part is more likely to fracture with the canine and incisive parts. This suggests that fractures of these two regions may require different fixation strategies. For example, fractures of the molar part of the mandible typically do not have enough substantial teeth caudal to the fracture line to support an interdental wire and composite splint (16). Therefore, internal fixation emerges as a better option. However, if the ramus of the mandible is also fractured, and especially if it is fractured in multiple locations, placing internal fixation is more challenging owing to the very thin nature of the bone and overlying soft tissue (17). In addition, even if identification of concurrently fractured regions does not result in an immediate change in fixation strategy, it may necessitate the need for future monitoring of tooth vitality given that the mandible is largely a tooth-bearing bone.

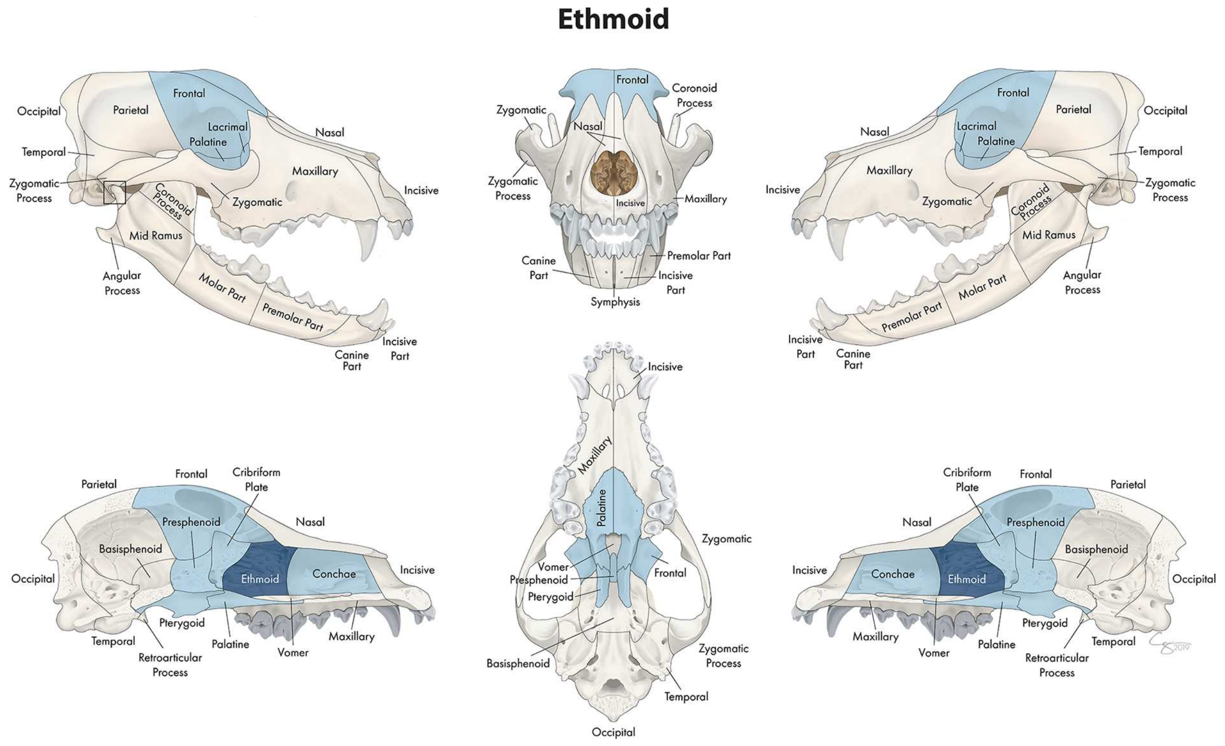

FIGURE 10 | Significant bones or regions fractured concurrently with the ethmoid bone. When the ethmoid bone was the region of interest, the conchae, cribriform plate, presphenoid bone, and bilateral pterygoid, frontal, lacrimal, and palatine bones fractured concurrently.

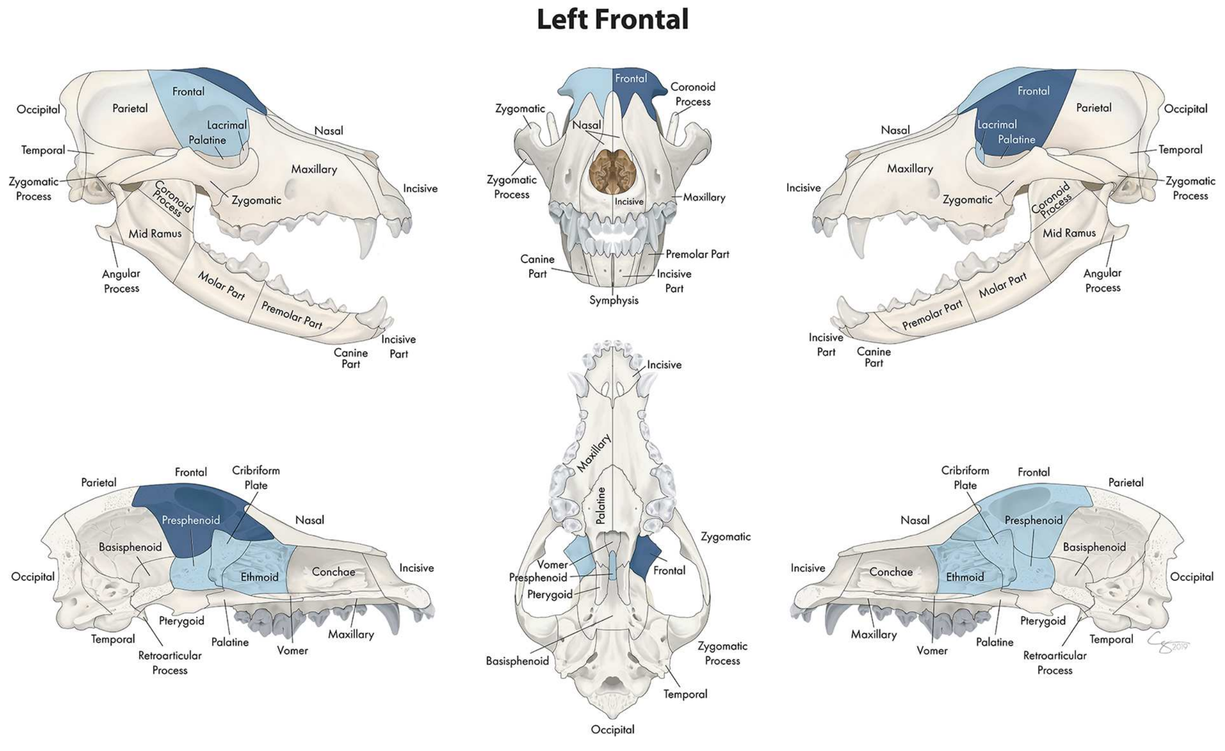

FIGURE 11 | Significant bones or regions fractured concurrently with the frontal bone. When the frontal bone was the region of interest, the cribriform plate, ethmoid bone, presphenoid bone, ipsilateral lacrimal bone, and contralateral frontal bone fractured concurrently. Although the left frontal bone is shown, when the right frontal bone was the region of interest, a mirror image of concurrently fractured locations applied.

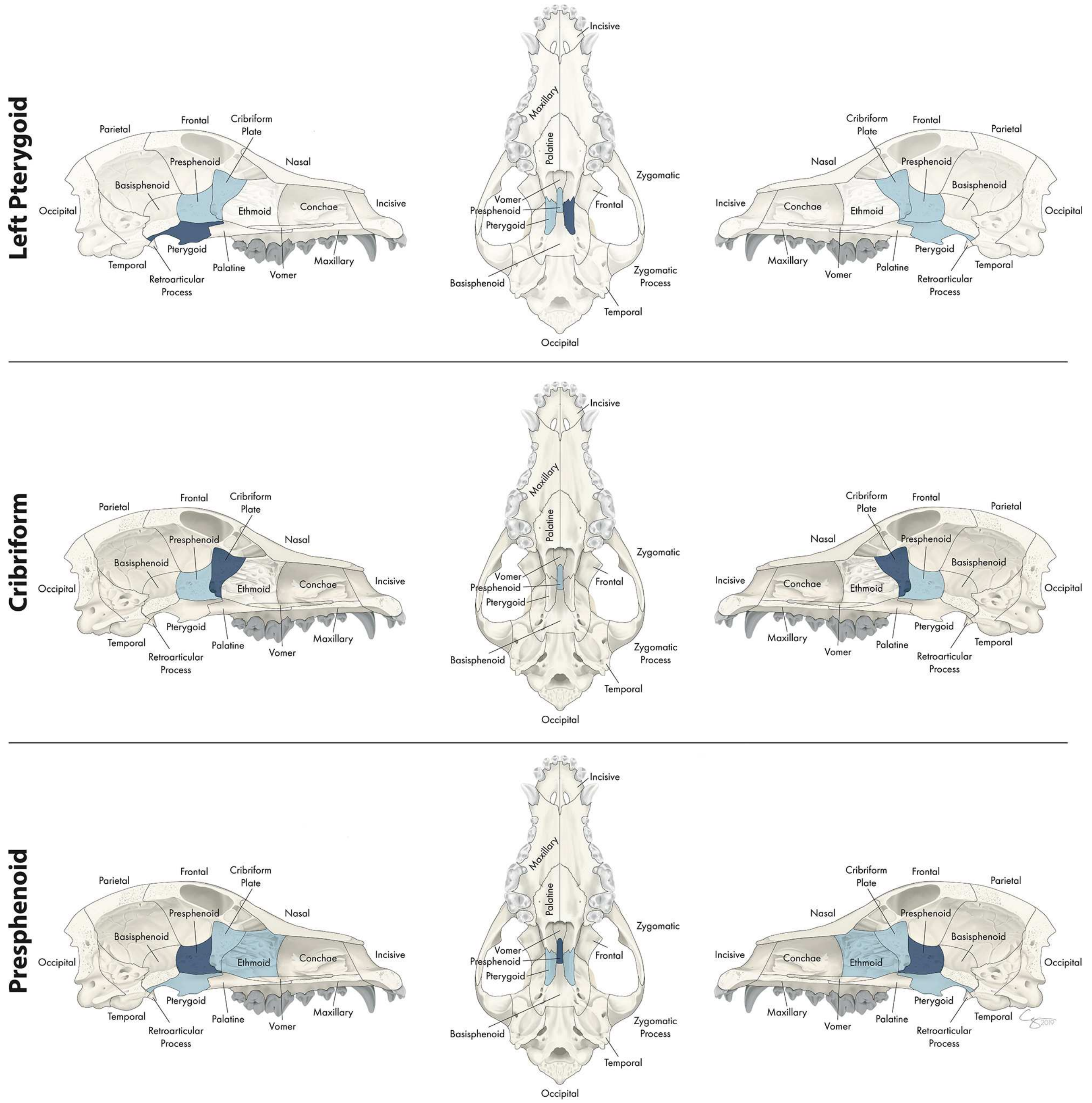

FIGURE 12 | Significant bones or regions fractured concurrently with the pterygoid, cribriform plate, and presphenoid bone. When the pterygoid bone was the region of interest, the cribriform plate, presphenoid bone, and contralateral pterygoid bone fractured concurrently. When the cribriform plate was the region of interest, the presphenoid bone fractured concurrently. When the presphenoid bone was the region of interest, the cribriform plate, ethmoid bone, and bilateral pterygoid bones fractured concurrently. Although the left pterygoid bone is shown, when the right pterygoid bone was the region of interest, a mirror image of concurrently fractured locations applied. Only views of the skull that demonstrate a fractured region are shown.

When examining the concurrently fractured locations with the zygomatic bone, it was apparent that the various skeletal structures comprising the orbit tend to be affected simultaneously. The zygoma, frontal bone, and lacrimal bone, which together form the majority of the orbit, (12), all tend to be affected. As is well-established in humans, reconstructing the orbital dimensions has significant clinical and cosmetic implications. While the cosmetic implications are not a primary goal in veterinary medicine, clinical implications such as diplopia, muscle entrapment, and impingement

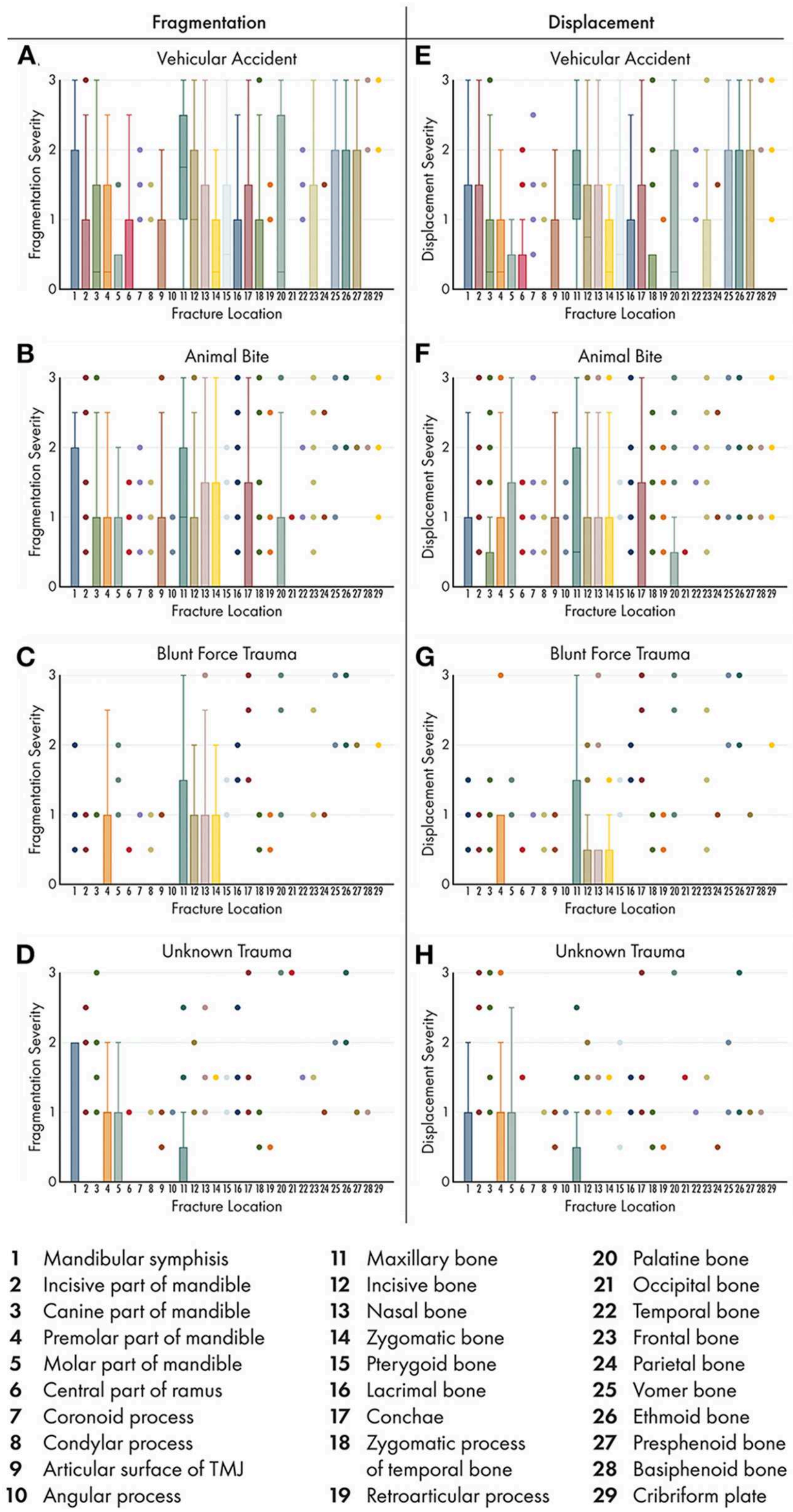

FIGURE 13 | Box and whisker plots of fracture morphology by trauma etiology and fracture location. Box and whisker plots were used to demonstrate the severity of fragmentation **(A–D)** and displacement **(E–H)** of fractures occurring at each location based on trauma etiology. As in part I of this study, only those trauma etiologies that occurred in >20 cases were included for analysis. Boxes and whiskers represent the majority of the fractures recorded. Colored dots indicate outliers in the data. Therefore, locations with only colored dots visible indicate that the fragmentation and severity score was 0 (the locations were not fractured) the majority of the time. No attempt was made to determine significance in patterns across data.

on neurovascular structures are clinically important. This underscores the need for thorough examination all of bones concurrently with appropriate imaging. Although dogs, lacking an orbital floor equivalent to that in humans, are less prone to muscle entrapment and similar clinical complications, fixation should nonetheless focus on retaining/reconstructing the orbit as much as possible. In addition, involving an ophthalmologist in the case when there are fractures of the orbit may be an important step in patient management.

Fractures of the conchae, vomer bone, and nasal bones tended to co-occur. Importantly, nasal bone fractures may be amenable to and may even require fixation to help reconstruct the nasal cavity to maximize airflow and prevent formation of a sequestrum or other complication (17). Although the conchae and vomer bones may not be surgically addressed when fractured, the presence of fractures in this region is important to diagnose and monitor, as it can predispose the patient to chronic rhinitis, fungal disease, or stenosis. In some cases, surgical exploration and stenting of the nasal cavity is indicated to prevent these complications (18, 19). Therefore, finding that the conchae and vomer tend to fracture concurrently with the nasal bone has clinical significance in that if a nasal bone fracture is identified, the underlying bones and deeper structures should be examined to optimize treatment.

As was demonstrated in Part I of this study, the maxillary bone was the most commonly fractured location regardless of trauma etiology. In this part of the study, we found that the maxillary bone also tends to fracture concurrently with the highest number of other bones or regions, including the midface, skull base, and cranial vault. Therefore, when a maxillary fracture is noted on physical examination or diagnostic imaging, this should prompt the clinician to thoroughly evaluate not only neighboring bones but also those in distant locations. Interestingly, the mandibles and maxillary bones did not tend to fracture concurrently in the majority of cases, but this should not preclude complete evaluation of both regions.

We found that trauma etiology is also associated with fracture morphology in dogs, which is consistent with the human medical literature (1, 20, 21). In our study, severely fragmented or displaced fractures were less common overall than those that were non-displaced. However, vehicular accidents, which often involve a higher velocity impact than the other trauma types examined, resulted in fractures with a higher degree of displacement and fragmentation. The potential importance of this for veterinarians lies in the need to properly visualize all fragments and their spatial relationship. Tridimensional (3D) imaging is especially important in these cases so that surgical planning can take into account the relative locations, sizes, and shapes of fracture fragments. In the human literature, it is well-accepted that 3D imaging (2, 22, 23) is superior for treatment planning related to CMF trauma and is commonly being used for intraoperative visualization as well (24).

The limitation of this study is inherent to its retrospective design. In addition, the dogs included in this study were assessed at a tertiary referral institution, which could have affected the types of CMF trauma included in the study. For example, very mild cases may not have been referred to our institution if the primary veterinarian felt capable of treating the patient. Likewise, very severe cases may have died or been euthanized prior to referral. Because several of the trauma etiologies (crush injuries, fall from height, and ballistic traumas) occurred infrequently, the sample size for those etiologies was too small to draw any conclusions from the associated data. Although not specifically addressed in this study, documentation and treatment of dentoalveolar trauma is of clear importance when caring for any CMF trauma patient and has been thoroughly discussed elsewhere (8). A limitation of particular relevance to part II of this study is that skull conformation was not specifically included as a variable. For example, it is possible that concurrent fracture locations in brachycephalic dogs may be different than those in dolichocephalic dogs. However, precise determination of skull conformation requires measurements between fixed points in the CMF skeleton which are inherently disrupted and potentially distorted when fractured.

In conclusion, this study further elucidates the relationships of fracture etiology, location, and morphology in dogs that sustained CMF trauma. We highlight the importance of CT evaluation of the entire CMF region in CMF trauma in dogs. In addition, this research underscores the need for thorough systemic evaluation of CMF trauma cases to ensure that there are not more pressing concurrent injuries (e.g., traumatic brain injury) that require immediate treatment. Finally, we laid the foundation for future studies to address classification of CMF fractures and trauma. As with development of the human AOCMF classification system (6), developing a classification system in dogs will be an iterative process and will require multi-institutional cooperation for validation. Therefore, a classification system was not proposed based solely on the results of this study. However, it is important to note that although certain bones or regions do tend to fracture concurrently, there are a large number of bones which fracture independently, and this finding must be accounted for when developing possible classification systems.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be made available from the authors upon request.

ETHICS STATEMENT

Ethical review and approval was not required for the animal study because the study is retrospective in nature and included clinical cases; hence, it is exempt from IACUC requirements. Written informed consent for participation was not obtained from the owners because the study is retrospective in nature and hence, it is exempt from written informed consent.

AUTHOR CONTRIBUTIONS

MD: study concept and design, image analysis, data acquisition, analysis, interpretation, drafting of the manuscript, and final approval of the version to be published. RP: study concept and design, image analysis, drafting of the manuscript, and final approval of the version to be published. BA and FV: study concept and design, data interpretation, drafting of the manuscript, and final approval of the version to be published. PK: data analysis and interpretation, drafting of the manuscript, and final approval of the version to be published.

ACKNOWLEDGMENTS

We would like to thank Chrisoula Toupadakis Skouritakis for providing the illustrations and images used in this study. We would also like to thank Daria Farkondeh for her assistance with coding demographic data from the medical records.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Veterinary Science

ORIGINAL RESEARCH
published: 13 August 2020
doi: 10.3389/fvets.2020.00506

Variability in Recommendations for Cervical Lymph Node Pathology for Staging of Canine Oral Neoplasia: A Survey Study

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There is no clear guideline regarding the indication for routine lymph node extirpation and pathologic evaluation during staging of canine oral tumors, despite a relatively high reported nodal metastatic rate for select tumor types. It is particularly unclear if clinicians recommend removal of lymph nodes only when there is confirmation of metastasis, defined as the N+ neck, or if elective neck dissection (END) is routinely recommended to confirm the true pathologic metastatic status of lymph nodes in the clinical N0 neck (no evidence of metastasis on clinical staging with diagnostic imaging or cytology). When clinicians are recommending END as a staging tool to confirm nodal status, there is also ambiguity regarding the surgical extent for subsequent histopathologic evaluation. The objective of this cross-sectional survey study was to determine the current recommendations given by practicing specialists regarding lymph node removal for dogs with oral tumors. Overall, 87 responses were obtained from 49 private practices (56%) and 38 academic institutions (44%). Respondents identified as oncologists (44%, $N = 38$), soft tissue surgeons (40%, $N = 35$), and dentists (16%, $N = 14$). Regardless of tumor type and stage, extirpation and histopathology were most commonly recommended in the clinical N+ neck only. The recommendation to routinely perform END in the N0 neck was significantly associated with tumor type. Bilateral removal of the mandibular and retropharyngeal lymph nodes was recommended more often for oral malignant melanoma (OMM) than for oral squamous cell carcinoma (OSCC; $p \leq 0.0039$) or for oral fibrosarcoma (OFSA; $p \leq 0.0007$). The likelihood of recommending END increased with increasing tumor size. Academic clinicians were significantly ($p < 0.01$) more likely to recommend END compared to private practitioners for canine T1–T3 OMM, T3 OSCC, T2 OFSA, and MCT. This study highlights the variability in recommendations for lymph node pathology for dogs with oral tumors. While tumor type and size influenced the decision to pursue END, it was not routinely recommended, even for tumor types with a known propensity for metastasis. Prospective studies are warranted to determine the potential diagnostic and therapeutic value of END in the N0 neck in veterinary patients such that a consensus approach can be made.

Keywords: lymph node (LN), lymphadenectomy, oral tumor, staging, neoplasia, biologic behavior

OPEN ACCESS

Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Veterinary Dentistry and Oromaxillofacial Surgery, a section of the journal Frontiers in Veterinary Science

Received: 03 May 2020
Accepted: 03 July 2020
Published: 13 August 2020

Citation:
Congiusta M, Lawrence J, Rendahl A and Goldschmidt S (2020) Variability in Recommendations for Cervical Lymph Node Pathology for Staging of Canine Oral Neoplasia: A Survey Study. Front. Vet. Sci. 7:506. doi: 10.3389/fvets.2020.00506

INTRODUCTION

Tumor staging including the evaluation of locoregional draining lymph nodes for metastasis is a critical step in the oral oncologic work-up. For tumors of the head, presence of lymph node metastasis can affect treatment recommendations and may negatively impact outcome (1–7). Arguably, failure to properly identify the lack of lymph node involvement could also result in unnecessary treatment that fails to improve outcome. Lymphatic drainage from the head can be complicated making prediction of the most likely lymphocenter for metastasis challenging (3, 8–11). Furthermore, relying on lymph node size alone has been shown to be unreliable for accurate identification of metastatic disease, with palpably normal nodes commonly harboring metastasis (12, 13). While the mandibular, retropharyngeal and parotid lymphocenters represent the major draining sites for oral and maxillofacial tumors, the mandibular lymph nodes (MLN) are the most peripherally accessible for fine needle aspiration (FNA) and therefore the most commonly sampled (14). Not only may the tumor metastasize to nodes other than the MLN, but dogs can have multiple MLNs and it is not clear that all are evaluated during staging (3, 14). Importantly, assessment of only one MLN is unreliable at ruling out locoregional metastasis (8, 11). Similar to assessment of size, imaging alone is insufficient for prediction of metastasis, even with the use of computed tomography (CT), and is not recommended as a reliable tool without concurrent cytologic or histopathologic evaluation (15). Ultrasound or CT guidance may aid in the cytologic sampling of the less accessible retropharyngeal and parotid lymph nodes (RLN and PLN, respectively), depending on operator experience and comfort (10). Conversely, several veterinary oncologic studies have shown that cytology alone also lacks sensitivity and specificity in the evaluation of potential metastasis to regional lymph nodes (3, 16, 17). Thus, histopathologic evaluation of the draining regional lymph nodes at risk for metastasis remains the gold standard diagnostic staging tool for dogs with oral tumors (3, 8, 10, 11, 16, 17).

Similar concerns are noted for accurate detection of cervical metastatic disease in human oncology, with palpation, diagnostic imaging, and cytologic evaluation failing to replace elective neck dissection (END) for patients with the clinically negative (cN0) neck. The cN0 neck is defined as having no identified metastatic nodal disease with clinical staging utilizing palpation, diagnostic imaging, and/or cytologic sampling (18–20).

END by definition is removal of lymph nodes to confirm the true pathologic metastatic status in the clinical N0 neck. It may include ipsilateral or bilateral removal of the draining cervical lymphocenters, encompassing either a subset or all of the possible draining nodes. Conversely, therapeutic neck dissection refers to the removal of known metastatic lymph nodes (N+ neck), used to decrease tumor burden and improve discomfort associated with bulky nodal disease (21).

In early stage human head and neck tumors, where squamous cell carcinoma (SCC) is the predominant tumor type, an early recommendation was for END to be performed to confirm pathological node-negative disease (pN0) when the estimated risk of occult metastases from the tumor exceeds 20% (22). This risk analysis was based on the fact that pathologically detected occult metastases decreases 5 year survival by 50% (21, 23–26); undetected cervical metastasis therefore carries significant therapeutic and prognostic implications.

Human head and neck cancer data has identified tumor specific risk factors associated with cervical metastasis. Specifically, tumor location (tongue, floor of mouth), tumor size (>2 cm), and depth of invasion (>4 mm) have been repeatedly associated with an increased risk of nodal metastasis, leading to published guidelines outlining clear indications for END (21, 27–30). Additional data has shown that END reduces regional nodal recurrence and improves disease-specific survival for cN0 head and neck carcinoma, even for small tumors (20, 21, 23, 27, 30–35), and that up to 40% of patients without obvious nodal metastases at presentation will have occult metastases following dissection (36).

Critics of END in human oncology focus on the potential over-treatment argument and highlight that a large percentage of patients with clinically N0 neck assessment undergo unnecessary surgery, and it is not always clear that benefits outweigh the postoperative morbidity (21, 24, 26). Risks associated with END include prolonged anesthesia time, and postoperative changes including neck pain, fibrosis, reduced shoulder mobility and strength, and nerve damage, all of which can negatively affect patient quality-of-life measures (24, 32, 37, 38). As rapid advances in CT, MRI, and PET technology better detect nodal changes during cancer staging, it is possible that close observation with salvage neck dissection, defined as removing bulky metastatic disease at the time of nodal progression, may yield similar outcomes to END (24).

Sensitive methods for sentinel lymph node (SLN) mapping and biopsy, may also provide less invasive methods for some patients while still conferring the benefit of accurate staging with pathologic evaluation of the lymph nodes. SLN mapping involves identification of the first draining lymph node from the oral tumor, termed the SLN, and histopathologic evaluation in order to predict the status of the entire cervical region. SLN mapping techniques have been validated to accurately predict the entire lymphatic basin over 90% of the time, and have been shown to be a reliable staging tool for early stage human SCC (39–41). Studies directly comparing disease free survival time between SLN mapping with biopsy vs. END for the cN0 neck suggest SLN mapping may be a promising substitute in some circumstances to END (41–43).

Contrary to humans, tumor specific risk factors for cervical metastasis are not clearly defined in dogs; thus, it is difficult to understand if common guidelines lead clinicians to recommend

Abbreviations: SLN, Sentinel Lymph Node; RLN, Retropharyngeal Lymph Node; MLN, Mandibular Lymph Node; PLN, Parotid Lymph Node; END, Elective Neck Dissection. In this survey END included bilateral removal of RLN, MLN, and PLN, bilateral removal of RLN and MLN, or ipsilateral removal of MLN and RLN; cN0 neck, Cervical lymph nodes have staged clean with clinical assessment performed by palpation, diagnostic imaging, and/or cytology; pN0 neck, Cervical lymph nodes are confirmed to not have metastatic deposits by pathologic evaluation; N+ neck, Cervical lymph nodes have been shown to be metastatic or are highly likely metastatic based on clinical assessment with palpation, diagnostic imaging, and/or cytology; OMM, Oral Malignant Melanoma; OSCC, Oral Squamous Cell Carcinoma; OFSA, Oral Fibrosarcoma; MCT, Mast Cell Tumor.

END as a component of thorough staging protocol for oral tumors. Several recent studies have highlighted efficient and thorough methods for END that permit surgical staging for regional metastasis (10, 11, 44–46); but, a lack of clarity exists regarding which techniques clinicians are utilizing, and when they are recommending END, given the potential morbidity associated with this procedure. Possible surgical complications associated with END include seroma formation, infection at the lymphadenectomy site, and laceration of major vessels in the area, all of which could significantly impact the quality of life of the patient. Although, most complications are anecdotally reported as rare, the exact complication rates associated with END in dogs are not reported in the literature (10, 11, 44–46). Consideration also needs to be given to the additional surgical skill and time required to perform this procedure, as well as the associated cost to the client for additional surgery that may not result in a difference in overall survival time (46). Lastly, and potentially most impactful, the true prognostic effect of cervical metastasis on median survival time is unknown due to inconsistencies in both staging methodology as well as long term oncologic surveillance in canines. Thus, it is unclear at this time, if the benefit of END to confirm true pathologic status of the cervical lymph nodes will result in improved disease-free or overall survival, as it does in humans, where outcome is often evaluated in 3 or 5 year increments.

Standardization of approach for pathologic evaluation of cervical lymph nodes will minimize differences between studies regarding metastatic rate and outcome, thereby improving current knowledge to alter care paradigms and allowing evaluation of tumor-specific risk factors for cervical metastasis. Therefore, the purpose of this study was to assess current practice amongst veterinary specialists who routinely stage and treat canine oral tumors. The goal was to determine the variability in staging and therapeutic recommendations currently made for common canine oral tumors. We hypothesized that specialists would recommend END in oral malignant melanoma (OMM) and large (>4 cm) oral squamous cell carcinoma (OSCC) more frequently than for other tumors, based on the current body of veterinary literature on metastatic risks.

MATERIALS AND METHODS

Survey Questions

A link to an electronic questionnaire formulated in Microsoft Outlook (available in **Appendix 1**) was provided to members through distribution via the email list server (listserv) for the American Veterinary Dental College (AVDC), the Veterinary Society of Surgical Oncology (VSSO), the American College of Veterinary Surgeons (ACVS), the American College of Veterinary Internal Medicine-Oncology (ACVIM-Oncology), and the American College of Veterinary Radiology-Radiation Oncology (ACVR-Radiation Oncology). The survey was accessible for a 4 month duration, and three reminders were emailed to request completion. This study was exempt from review by the University of Minnesota Institutional Review Board (IRB).

TABLE 1 | Lymph node recommendation for tumor type and stage: guidelines when answering the survey.

1. Please indicate lymph node recommendations for the following oral neoplasms using the WHO TNM Classification scheme
2. For all staging questions regarding tumor size (T1–T3), assume there is no evidence of distant metastasis
3. For all patients, assume no patient has a concurrent comorbidity and that each client is not concerned about finances to provide optimal treatments to best manage clinical symptoms of their animal
4. While there is no clear consensus statement on proper lymphadenectomy procedure guidelines, please provide answers that fit with your strongest recommendation

TABLE 2 | List of possible survey responses.

1	All	Recommend removing retropharyngeal, mandibular, and parotid lymph nodes bilaterally regardless of normal appearance on diagnostic imaging
2	All	Recommend removing retropharyngeal and mandibular lymph nodes bilaterally regardless of normal appearance on diagnostic testing
3	All	Recommend removing retropharyngeal and mandibular lymph nodes ipsilaterally regardless of normal appearance on diagnostic imaging
4	All	Recommend removing a regional lymph node only if it is suspicious on diagnostic imaging and/or suspicious/positive for metastasis following cytologic assessment
5	All	Other
6	Stage 4 only	Recommend removing the regional lymph node(s) depending on the goals of the client and on the clinical signs and/or quality of life of the pet (i.e., palliative radiation therapy to primary tumor/regional LN and/or medical management may be elected in lieu of surgery)

Responses were listed as multiple choice options (1-5) that pertained to all tumor questions. For stage 4 dogs, respondents were also provided with an additional option (6).

The questionnaire requested that respondents follow guidelines (**Table 1**) and make particular assumptions prior to taking the survey to permit standardized results. Tumor types queried with respect to clinical staging were limited to OMM, OSCC, and oral fibrosarcoma (OFSA). Questions regarding tumor stage utilized the World Health Organization staging scheme describing the primary tumor (T), status of regional lymph nodes (N) and presence or absence of distant metastasis (M) (47). Specifically, T1 referred to tumors $\leq$ 2 cm in diameter, T2 referred to tumors 2–4 cm in diameter and T3 referred to tumors >4 cm in diameter (47).

OSCC, OMM, OFSA was queried separately for T1–T3 as well as stage 4. All questions pertaining to the T1–T3 tumor types had the same 5 multiple choice options to choose from **Table 2**. For stage 4 tumors, defined as presence of distant metastasis, respondents were also provided an alternative option regarding lymph node extirpation to clarify if they recommend removal if it aligns with the client goals and/or improves quality of life of the patient. For stage 4 tumors, it was assumed that distant metastasis was confirmed with diagnostic imaging, although this was not clearly defined within the survey.

Within the survey, END was defined as either bilateral removal of RLN, MLN, and PLN, bilateral removal of RLN and MLN, or ipsilateral removal of MLN and RLN when there was no evidence of lymph node metastasis on cytology or diagnostic imaging (cN0). Conversely, removal of a single node or nodes based on high suspicion or confirmation of metastasis was considered to be therapeutic, not elective, neck dissection.

Lymph node management for uncommon tumor types were queried via additional questions without regard to tumor size. Uncommon tumor types queried included osteosarcoma, chondrosarcoma, and mast cell tumor (MCT). For osteosarcoma and chondrosarcoma, respondents were queried yes or no if their recommendations are the same for both these tumors as they are for OFSA. For MCT respondents were given the same 5 multiple choice options as common oral tumors (**Table 2**).

To gather information about alternative methods of cervical node management, the survey also asked respondents if SLN mapping was routinely performed for head and neck tumors and to define the protocol if one existed. This section on SLN mapping was free text to allow clinicians to specify the protocol used to identify the first draining lymph node from the oral tumor, which is presumed to be the sentinel lymph node (although the use of SLN biopsy to predict the metastatic status of the entire basin has not been validated in canines). Respondents were also asked to subjectively report how often the mapping technique accurately identified the first draining lymph node.

Additional information regarding subjective complications following END was also recorded. Specifically, respondents were asked to report approximate occurrence of postoperative seroma as stratified answers of: often (>75% of the time), frequent (50% of the time), rarely (<25% of the time), or other. Respondents were also queried about postoperative infection and given the same possible responses as for seroma.

Statistical Analysis

To assess overall differences in response by practice type (academia vs. private practice) and specialty, Fisher's test was performed using the algorithm in the fisher.test R function. This was done separately for each question. To follow-up statistically significant differences, pairwise Fisher's tests were performed, for each response separately against all others. For specialty, this was done pairwise between the three specialties, with the Bonferroni-Holm adjustment for multiple comparisons. Adjustment, however, was not performed across the possible different answers for each question. First, because the overall Fisher's test controls the overall Type I error, and second, because the responses are necessarily correlated (that is, if there are more of one response, there must be less of another). To assess overall differences in response between the three common tumor types (OMM, OSCC, and OFSA), a different method was needed, because these were repeated responses from the same individuals. Therefore, a permutation-based chi-squared test was used, with permutations formed by permuting the three responses within each respondent. This was done separately for (T1–T3), and 20,000 permutations were performed for each. To follow up statistically significant differences, pairwise McNemar's tests were performed, with p-values computed using the exact binomial test. Adjustment for multiple comparisons was performed similarly to the adjustments for the specialties. Lastly, for each possible response separately, pairwise McNemar's tests was performed between all 11 groups (T1, T2, T3 OMM, SCC, FSA as well as mast cell tumor and lymphoma) with p-values corrected using the Bonferroni-Holm method. For each of the 11, significant difference between responses was defined at the $p < 0.05$ level. This was repeated with responses 1–3 combined to represent recommendation for any form of END. All computations were performed with standard statistical software.[1]

RESULTS

Overall, 87 surveys were completed. Fifty-six percent (49/87) of the responses were from private practices and 43% (38/87) were from academic institutions. None of the survey responses were excluded from analysis. However, information on lymph node management for epitheliotrophic lymphoma as well as one survey question on SLN mapping were removed from analysis due to ambiguous wording and inconsistent responses (**Appendix 1**). Respondents identified as practicing oncology (44%, $N = 38$), soft tissue surgery (40%, $N = 35$), or dentistry and oral surgery (16%, $N = 14$). No respondent identified as a non-boarded specialist when queried on their specific specialty in the survey.

Lymph Node Recommendations for Common Oral Tumors

Clinicians most commonly recommend lymph node extirpation for histopathologic analysis only when there is clinical suspicion of metastasis (N+ neck), regardless of tumor type (**Figure 1**). Accordingly, the majority of clinicians do not routinely recommend END (**Table 3**) for cN0 oral tumors in dogs. When END is recommended, it is recommended more frequently for OMM compared to OSCC and OFSA for similar clinical stages (**Figure 2**).

Within this study population it was identified that when END is recommended, parotid lymph node extirpation is rarely included (**Figure 2**). Bilateral removal of RLN and MLN is more commonly recommended for OMM, while ipsilateral or bilateral extirpation is recommended with similar frequency for OSCC and OFSA. Notably, bilateral extirpation of the MLN and RLN in the cN0 neck is recommended significantly more frequently for OMM than for OSCC and OFSA, and more often ($p < 0.01$) for T3 OSCC than for T3 OFSA (**Figure 2**). Selective extirpation of only a suspicious or confirmed metastatic node is performed significantly more frequently ($p < 0.05$) for OFSA compared to OSCC and OMM, as well as OSCC compared to OMM ($p < 0.01$) (**Figure 2**).

As tumor size increased, regardless of tumor type, clinicians recommend END more often, although this difference was often

[1]R Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna (2019). Available online at: https://www.R-project.org/.

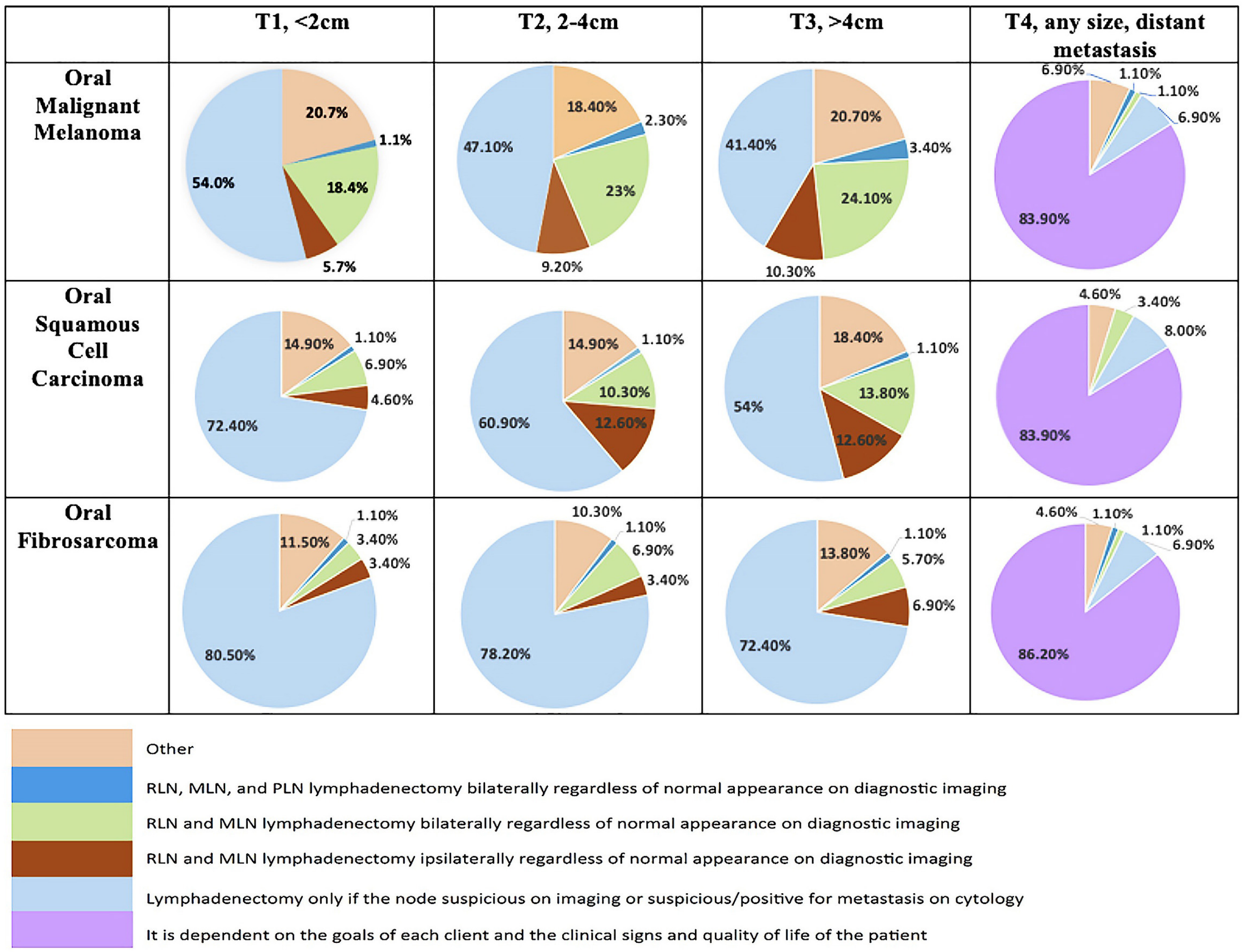

FIGURE 1 | All survey responses for clinician recommendations regarding performing lymph node pathology for common tumor types separated by stage.

TABLE 3 | Overall recommendations for any form of elective neck dissection (END) for common canine oral tumors (OMM, OSCC, OFSA) for clinically neck negative (N0) disease.

OMM			OSCC			OFSA		
T1	**T2**	**T3**	**T1**	**T2**	**T3**	**T1**	**T2**	**T3**
22 (25.2%)	30 (34.5%)	33 (37.9%)	11* (12.6%)	21 (24.1%)	24* (27.6%)	7 (8.0%)	10 (11.5%)	12 (13.8%)

*In this study, any form of END included bilateral removal of RLN, MLN, and PLN, bilateral removal of RLN and MLN, or ipsilateral removal of MLN and RLN. Data are presented as the number of respondents who elected END with the percentage of respondents in parentheses. *Significant ($p < 0.01$) difference between T1 and T3 OSCC.*

not significant (**Table 3**). No significant difference was identified in the likelihood of END recommendation for dogs with OMM or OFSA with increasing tumor size. For OSCC, clinicians are significantly ($p < 0.05$) more likely to recommend END for T3 tumors compared to T1 tumors.

Regardless of tumor type, once there is evidence of distant metastatic spread, END is only recommended in 2–4% of cases (2.2% OMM, 3.4% OSCC, 2.2% OFSA) (**Figure 1**).

Lymph Node Recommendations for Less Common Oral Tumor Types

Osteosarcoma and Chondrosarcoma

When queried as a yes or no response, 77% of respondents replied that they make the same recommendations for both osteosarcoma and chondrosarcoma (67/87) regarding lymph node extirpation for histopathologic analysis as they do for OFSA.

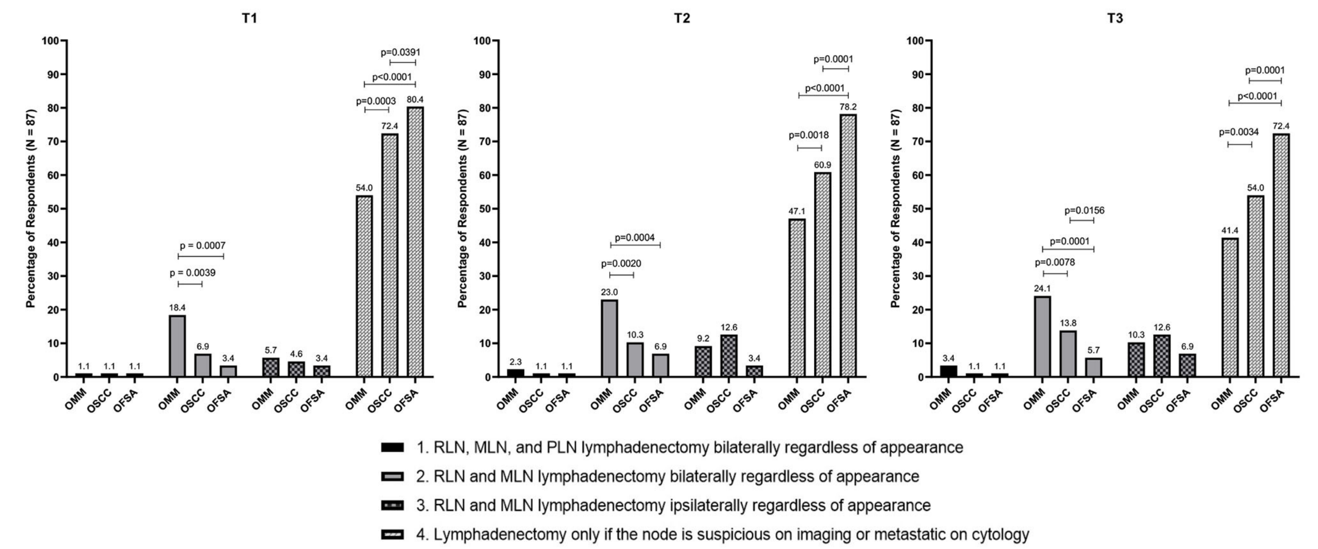

FIGURE 2 | Recommendation to perform END in the N0 neck vs. extirpation in the N+ neck only for T1–T3 OMM, OSCC, OFSA. Significant differences between specific recommendations per tumor type are highlighted.

Oral/Labial Mast Cell Tumor (MCT)

Twenty seven percent of clinicians responded that END is recommended for dogs with oral MCT (**Figure 3**). There was no significant difference in the frequency of some form of END recommendation in dogs with MCT compared to T1–T3 OMM, T2–T3 OSCC, and T3 OFSA. When END is recommended, bilateral removal of the MLN and RLN is the most common (58.3%) surgical recommendation.

Use of SLN Mapping

Thirty-three percent (29/87) of respondents reported use of SLN mapping techniques for head and neck tumors. Twenty- one respondents reported the exact technique utilized, with some reporting more than one technique that is utilized at their institution. Reported techniques included CT lymphangiography +/– methylene blue at surgery (19/21), intra-operative use of methylene blue alone (1/21), lymphoscintigraphy (3/21), and intraoperative near infrared fluorescence lymphography (4/21). Of those that responded that they perform SLN mapping, the majority (66.7%) subjectively reported they are able to accurately identify the first draining LN (presumed SLN) >50% of the time. Specifically, 23.3% of respondents claimed a 50–75% success rate, and 43.3% reported a 75–100% success rate using their respective mapping protocols. Only 8 respondents reported they request additional pathologic information on the removed "sentinel lymph node." Reported written requests included: breadloafing (2/8) immunohistochemistry (2/8), polymerase chain reaction (1/8), and bivalving the SLN through the hilum (2/8). Kiupel mast cell tumor staging (1/8) was also listed, however due to the anonymous nature of the survey, the authors were unable to determine if this was meant to reflect a request for grading of the primary tumor, or if the respondent uses a unique lymph node evaluation method with criteria similar to the Kiupel grading system (48).

Complications With Elective Lymphadenectomy

The self-reported complication rate of seroma formation following END was low, with 6.9% reporting it is never seen and 47.1% reporting it is seen rarely (<25% of the time). The remainder reported it was seen ∼50% of the time (19.5%), >75% of the time (16.1%), 100% of the time (3.4%) or reported the question was not applicable to them.

The self-reported infection rate following END was also low with 24.1% reporting this complication was never seen and 56.3% reporting it is seen rarely (<25% of the time). No respondents reporting that the complication was always or commonly (>75%) seen. The remainder reported that it was seen 50% of the time (2.3%) or that the question was not applicable to them.

Less common complications reported included facial swelling, regional edema, dehiscence, hemorrhage, and muzzle edema. Respondents were not queried on if they saw complications more commonly with specific forms of END compared to other surgical techniques.

Recommendation Differences Based on Practice Type or Specialty

Clinicians in academia were significantly ($p < 0.01$) more likely to recommend routine bilateral END (RLN and MLN) for T1–T3 OMM, T3 OSCC, T2 OFSA, and oral MCT compared to clinicians in private practice. Conversely, clinicians in private practice were significantly ($p < 0.01$) more likely to make recommendations for solitary lymph node extirpation based on suspicion of metastasis. Academic clinicians were significantly ($p < 0.01$) more likely to perform SLN mapping compared to

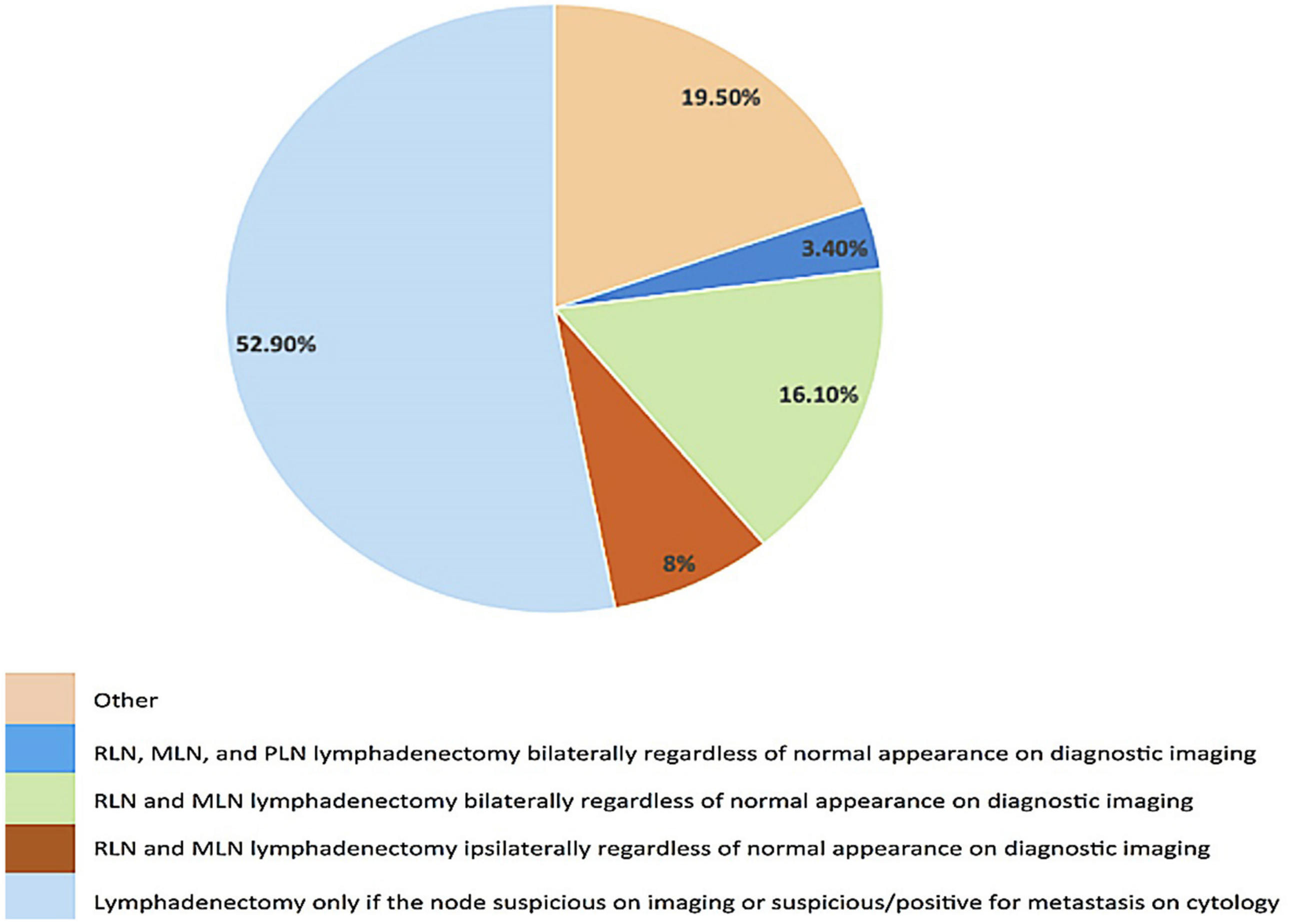

FIGURE 3 | Survey responses for clinician recommendations regarding performing lymph node pathology for oral/labial mast cell tumor.

private practices. No significant differences in recommendations between specialties were found for dogs with OMM, OSCC, OFSA, or oral mast cell tumors.

DISCUSSION

This is the first study to investigate current practice for the management of regional lymph nodes in dogs with oral tumors. There is no current consensus for lymph node management or neck dissection in the clinically negative (cN0) neck or in the metastasis-positive or suspected positive (N+) neck. The lack of standardized approach among board certified small animal oncologists, dentists, and surgeons to the determination of lymph node status prohibits the realization of the true prevalence of metastasis for canine oral tumors and evaluation of tumor specific risk factors for cervical metastasis. This study therefore sought to determine recommendations provided by specialty clinicians who routinely stage and treat canine oral tumors.

It was identified that clinicians are most likely to recommend bilateral END for OMM compared to other tumor types. However, a large proportion of clinicians (41–80% of respondents pending tumor type and stage) elect to remove only a suspicious or cytologically confirmed metastatic lymph node for dogs with oral tumors.

This finding was not surprising for canine OFSA given the low likelihood of lymph node metastasis (6, 49–51); indeed, even for T2 and T3 tumors, clinicians only recommend END in 11 and 14% of cases, respectively. Furthermore, the true prognostic effect of cervical metastasis with OFSA is elusive due to limited data. In one study of OFSA, only 3% of dogs had lymph node metastasis at diagnosis and it was not associated with outcome (51). A second study of OFSA in 29 dogs did not detect any nodal metastasis at diagnosis while local recurrence and distant metastasis were the documented causes of death (50). Overall, the risks of node extirpation unlikely outweigh any potential benefits. Similar to canine OFSA, respondents largely reported similar lymph node management for uncommon oral sarcomas such as osteosarcoma or chondrosarcoma.

Unexpectedly, only 25–38% of clinicians recommended END in the N0 neck for dogs with OMM, despite the high (up to 74%) propensity of these tumors to metastasize to regional lymph nodes, often to multiple lymphocenters (7–9, 11, 13, 17, 52). This was especially surprising given that existing literature suggests that lymph node size, imaging appearance, and cytologic evaluation are limited in accurately determining nodal metastasis for canine OMM (3, 8, 9, 13, 15, 17). It is possible that respondents have factored in several findings in their clinical management strategies, such as: the uncertainty in the true rate of cervical lymph node metastasis in canine OMM in part due to lack of standardized assessment, that the literature has not always shown lymph node metastasis to be a negative prognostic factor, and that distant metastasis is particularly problematic for many cases (7, 53–56). The risk of concurrent distant metastasis may also explain why END was not increasingly performed with advancing tumor size for OMM. It is well-documented that as tumor size increases, the risk of distant metastasis concurrently increases; thus, even with the lack of confirmed metastatic deposits in the thorax, the morbidity of performing lymphadenectomy may not be justified given the potential for decreased median survival time (7, 52). This is a controversy that surrounds human melanoma as well and forms the basis of the primary argument against END for staging human head and neck cutaneous melanoma. This argument stems around the "marker hypothesis," which supposes that the presence of metastatic deposits in the cervical lymph node(s) act only as a marker for distant dissemination and removal of the tumor burden in the nodal bed carries no therapeutic value itself (57, 58). Thus, END used solely for staging and prognostic purposes, without a positive effect on survival, cannot be justified in the face of its associated morbidity; this may be particularly relevant when considering tumors like metastatic melanoma that are challenging to treat systemically (57, 58).

Recommendations made for OSCC were in accord with the majority of available veterinary literature suggesting overall low rates (9–13%) of nodal metastasis at diagnosis (2, 6, 8, 10) and increasing risk of metastasis with larger tumors (5, 59, 60). Notably, clinicians were significantly more likely to recommend END for T3 tumors compared to T1 tumors. Although the recommendation for END increased as tumor size increased, within each T stage, clinicians were still more likely to recommend bilateral removal of the MLN and RLN for dogs with OMM compared to OSCC. This recommendation mirrors the reported biologic behavior of these two tumor types. However, when looking at a recommendation for any form of END (not just bilateral MLN and RLN removal), there was no significant difference in the likelihood to recommend END for a T2/T3 OSCC or OMM. This suggests that clinicians feel that larger OSCC may have a higher nodal metastatic rate. Indeed, a recent study did not identify significantly different percentages of cervical metastasis in dogs with OMM and OSCC following histopathologic evaluation (8). Notably, the majority of dogs with OSCC in that study had T2 or T3 tumors but none had suspicion of nodal metastasis with staging; however dogs with tonsillar SCC were also included in the study cohort, which impacted the reported metastatic rate (8). In a separate case series that studied efficacy of piroxicam and carboplatin in dogs with T3 non-tonsillar OSCC, all dogs had nodal metastasis (61). The identification of nodal metastasis is clinically significant for OSCC, as literature has reported that median survival time is reduced by 67–90% when nodal metastasis is present (1, 6). Furthermore, unlike melanoma, there is stronger evidence to suggest that carcinoma stalls in the lymphatic channels prior to hematogenous dissemination, which supports that lymphadenectomy may be therapeutic in addition to prognostic (21, 34). Recent literature surrounding T2–T3 OSCC in canines suggests that routine END might be justified, although its prognostic and therapeutic value requires further investigation.

Interestingly, despite the paucity of literature on oral MCTs (4, 62), respondents were equally as likely to recommend END (27.5%) for dogs with oral/labial MCTs as they were for OMM (approximately 32.5% overall). Unlike OMM, where distant metastasis poses a significant clinical challenge (5, 7, 52, 63), literature supports that adequate local control of the primary tumor and regional nodes is the most important facet for long-term control for dogs with MCTs (54–56). Thus, it was surprising that given the high propensity for oral MCT to metastasize to lymph nodes and the significant effect of nodal metastasis on median survival time, that END is not recommend more often for the N0 neck (54–56). Rather, in 53% of cases, nodes are extirpated only if suspicious for metastasis on imaging or cytologically confirmed as metastatic.

Overall regardless of tumor type, clinicians are most likely to recommend lymph node extirpation only if there is clinical suspicion or cytologic confirmation of metastasis. The survey was not designed to assess subsequent clinical decision making, so it could not accurately capture the frequency at which recommendations changed upon clinical staging, or what served as rationale behind this recommendation. Specifically, the survey asked that respondents choose between recommending removal of a lymph node only where there is suspicion of metastasis (N+) or various forms of END in the cN0 neck (or other); it was therefore challenging to capture clinical cases where, following identification of a suspicious lymph node during staging, END was performed to remove other cervical nodes at risk. Furthermore, the survey was not designed to determine if radiotherapy was planned in order to achieve local control following identification or confirmation of a suspicious lymph node, rather than surgical extirpation.

What was clear from the survey results, however, is that END is not routinely recommended in the cN0 neck for dogs with oral tumors. This may be due to the morbidity associated with lymphadenectomy without a clearly defined benefit in survival times, or a lack in understanding surrounding the importance of examining lymphocenters other than the MLN (8, 11).

Of the subset of respondents that routinely recommend END, the majority recommend bilateral removal of the RLN and MLN for OMM, while bilateral and ipsilateral removal of MLN and RLN is recommended with similar frequency for OSCC and OFSA. The canine literature points out the risk of lymph drainage in the head occurring on the contralateral side in up to 62% of cases (8, 10, 11). Thus, for complete evaluation of cervical metastasis, removal of the lymph nodes bilaterally should be performed especially if the tumor is at midline (8, 9, 45). There may be a propensity for tumors at certain locations to spread only ipsilaterally, but further evaluation of metastatic lymphatic patterns in the dog are required to identify when ipsilateral lymphadenectomy may be more appropriate. Until more robust data on lymph node metastatic drainage patterns is available, it seems prudent to perform bilateral RLN and MLN for high risk oral tumors (9, 10, 45).

It is also important to note, that dogs can have differing numbers of mandibular nodes (range of 2–4 is most common) as well as both a medial and lateral retropharyngeal lymph node (14). The survey was not designed to specifically question how clinicians manage aberrant anatomy. Ideally, CT scan with IV contrast should be performed prior to END to highlight aberrant anatomy and guide surgical planning. Failing to remove all possible draining lymph nodes for pathologic evaluation may hinder END performance in the cN0 neck, as the first draining node of the lymphatic basin may not be pathologically evaluated. In the future, the use of SLN mapping techniques may help highlight which node(s) are most important to sample for staging purposes.

Of respondents performing END, the self-reported complication rate was low, with the majority reporting infection and seroma as potential complications. While the survey was not designed to address specific complications and severity, in retrospect, a critical flaw was that the survey did not differentiate transient cervical edema from true seroma formation. This potentially overestimates the reported frequency of seroma formation and underestimates cervical edema formation in our study. Furthermore, the questions surrounding complications also queried oncologists, which may not be an accurate reflection of the complication rate as they most likely are not overseeing the follow up for these patients postoperatively. Additional study is needed to characterize adverse events associated with END procedures, particularly as seroma and postoperative infection cause discomfort and may delay adjuvant therapy in some dogs. For example, postoperative seroma may delay the start of treatment or require adaptive planning (re-planning due to tissue changes in the radiation field) (64, 65). Decreases in seroma volume or resolution of a postoperative abscess after CT simulation for radiation planning and/or during treatment negatively affect the quality of the radiation plan and may significantly increase the volume of and dose to normal tissue if uncorrected, thus increasing the likelihood that adverse events will develop (65, 66). While adaptive planning techniques are routine in some human radiation oncology departments, the use of adaptive planning in veterinary oncology is not yet standard and carries financial and time commitment implications for pet owners.

Given the infrequency in which END is currently recommended, and the potential for morbidity with neck dissection, it raises the question of whether or not the potential benefit of END is worth the associated morbidity in the canine N0 neck.

This same argument exists in human literature, where the true necessity of END still remains uncertain for early stage disease OSCC, despite updated meta-analyses and guidelines (21, 22, 24, 32, 36, 67). In favor of END is literature that has shown that occult metastases can be detected in 10–45% of cases despite a N0 clinical assessment and the fact that the presence of nodal metastasis carries clear prognostic implications decreasing 5 year survival by up to 50% (21, 23–26). Based on this, it has been historically recommended that END is performed when the cervical metastatic risk of the tumor exceeds 20%. However, in opposition to END is the fact that this means that up to 80% of patients are receiving unnecessary neck dissection, which can be associated with significant morbidity. Due to the controversy surrounding END, SLN mapping, and biopsy was introduced and is regarded as an alternative staging method to END with similar long-term survival outcomes (41).

In this study, 30% of respondents reported that they routinely recommend and perform SLN mapping techniques in canines to identify the first draining lymph node for oral tumors. This low percent was unsurprising since SLN techniques have not been standardized or validated in veterinary medicine. While a comprehensive review of SLN techniques is beyond the scope of this study, the reader can be directed toward a recent review of promising methodologies (68). Briefly, SLN mapping techniques primarily include peritumoral injection of either iodinated contrast, methylene blue dye, or radioactive isotopes and then evaluation of which node the injected material drains to first, utilizing either preoperative diagnostic imaging, a gamma probe, or visual detection surgically. This first draining lymph node is referred to as the SLN, which has the potential to predict the status of all other nodes in the basin; thus, negating the need for END for confirmation of pathologic N0 status. In canines, several mapping techniques to identify the first draining lymph node in the neck have been reported (68–70). However, in dogs, it is not yet clear if pathology within the first draining lymph node accurately reflects the remainder of the lymphatic basin so further validation is needed for SLN mapping to replace END to confirm pN0 status.

One limitation of the survey study is that it did not clearly define what was meant by SLN mapping nor "success" of the SLN mapping procedure. Of those that responded they perform SLN mapping, 67% stated that they accurately identify the SLN the majority of the time, but it wasn't clear if success was defined by histopathologic assessment of metastasis as the gold standard. One key component to validating novel SLN mapping techniques requires histopathologic confirmation that the "sentinel node" identified accurately predicts the remaining lymph node basin, and thus can reliably be used in place of END. It is therefore essential to capture the false negative rate of SLN biopsy to validate the technique. This supports the establishment of recommendations for END as the gold standard for canine oral tumors at moderate to high risk of metastasis until validation of SLN techniques has been performed (71). Surprisingly, for those respondents who currently recommend or perform SLN mapping and biopsy, only 6% specify how they would like nodes to be evaluated histologically. One of the primary benefits of SLN mapping is that if only one or two nodes are submitted, more rigorous pathologic evaluation may be feasible. For example, thin serial sectioning of each node with immunohistochemistry (such as AE1/AE3) has been shown to significantly increase detection of occult metastases in humans (72–75), and these techniques may also be beneficial in canine tumors.

At our institution, we perform SLN mapping with CT lymphangiography followed by END (bilateral MLN and RLN) for tumors with a reported cervical metastatic risk of 20% or greater based on the current body of veterinary literature (2, 5, 7–11, 13, 17, 52, 59, 60), similar to early recommendations in human OSCC (22). Currently, we perform SLN mapping and END for T1–T3 OMM (excluding well-differentiated T1 melanomas with a mitotic index of 0–1), T2–T3 OSCC, and mucosal/oral mast cell tumors. However, the authors do acknowledge that the true cervical metastatic rate is not clearly defined and the treatment paradigm we have instituted will be updated as new literature is available.

It is our hope that with more data on the true metastatic risk of canine oral tumors and the prognostic effect of cervical metastasis, a more appropriate risk analysis and guideline for when to perform END could be adopted. By corroborating cervical metastatic status with factors such as tumor size, location, and pathologic features such as depth of invasion, mitotic index, and perineural invasion, specific risk factors may highlight dogs with oral tumors that most benefit from END approaches.

It is important to note that risk analysis comparing the benefit vs. risk of recommending routine END cannot be accurately performed within the canine population without knowledge of the true cervical metastatic rate of oral tumors as well as the prognostic implication of cervical metastasis. Further work needs to be done to characterize the rate of occult metastasis in the canine clinical N0 neck, document the rate of complications following END procedures, compare progression-free survival between END and observation or nodal irradiation, and determine the clinical case scenarios that unequivocally benefit from END.

As detailed, there were several limitations in the survey study, including inherent bias with self-reporting, low response rate which may have selected for clinicians with strong opinions for or against END, lack of definition or clarification of individual postoperative complications with END, lack of definition of a "successful" SLN mapping procedure, and the inability for respondents to rationalize their responses. However, it provides the first study to highlight the variability that exists across clinicians who stage and treat canine oral tumors. The recognition of this variability will hopefully encourage thoughtful protocols that improve our clinical decision-making.

CONCLUSIONS

This is the first study to describe variability in lymph node management across veterinary specialty practices that routinely stage and treat canine oral tumors. Current practice is such that the majority of specialists who stage and treat canine oral tumors remove one or several cervical lymph node(s) for histopathologic evaluation based on clinical suspicion of metastases (i.e., pathology only performed in the N+ neck). For the cN0 neck, observation is more commonly recommended than END, although select practitioners recommend END for T2 and T3 canine OMM and OSCC, as well as mucosal MCT, consistent with their known higher risk of local metastases.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

MC, JL, and SG: study concept and design, contributed to manuscript revision, and approved the submitted version. MC: data acquisition and initial draft of manuscript. AR: statistical analysis. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

We would like to thank the University of Minnesota for support of this project as well as our medical oncologists, Dr. Amber Wolf Ringwall, who reviewed the survey prior to dissemination.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2020.00506/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Veterinary Science

ORIGINAL RESEARCH
published: 13 August 2020
doi: 10.3389/fvets.2020.00482

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Temporomandibular Joint Gap Arthroplasty in Cats

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Temporomandibular joint (TMJ) ankylosis is defined as fibrous or bony fusion of the mandibular head of the condylar process and the mandibular fossa of the squamous part of the temporal bone. Ankylosis of the TMJ may be intraarticular, extraarticular, or both. The objective of this report is to describe the surgical planning, technique, and outcome of gap arthroplasty for extensive TMJ ankylosis in cats. Client-owned cats ($n = 7$) were examined clinically and surgical planning included the use of cone-beam computed tomography (CBCT) and tridimensional (3D) printed models. In six of the seven cats, temporary tracheostomy intubation was required. Gap arthroplasty included zygomectomy, coronoidectomy, condylectomy, as well as fossectomy (removal of the mandibular fossa of the temporal bone) and was performed using a piezosurgical unit. In all seven cats, gap arthroplasty was performed without surgical complications. In addition, a clinically acceptable mouth opening was achieved in all cases. However, a noticeable mandibular instability was observed. Medium-term follow-up demonstrated acceptable quality of life with one case of recurrence of ankylosis requiring repeated bilateral surgery, and a second case with recurrence of ankylosis not requiring surgical intervention at the time of manuscript preparation. We concluded that TMJ gap arthroplasty in cats is a salvage procedure indicated in cases of severe intraarticular and extraarticular ankylosis. Diagnostic imaging by means of CBCT and 3D printing are essential for precise surgical planning. The use of a piezosurgical unit allows for safe and precise ostectomy. Clinically, despite the resulting mandibular instability, appropriate prehension of food and water was possible.

Keywords: temporomandibular joint, ankylosis, pseudoankylosis, cat, gap arthroplasty, piezosurgery, cone-beam computed tomography

OPEN ACCESS

Edited by:
Jason W. Soukup, University of Wisconsin-Madison, United States

Reviewed by:
Milinda Jean Lommer, Aggie Animal Dental Center, United States
Kendall Taney, Center for Veterinary Dentistry and Oral Surgery, United States

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Specialty section:
This article was submitted to Veterinary Dentistry and Oromaxillofacial Surgery, a section of the journal Frontiers in Veterinary Science

Received: *24 April 2020*
Accepted: *29 June 2020*
Published: *13 August 2020*

Citation:
Aghashani A, Verstraete FJM and Arzi B (2020) Temporomandibular Joint Gap Arthroplasty in Cats. Front. Vet. Sci. 7:482. doi: 10.3389/fvets.2020.00482

INTRODUCTION

Temporomandibular joint (TMJ) ankylosis and pseudoankylosis is an uncommon but severely debilitating condition that can be seen in various species (1–4). A rapidly progressive inability to open the mouth is most commonly noted, often with a history of maxillofacial trauma (5). Ankylosis of the TMJs prevents basic and important functions such as prehension of food, grooming, vocalization, thermoregulation, and adequate water intake. Additional implications of TMJ ankylosis may be skeletal and/or dental malocclusion, periodontitis, and mucosal ulceration (2, 6–8). Cats with TMJ ankylosis may be presented on an urgent or emergent basis due to tongue entrapment and secondary swelling, which can result in distress and death.

TMJ ankylosis is defined as fibrous or bony fusion of the mandibular head of the condylar process and the mandibular fossa of the squamous part of the temporal bone. Ankylosis can be further defined as intraarticular, extraarticular, or a combination of both (4, 6, 9). Intraarticular ankylosis involves destruction of the fibrocartilaginous disc, narrowing of the joint space, and flattening of the mandibular head (9). Extraarticular ankylosis occurs due to encapsulation of the joint or structures near the joint (i.e., the zygomatic arch and coronoid process of the mandible) with little or no involvement within the joint (5, 9). Extraarticular ankylosis may be termed "pseudoankylosis" due to mechanical obstruction or fusion of bones surrounding the joint (4, 6). Often times, severe cases have a combination of intraarticular and extraarticular ankylosis (9).

Cats most commonly are presented at a young age with a known history of maxillofacial trauma, typically secondary to falling, having been bitten by a dog, or vehicular trauma (10, 11). If there is no known history of trauma, diagnostic imaging is often suggestive of historic trauma. Other differential diagnoses include infection (i.e., osteomyelitis or retrobulbar disease), a congenital malformation, or less likely, neoplasia (1, 2, 6–8, 12, 13). Depending on age at the time of trauma, there may be discordant maxillofacial growth or abnormal tooth eruption (1, 4, 10). Oral examination of these cats is often unrewarding due to the limited ability to open the mouth. In some cases, proliferative abnormal bone growth may be palpable in the region of the TMJs. Close attention should be paid to the actual range of motion of the jaws as this will likely to have implications for future anesthesia and surgical treatment of this condition.

The accurate and precise diagnosis of lesions of the TMJ requires the use of advanced imaging. Computed tomography (CT) and cone-beam CT (CBCT) are considered the gold-standard for imaging for the TMJ (1, 3, 14). The use of CT images with 3D reconstruction is a helpful tool to aid in anatomic understanding and surgical planning (4, 15). In addition, the use of 3D printed skulls has been advocated as a surgical planning tool and for reference in the operating room (4, 15).

This retrospective study describes the clinical features, surgical treatment, and outcome of a gap arthroplasty procedure in a series of seven cats affected by extensive intraarticular and/or extraarticular TMJ ankylosis. We further aim to exemplify the use of 3D printing and piezosurgical technique for precise surgical planning and execution.

MATERIALS AND METHODS

Case Inclusion

Seven client-owned cats were presented to the Dentistry and Oral Surgery Service at the William R. Pritchard Veterinary Medical Teaching Hospital, University of California, Davis, for assessment and treatment of intraarticular and/or extraarticular TMJ ankylosis between September 2017 and December 2019. The cats were between 5 and 18 months old (mean age of 7.7 months). All cats experienced a severe restriction in opening of the mouth which was subsequently treated with gap arthroplasty (**Table 1**).

Medical Records Review

Information was obtained from the medical records including history, physical examination, laboratory diagnostics, diagnostic imaging results, surgical treatment, and outcome, along with follow-up care. CBCT images were reviewed and 3D printed skulls were examined for all included cases. Follow-up was obtained from medical records and telephone interviews with owners.

Cone-Beam Computed Tomography (CBCT)

Preoperative CBCT scans were obtained to allow for the evaluation of anatomical and structural features of the TMJs along with the rest of the skull and dentition. Six of seven cats were heavily sedated while the last cat was anesthetized for preoperative CBCT scans (**Table 2**). The TMJs were evaluated using a CBCT unit (NewTom 5G CBCT scanner, NewTom, Verona Italy). The field of view was 15 × 12 cm, and serial sections of the skull were obtained with a scan time of 18 s, which resulted in a voxel size (slice thickness) of 150 μm. The images were evaluated with 3D rendering using Invivo5 software (Anatomage, San Jose, CA) by two experienced board-certified veterinary dentists and maxillofacial surgeons (BA and FV) and a board-certified radiologist. The location and extent of the anatomic changes associated with the intraarticular and/or extraarticular ankylosis were documented.

3D Model Printing

For initial surgeries in all cats, 3D volume renderings, and 3D printing of the CBCT images were created for surgical planning purposes as previously described (15). The CBCT was compiled into a Standard Tessellation Language mesh. A 3D model was then printed to scale using an Object Connex 260V Polyjet Printer (Object/Stratasys, Rehovot, Israel). The 3D model was used to mark the sites of planned osteotomy prior to surgery and as a visual aid during surgery (**Figures 1A–C**).

Anesthesia

All cats were placed under general anesthesia for gap arthroplasty using variable anesthetic protocols as described in **Table 2**. All cats were given anti-emetics prior to anesthesia as cats with limited opening of the mouth are prone to aspiration pneumonia. Temporary tracheostomy was performed without complication in six of the seven cats as described elsewhere (16–18). In the cat with staged surgery, a temporary tracheostomy for the first surgery and a routine oral intubation for the second surgery were performed. In the cat with recurrent ankylosis, the cat had a temporary tracheostomy for the first surgery and endoscopic-guided endotracheal tube placement for the second surgery. In one cat, the mouth was able to open wide enough for a stylet to be guided down the airway and an endotracheal tube to be passed over the stylet.

Surgical Technique

All affected cats were clipped on both sides of the head and aseptically prepared for surgery. The cats were placed in lateral recumbency with the face and cervical region slightly elevated. A lateral approach to the TMJ area was performed as recently described (6). Briefly, a full-thickness incision along the length of the zygoma on the ventral aspect was performed. A periosteal elevator was used to elevate the periosteum from the zygomatic arch, coronoid process, condylar process, and around

TABLE 1 | Case information, TMJ involvement, complications, and follow-up (including recheck appointments with or without diagnostic imaging).

Cat	Age (months)	Sex	Weight at diagnosis (kg)	Known history of trauma	Unilateral or bilateral treatment	Complications with surgery or during recovery	Duration of Follow-up (months)
1	6	F	3.3 kg	High-rise fall	Bilateral	Fracture line into the calvarium with gas tracking intracranially Postoperatively developed left mandibular drift and malocclusion	13
2	18	FS	2.7 kg	Dog bite injury	Bilateral (staged surgery right side first)	6-months postoperative left-sided pseudojoint formation with substantial narrowing of gap	10
3	5	F	2.0 kg	Hit by car	Bilateral	Lack of blink of the left eye	3 (by referring veterinarian)
4	7	FS	3.0 kg	Unknown	Bilateral	A small portion of the left retroarticular process remaining postoperatively	6
5	5	MC	3.4 kg	Unknown	Bilateral	None	4
6	6	F	2.1 kg	Historic jaw fracture (unknown etiology)	Unilateral (left sided surgery)	Persistent malocclusion postoperatively with secondary trauma	2
7	7	MC	2.7 kg	Unknown	Bilateral (for first and second surgery)	Left-sided incision partial dehiscence 10-days postoperatively with resolving subcutaneous emphysema Suture removal for second surgery—cat had ∼50% of normal range of motion	4

TABLE 2 | Sedation and anesthetic protocol.

Case	Diagnostic imaging	Surgical anesthetic protocol	Temporary tracheostomy	Suture removal
1	Heavy sedation with ketamine, midazolam, and dexmedetomidine. No intubation performed	Ketamine, midazolam, and dexmedetomidine premedication. Induction with alfaxalone	Yes, no complications	Sedation with dexmedetomidine IM
2	Heavy sedation with butorphanol, midazolam, and dexmedetomidine. No intubation performed	Methadone and atropine premedication. Induction with ketamine and midazolam (same protocol for both surgeries)	Yes (right side), no complications Second surgery: normal intubation for the left side.	No sedation needed
3	Heavy sedation with midazolam and dexmedetomidine with intermittent propofol administration. No intubation performed	Methadone premedication. Induction with ketamine and midazolam	Yes, no complications	Not performed at UC Davis
4	Heavy sedation with alfaxalone, butorphanol, and midazolam. No intubation performed	Methadone, atropine, and dexmedetomidine premedication. Induction with ketamine and midazolam	Yes, no complications	Not performed at UC Davis
5	Anesthesia with oral intubation using a stylet. Premedication with butorphanol and atropine. Induction with alfaxalone and midazolam	Alfaxalone, hydromorphone, atropine premedication. Induction with alfaxalone and midazolam	No, endotracheal tube passed using a stylet	No sedation needed
6	Heavy sedation with butorphanol and dexmedetomidine. No intubation performed	Dexmedetomidine and hydromorphone premedication. Induction with ketamine and midazolam	Yes, no complications	No sedation needed
7	Heavy sedation with dexmedetomidine. No intubation performed	Methadone and dexmedetomidine premedication. Induction with ketamine and midazolam (same protocol for both surgeries)	Yes, no complications Second surgery: endotracheal tube passed via endoscopic guided stylet placement	Sedation with gabapentin orally for both suture removal appointments

**In all cases standard drug doses were used.*

the mandibular fossa of the squamous part of the temporal bone. In all cases, a piezosurgical unit (Implant Center 2® or Piezotome® Cube, Acteon, Mérignac, France) with a bone cutting (osteotomy) tip (BS1S or BS1L Acteon, Mérignac, France) was used to perform the ostectomies, avoiding damage to blood vessels and nerves (19, 20). The first ostectomy involved removal

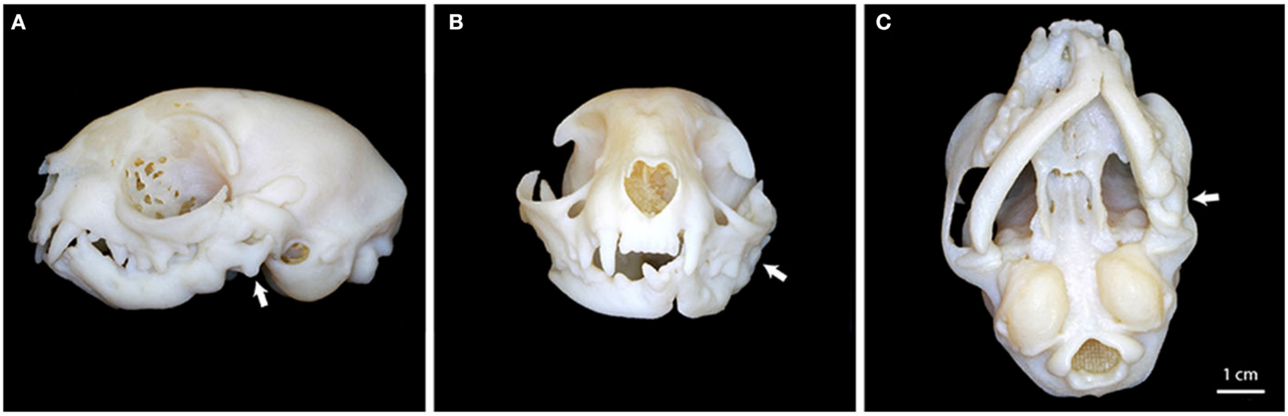

FIGURE 1 | Three-dimensional printed model of the cat depicted in **Figure 3**. **(A)** Left lateral view depicting ankylosis of the left TMJ (white arrow). **(B)** Significant asymmetry and malocclusion of the mandibles, along with left TMJ ankylosis (white arrow). **(C)** Ventral view depicting mandibular asymmetry and the region of ankylosis (white arrow).

of the majority of the zygomatic arch (i.e., zygomectomy) just caudal to the orbital ligament, and extending up to the mandibular fossa (**Figure 2A**). This allowed for exposure of the caudal mandible in preparation for the second ostectomy. The second ostectomy involved removal of the coronoid process and condylar process just above the level of the mandibular foramen (**Figure 2B**). The aim of the second ostectomy was to cut rostrally in a direct line to avoid the mandibular foramen and associated neurovascular bundle. The third ostectomy was made on the medial aspect of the mandibular fossa of the squamous part of the temporal bone (i.e., fossectomy; **Figure 2C**). This final ostectomy was made at the level of the retroarticular process, with care to direct the piezotome away from the calvarium.

Once the cut bones were removed, the bone edges were smoothed as needed with the piezotome using a curved scalpel tip (BS6 Acteon, Mérignac, France). The surgical site was flushed with sterile saline and a 3-layer closure was performed. The drapes were removed, and the range of motion of the jaws was evaluated and measured. If disease was unilateral and the cat had normal range of motion, the decision was made to stop with unilateral surgery. If range of motion was inhibited or restricted the cat was turned and the same procedure was performed on the other side for bilateral surgical treatment.

Postoperative Imaging

All cats had immediate postoperative CBCT performed to assess the ostectomies and to document that an appropriately sized gap had been made between the cut segments of bone. All postoperative imaging was performed with the cat's mouth in an open position to evaluate and document the mouth opening (i.e., immediate surgical outcome).

Postoperative Considerations

Cats with a temporary tracheostomy were recovered in an intensive care unit to allow for safe extubation and monitoring of respiratory status upon anesthetic recovery. The tracheostomy sites were left to heal by second intention without the placement of sutures or neck bandages.

IV fluid therapy was continued postoperatively until the cats were eating independently. Pain management was achieved using a multimodal approach. Postoperative pain was assessed using the Colorado State University Feline Acute Pain Scale (a 4-point scale). Non-steroidal anti-inflammatory drugs were administered via injection or by mouth for 1–3 days duration. Injectable opioid pain medications were administered in the first 8–12 hours postoperatively. The cats were then transitioned onto a transmucosal pain management with buprenorphine for an additional 5–7 days. If additional pain control or sedation was needed, the cat was also prescribed gabapentin. All cats were discharged with an Elizabethan collar and skin sutures that required removal in 10–14 days. All cats were also discharged with a 10–14 day course of postoperative antibiotics (amoxicillin trihydrate/clavulanate potassium).

RESULTS

On initial presentation, all cats were slightly underweight with unkempt fur but otherwise in good physical condition. Only one cat was estimated to be ∼5% dehydrated on initial physical examination. The results of hematological, and serum biochemical analysis were considered within normal limits for young cats. All cats were blood-typed and cross-matched in preparation for surgery (5 type A and 2 type B).

Mouth Opening

At initial presentation, all cats had severely restricted mouth opening; six of seven cats had only 2–3 mm of mouth opening from maxillary incisor to mandibular incisor teeth. Cat #5 was noted to have 18 mm of mouth opening, the least restricted of all cats. Two cats (#3 and #6) exhibited severe tongue entrapment, making these cases more urgent in nature due to concerns of upper airway obstruction (**Figure 3**).

Preoperative CBCT

In all seven cats, CBCT confirmed the clinical diagnosis of intraarticular and/or extraarticular TMJ ankylosis. Disease was

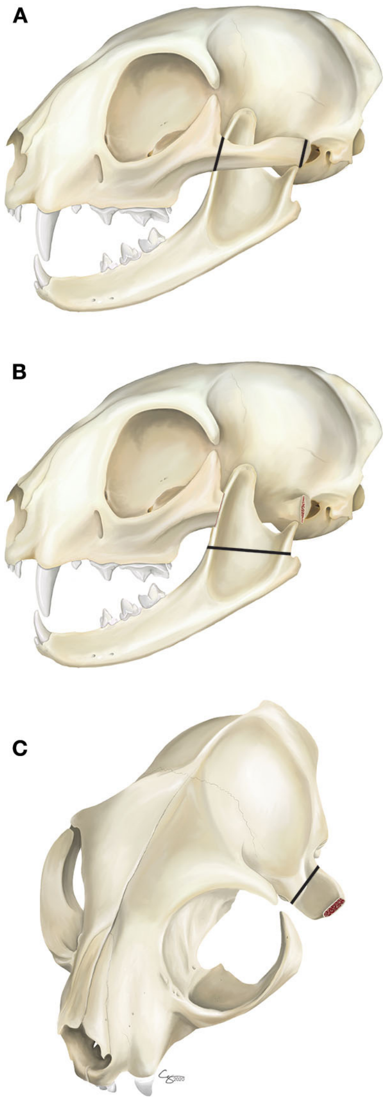

FIGURE 2 | Gap arthroplasty surgical ostectomies, **(A)** The first ostectomies are made on the zygomatic arch caudal to the orbital ligament and just rostral to the TMJ (i.e., zygomectomy). **(B)** The second ostectomy involves removal of the coronoid process and condylar process just above the level of the mandibular foramen. **(C)** The third ostectomy is made on the medial aspect of the mandibular fossa of the squamous part of the temporal bone (i.e., fossectomy).

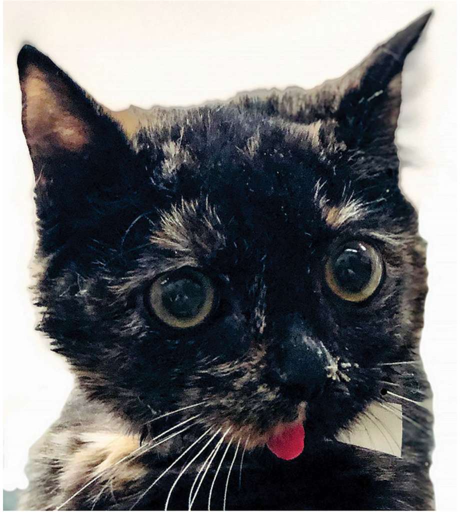

FIGURE 3 | A 6-month-old cat that was presented with tongue entrapment secondary to unilateral TMJ ankylosis. The tongue is trapped in between the mandibular and maxillary canine and incisor teeth.

severe and bilateral in five of the seven cases. In cats #6 and #7, both exhibited unilateral, left-sided TMJ ankylosis. All affected TMJs exhibited joint space narrowing to complete loss of the joint space. Loss of the joint space implied bridging bone across the joint resulting in complete immobility. The additional finding of an elongated and caudally curved coronoid process was documented in the abnormal joints as well (**Figure 4A**).

Other findings on CBCT included abnormal occlusion in three of the seven cats (a caudal crossbite and mandibular brachygnathism; **Figure 4B**). Impacted, non-vital, and odontodysplastic teeth were documented in two cats. Additional signs of trauma (previous fractures and a non-union fracture) were documented in two cats (**Figure 4C**).

Gap Arthroplasty

In six out of seven cases bilateral gap arthroplasty was performed under one anesthetic episode. One case had staged surgery due to progressive ankylosis of the contralateral side 1 month after the initial surgery. A second case had repeated bilateral gap arthroplasty 6 weeks after the initial surgery due to rapidly progressive intraarticular and extraarticular ankylosis resulting in severely restricted mouth opening. In all cases achieving the surgical goals were measured by the ability to open the patient's mouth without restricted range of motion.

Four cats were presented with pre-existing malocclusions secondary to presumed historic maxillofacial trauma, and two of these cats had the additional finding of impacted and odontodysplastic teeth. Postoperatively, three cats developed a malocclusion resulting in soft tissue trauma to the hard palate that was subsequently treated with tooth extractions.

Postoperative CBCT

The results of all postoperative CBCT were interpreted by a board-certified veterinary radiologist along with two experienced board-certified veterinary dentists and oral surgeons. In all cases appropriate ostectomies (zygomectomy, coronoidectomy, condylectomy, and fossectomy) were documented (**Figures 5A,B**). Additionally, all cases except one had subjectively large enough gaps to prevent contact of the cut ends of bone. The exact location of each ostectomy was dependent on the individual cat, the location, and extent of ankylosis. Postoperative imaging was used to document the ostectomies, and determination of surgical success was based on the clinical ability to open the patient's mouth postoperatively.

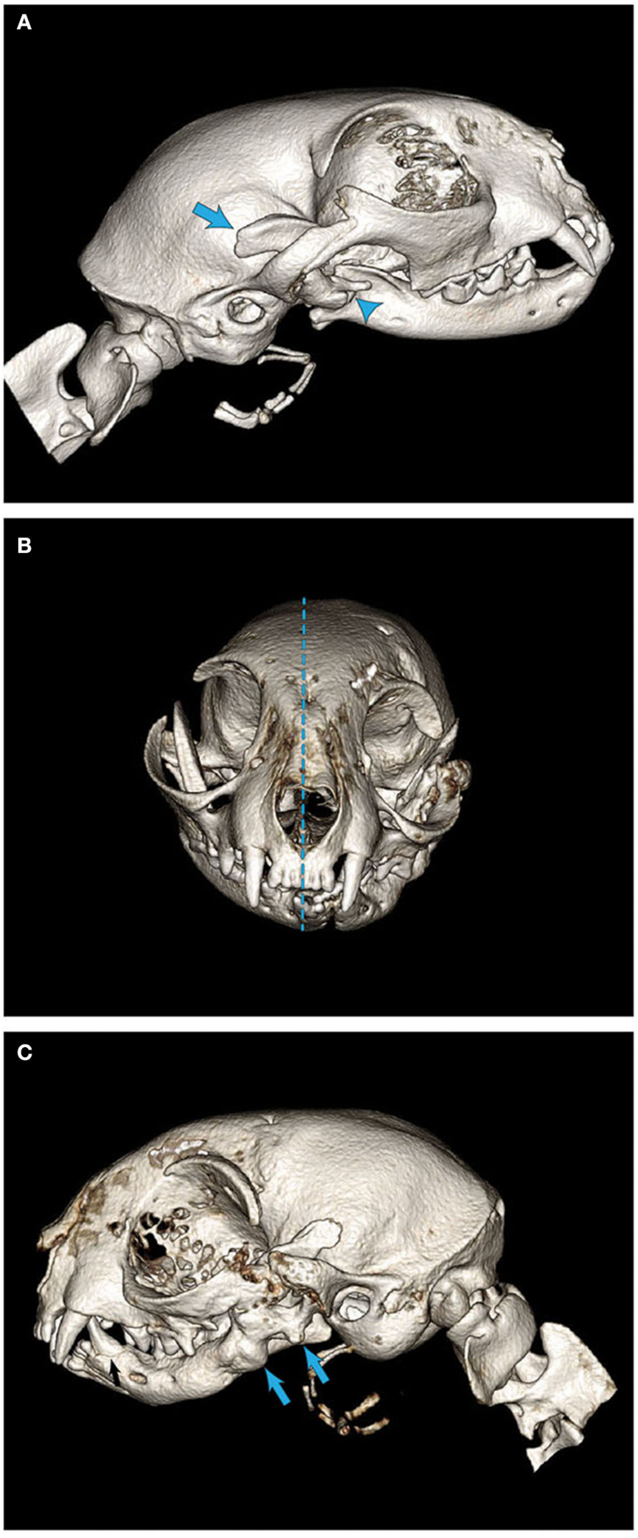

FIGURE 4 | Three-dimensional CBCT examples of skull alterations noted in cats with intra- and extraarticular ankylosis. **(A)** An example of a cat with an elongated and caudally curved coronoid process (arrows). **(B)** Significant asymmetry and malocclusion as demonstrated by the deviation of the mandibular symphysis from midline (dotted line). **(C)** A healed mandibular fracture with bony callus formation (arrows) seen in a cat with historical maxillofacial trauma.

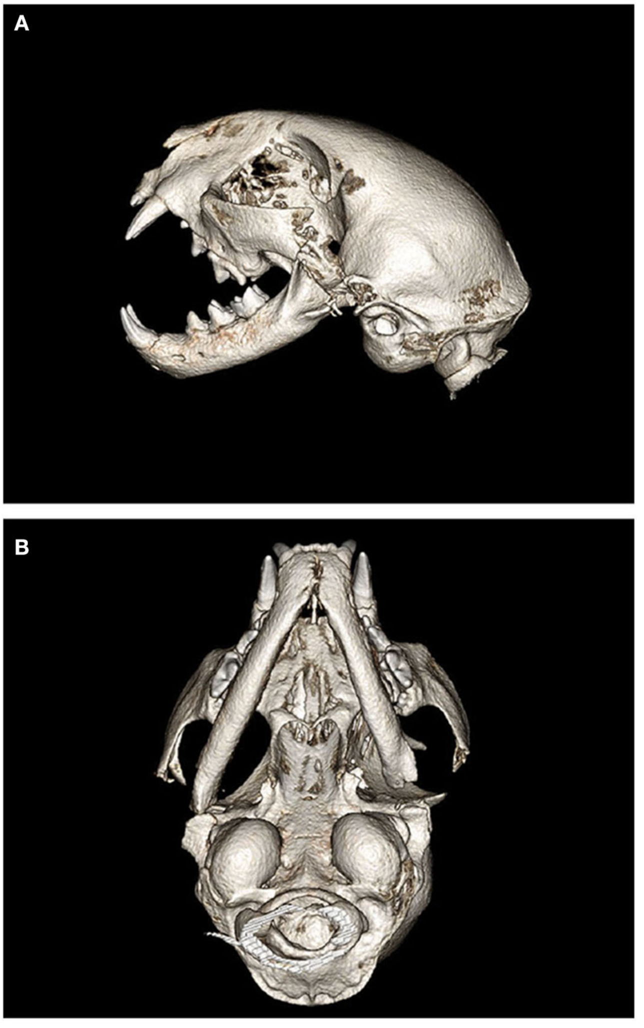

FIGURE 5 | Postoperative 3D CBCT images of cat #7 after bilateral gap arthroplasty. **(A)** Left lateral view depicting the ostectomies of the zygoma, coronoid process, condylar process, and mandibular fossa resulting in a "gap." **(B)** Ventral view depicting the left sided gap arthroplasty with more conservative ostectomies on the right side.

Gap size was dependent on the extent of ankylosis, patient size, and surrounding anatomic structures.

Immediate postoperative CBCT documented 1 case in which fragments of the zygomatic and retroarticular processes were remnant with a linear osteotomy line at the base of these two structures. The osteotomy line breached partial thickness of the calvarium and a small amount of focal gas accumulation was noted within the calvarium. The cat was monitored closely for any neurological signs postoperatively (none occurred) and the owner was also warned to monitor for any neurologic signs once the cat was discharged from the hospital (none reported). Importantly, all cats ate enthusiastically the same day of surgery. Cats were offered canned food or a slurry and were able to immediately lap up the food without signs of discomfort. One cat was noted to be grooming the day of surgery. All cats were discharged within 24–48 hours.

Complications

Complications after surgery were minimal. Immediately after anesthetic recovery one cat was noted to have a constricted left pupil, along with exophthalmos, and an incomplete blink in the left eye. Two additional cats recovered and were discharged with an incomplete blink and required topical ophthalmic lubrication. These signs all resolved within 2–3 weeks and were suspected to be secondary to postoperative swelling and inflammation.

Follow-Up

At the 10–14 day recheck appointment for suture removal, three cats were reported to be thriving at home while the remaining three cats presented with improved but persistent difficulty with prehension of food. The three cats with reported difficulty with prehension were documented to have malocclusions presumably secondary to historic trauma or recent surgical intervention. The follow-up of one cat was performed by the referring veterinarian, the cat was clinically assessed to have no restriction of mouth opening at 3 months postoperatively.

At the 10 day recheck appointment, one cat was presented with mild focal dehiscence of the left surgical incision. It was also noted that this cat had a mild amount of subcutaneous emphysema in the thoracic region due to the recent tracheostomy. The cat was eupneic and the owner was instructed to continue antibiotics for 10-additional days, continue Elizabethan collar usage, and monitor for worsening of subcutaneous emphysema or respiratory distress. The cat continued to recover without complication.

The duration of patient follow-up (including recheck appointments without diagnostic imaging) ranged from 2 to 13 months. Recheck appointments without diagnostic imaging were performed to assess the patient's function (ability to eat, groom, and overall quality of life), as well as to assess the range of motion of the jaws.

Follow-up with recheck CBCT was recommended for all cats, but only performed in three. One cat had a 4 month follow-up CBCT after bilateral surgery along with periodontal treatment with multiple extractions to treat a combination of periodontal disease, endodontal disease, and feline resorptive lesions. CBCT showed a moderate amount of smooth new bone formation along both of the mandibular ostectomy sites growing toward the remaining portion of temporal bone, resulting in bilateral pseudoarthrosis of the TMJs. Despite the findings on imaging, the cat had normal range of motion at that visit and was lost to further follow-up.

A second cat was presented 6 weeks after initial bilateral gap arthroplasty with acute onset of restricted mouth opening. On examination the cat was noted to have <10 mm of mouth opening with stable mandibles and loss of a palpable gap bilaterally. This cat underwent general anesthesia for diagnostic imaging with plans for immediate surgical revision of previous gap arthroplasty. This cat required endoscopic-guided endotracheal intubation. CBCT revealed extensive new bone formation bilaterally resulting in pseudoarthrosis and pseudoankylosis (**Figures 6A,B**). This cat had repeat bilateral gap arthroplasty with routine recovery. At the suture removal appointment 14-days postoperatively, the cat showed ∼50% of normal range of motion, which was suspected to be secondary to continued bone formation and muscle fibrosis. Despite restricted mouth opening, the cat was reported to be thriving at home. This cat was rechecked again at 6 weeks and showed continued reduction of range of motion resulting in ∼25% of normal mouth opening. Despite this further reduction, the cat was reported to be doing well at home but was no longer able to consume large kibble. The owners were instructed to monitor closely for any further restriction as additional surgical intervention may be required.

The third cat had a 6-month follow-up CBCT after staged bilateral gap arthroplasty. The right TMJ region had a palpable gap with mandibular mobility. The left TMJ had loss of palpable gap and the mandible was stable on palpation. The mouth opening from maxillary to mandibular incisor teeth while awake was restricted to ∼25 mm. CBCT demonstrated that the left gap

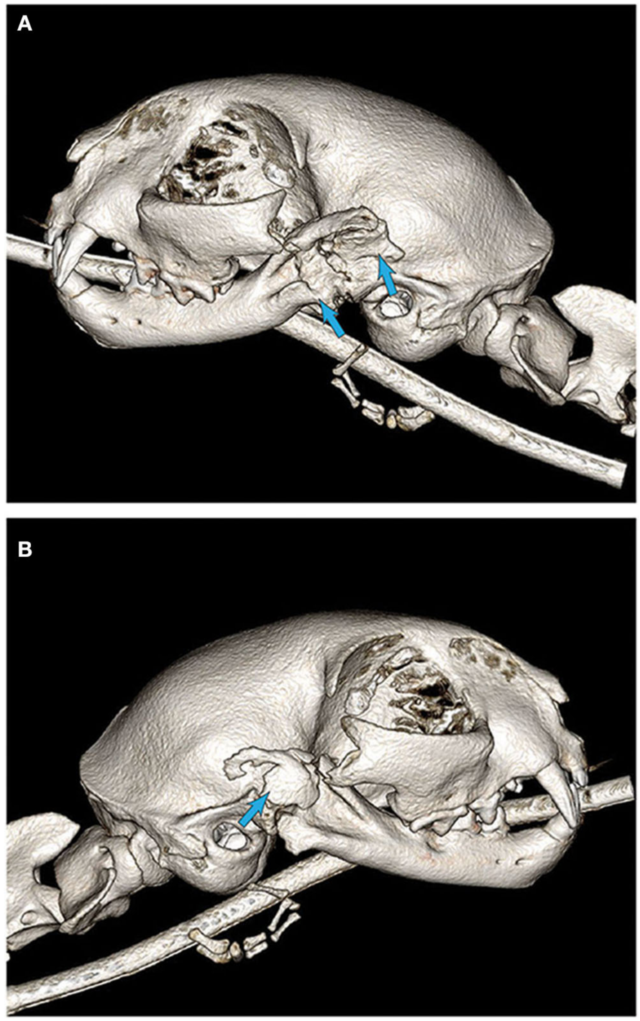

FIGURE 6 | 3D CBCT images of cat #7 6 weeks after initial surgery. **(A)** Note the substantial new bone formation stemming from the previous ostectomy sites (arrows). **(B)** New bone formation that is partially organized, at the location of the historically excised coronoid process (arrow).

arthroplasty site had formed a pseudoarthrosis with amorphous new bone formation with near loss of the previous gap. The cat was discharged to the owner with instructions to monitor for any further restrictions in mouth opening. If further restriction occurred, surgical revision would be recommended.

DISCUSSION

To the authors' knowledge, this is the first case-series report of gap arthroplasty performed in cats. This report demonstrates several clinically relevant aspects in cases of intraarticular and extraarticular TMJ ankylosis with subsequent treatment with gap arthroplasty. First, all cats were young with suspected or documented maxillofacial trauma. Second, diagnostic imaging by means of CBCT was essential for diagnosis, surgical planning, and follow-up. Third, the use of 3D printing was essential in precise surgical planning and execution. Fourth, gap arthroplasty is a delicate procedure, requiring precision ostectomy. Finally, dental malocclusion was noted and had a history of variable clinical presentations and consequences.

In all cases, historical maxillofacial trauma at a young age was suspected or known to be the cause of the underlying TMJ ankylosis. In humans, maxillofacial trauma is the leading cause of bony ankylosis of the TMJs (1, 12). TMJ ankylosis can be seen in human pediatric patients due to trauma during the birthing process, and less commonly due to an underlying congenital condition (1, 7, 8, 13). Some authors have suggested that traumatic injury of the TMJ in young human and animal patients can result in increased severity of ankylosis due to the excellent healing capacity noted in younger patients and due to the development of an exuberant bony callus (4, 10, 12). In this report, documented injuries included high-rise falls, dog attacks, and vehicular trauma. Therefore, it is essential that veterinarians and owners are aware that maxillofacial trauma in young cats, especially in the region of the TMJs may result in a life-limiting restriction of mouth opening later in life. Hence, as soon as restricted mouth opening is noted, diagnostic imaging should be performed, and early intervention is recommended.

Due to the size of a cat's skull and complexity of the local anatomy in the region of the TMJs, conventional radiography has been shown to be an inadequate imaging modality (14). Furthermore, conventional radiography is likely to underestimate the extent of ankylosis and true bony involvement. In fact, conventional CT was shown to be superior to skull radiographs when identifying specific anatomical structures of the feline skull (14). Therefore, diagnostic imaging by means of CT or CBCT are considered the gold-standard imaging modalities (1, 3, 4, 6, 10, 14, 21).

In order to enhance the understanding of two-dimensional (2D) imaging, the use of CT and CBCT is recommended with the use of 3D rendering. The use of 3D rendering is essential to allow the clinician to have a thorough understanding of the involved anatomic structures, to document the extent of ankylosis, and to assess the skull and jaws for any other signs of trauma or congenital abnormalities (4, 6, 15, 21). The use of CT or CBCT imaging can be employed to create a 3D printed model of the patient's skull, allowing the surgical team to precisely plan the sites of ostectomy (4, 6, 15). Based on the authors' experience, the use of anatomical landmarks in reference to planned ostectomy sites on the 3D printed model proved to be a valuable resource in the operating room.

In all cases, precision ostectomy has been performed using a piezotome with bone cutting tips. The piezotome was demonstrated to be an effective surgical tool that allows for accurate bone cutting with reduced risk of hemorrhage and damage to surrounding soft tissues and neurovascular structures (6, 19, 20). The piezotome is an innovative surgical instrument that uses high frequency (25–35 kHz) vibration of a metallic tip to selectively cut bone while sparing neurovascular structures and soft tissues (19). Use of a piezosurgical unit also results in more rapid healing (19, 20). The piezotome can also be used to perform osteoplasty with specialized bone surgery tips that allow for smooth shaping and contouring of bone margins. The disadvantages of the piezosurgical unit are that it tends to be slower than traditional oscillating saws or cutting burs and a learning curve exists for the operator of the instrument (19, 20). Regardless, given the delicate nature of the surgery and the challenging anatomic location, the authors recommend the use of a piezotome when performing gap arthroplasty in cats.

In the authors' perspective, gap arthroplasty is considered to be an invasive salvage procedure for cats that have poor quality of life or risk of death secondary to restricted opening of the mouth. Gap arthroplasty allows cats to have immediate mouth opening by removal of all intraarticular and extraarticular structures associated with the ankylosis. All cats were able to return to normal function in terms of prehension of food and water, grooming, and vocalizing within hours of surgery.

Creation of a subjectively appropriate gap is essential for reducing recurrence of ankylosis, along with immediate return to function as physiotherapy to prevent refusion (4, 6, 22, 23). In humans, the three surgical treatment options for TMJ ankylosis include: (1) gap arthroplasty; (2) interpositional arthroplasty; and (3) reconstruction of the articulation (22, 24). Interpositional arthroplasty involves the insertion of an interpositional material after resection of the ankylosis in hopes of preventing refusion, while reconstruction of the articulation can occur using autogenous or alloplastic grafts (22, 24). The current strategy in cats is aimed at removing of the ankylotic structures (i.e., salvage procedure) with the intention of immediate return to essential functions such as eating and drinking. Physiotherapy in feline patients can be challenging, therefore immediate return to hard food is recommended to encourage masticatory movement of the jaws. All clients were encouraged to engage in play with the cats postoperatively, specifically encouraging biting/chewing behaviors in an attempt to prevent any further ankylosis. Future options and advancements may become available in veterinary medicine to enhance the functionality of the TMJ.

Dental malocclusions and mandibular instability are expected consequences of gap arthroplasty. Malocclusions may be preexisting or may develop secondary to surgical treatment. In the authors' experience cats tolerate the mandibular instability well and were typically able to close their mouths using muscle tone within 30 minutes to 4 hours postoperatively.

Once cats healed from the initial surgery, regularly timed recheck examinations should reveal if there is a need for tooth extractions to prevent trauma secondary to malocclusion. An additional consideration is that the young cats with TMJ ankylosis seen in this report had more significant periodontal disease as compared to their normal counterparts. Therefore, oral and dental care such as a full periodontal treatment and regular tooth brushing should be initiated after healing from surgery.

Limitations of this study include a small sample size and follow-up time of <2 years in all patients. Lastly, only three of the seven patients had repeated diagnostic imaging performed to assess for recurrence of ankylosis. Therefore, the follow-up of the remaining four cats was based on clinical assessment of mouth opening and performing basic functions such as eating, drinking, and grooming.

In summary, gap arthroplasty is an invasive and delicate procedure that allows for immediate return to function. Proper surgical imaging and preparation are essential for a positive surgical outcome resulting in a cat that can have functional mouth opening. The downfall of gap arthroplasty is that despite extensive removal of bone and creation of a gap, there is a possibility of varying degrees of recurrence of ankylosis as demonstrated by two cats in this report. However, the risk of the surgery is outweighed by the alternative of euthanasia or deterioration of health and subsequent death for these cats. The immediate return to function allows for the restoration of acceptable quality of life as demonstrated in these cats by the immediate ability to eat, drink, and groom.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Ethical review and approval was not required for the animal study because the study is retrospective in nature and included clinical cases, hence, it is exempt from IACUC requirements. The standard written informed consent required for all procedures performed at the William R. Pritchard Veterinary Medical Teaching Hospital of the University of California, Davis was obtained from the owners. Written informed consent was obtained from the owners for the participation of their animals in this study.

AUTHOR CONTRIBUTIONS

AA: study concept and design, provision of study material or cases, manuscript writing, data analysis, and interpretation. BA and FV: study concept and design, provision of study material or cases, manuscript writing, data analysis and interpretation, and review of manuscript for important intellectual input. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

The authors thank Dr. Chrisoula Toupadakis Skouritakis for assistance with the figures.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Veterinary Science

REVIEW
published: 29 September 2020
doi: 10.3389/fvets.2020.575911

Electroporation-Based Treatments in Small Animal Veterinary Oral and Maxillofacial Oncology

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Electroporation is a method of inducing an increase in permeability of the cell membrane through the application of an electric field and can be used as a delivery method for introducing molecules of interest (e.g., chemotherapeutics or plasmid DNA) into cells. Electroporation-based treatments (i.e., electrochemotherapy, gene electrotransfer, and their combinations) have been shown to be safe and effective in veterinary oncology, but they are currently mostly recommended for the treatment of those solid tumors for which clients have declined surgery and/or radiotherapy. Published data show that electroporation-based treatments are also safe, simple, fast and cost-effective treatment alternatives for selected oral and maxillofacial tumors, especially small squamous cell carcinoma and malignant melanoma tumors not involving the bone in dogs. In these patients, a good local response to treatment is expected to result in increased survival time with good quality of life. Despite emerging evidence of the clinical efficacy of electroporation-based treatments for oral and maxillofacial tumors, further investigation is needed to optimize treatment protocols, improve clinical data reporting and better understand the mechanisms of patients' response to the treatment.

Keywords: electroporation, electrochemotherapy (ECT), gene electrotransfer, oral tumors, dogs, cats

OPEN ACCESS

Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Veterinary Dentistry and Oromaxillofacial Surgery, a section of the journal Frontiers in Veterinary Science

Received: *24 June 2020*
Accepted: *27 August 2020*
Published: *29 September 2020*

Citation:
Nemec A, Milevoj N, Lampreht Tratar U, Serša G, Čemažar M and Tozon N (2020) Electroporation-Based Treatments in Small Animal Veterinary Oral and Maxillofacial Oncology. Front. Vet. Sci. 7:575911. doi: 10.3389/fvets.2020.575911

BASIC PRINCIPLES OF ELECTROPORATION-BASED TREATMENTS AND GENERAL APPLICATIONS IN ONCOLOGY

Electroporation

Electroporation or electropermeabilization describes an increase in the permeability of the cell membrane due to the application of an electric field. The delivery of short high-voltage electrical pulses causes the formation of permeable structures in the cell membranes, thus allowing the passage of water-soluble ions and molecules into the cytosol (1). The key factor for successful permeabilization is the induced transmembrane voltage, which is generated in the presence of an external electrical field due to the difference in the electrical properties of the membrane and the external medium (2). The increase in cell membrane permeability may be transient (reversible electroporation) or may directly lead to cell death (irreversible electroporation), depending on the time of exposure of the cells to electrical pulses and the strength of the electric field. Cell death in irreversible electroporation may result from permanent disruption lysis of the cell membrane or the destruction of cellular homeostasis (3). Irreversible electroporation can be used in medicine as a sole treatment, and reversible electroporation can be combined as a delivery method for uploading molecules of interest (e.g., chemotherapeutics or plasmid DNA) into the cells (4, 5).

Several pulse generators (**Figure 1**) with different types of electrodes (**Figure 2**) are currently available on the market. When reversible electroporation is used for drug and gene delivery, electrical parameters must be adjusted for the delivery of the desired molecules into target tissues (1). Efficient cell membrane electroporation depends on establishing a sufficiently high electric field in the target tissue. In general, short high-voltage pulses are applied for the insertion of smaller molecules (e.g., chemotherapeutics), and larger molecules require pulses of longer duration that are either low-voltage or a combination of high- and low-voltage (6). There are two standard types of electrodes used for electroporation: penetrating (e.g., needle row and hexagonal) and non-penetrating (e.g., plate) (7) (**Figure 2**). The electrodes are selected individually, depending on the depth of the tumor nodule and the properties of the target tissue. In general, plate electrodes are used for superficial tumors (**Figure 3**), and needle or hexagonal electrodes are used for deeper tumors to achieve electroporation throughout the entirety of the tumor (8). In recent years, with the advancement of electroporation-based treatments, new types of electrodes are being produced (e.g., the single-needle electrode), which enables variable geometry specialized for electroporation of deep-seated tumors (9), and a multi-electrode array with pins for gene delivery to the skin (10).

Electrochemotherapy

Electrochemotherapy (ECT) is a local ablative method for the treatment of solid tumors that combines reversible electroporation and chemotherapy. Hydrophilic chemotherapeutics (bleomycin or cisplatin) can be administered either intravenously or intratumorally, and electric pulses are delivered directly to the tumor. Cells in the tumors become more permeable to chemotherapeutic agents that would otherwise have difficulty entering the cells (11, 12). This results in high concentrations of the intracellular chemotherapeutic agents and, consequently, up to 70 times (cisplatin) or thousands of times (bleomycin) more potent antitumor activity (13–17). Due to the increased cytotoxicity of bleomycin and cisplatin at the site of pulse delivery, low doses of chemotherapeutic agents are required, and a good local effect is achieved with no or minimal systemic toxicity. An antivascular effect of the treatment additionally occurs during the treatment; after the pulse application, the so-called "vascular lock" effect is observed, resulting in reduced blood flow and subsequent retention of the chemotherapeutics inside the tumor. Subsequently, the direct destruction of the endothelial cells of small vessels occurs (vascular disrupting effect), further accelerating tumor cell death (18, 19). ECT has also been shown to increase the efficacy of doxorubicin and mitoxantrone (20).

ECT triggers apoptotic and necrotic tumor cell death, and necrosis is expected in the treated area several days after the treatment. The dying tumor cells release extracellular tumor antigens and damage-associated molecular patterns (DAMPs) that trigger cytotoxic immune response against surviving neoplastic cells. This phenomenon is called immunogenic cell death and requires a combination of three DAMPs: calreticulin, which is exposed on the cell, and adenosine triphosphate (ATP) and high mobility group box 1 protein (HMGB1), which are released from dying tumor cells (21).

ECT has been established in more than 140 human clinical centers across Europe and is part of several national guidelines for the treatment of skin tumors in humans, such as the guidelines from the National Institute for Health and Care Excellence (NICE) (9). Recently, the use of ECT in oncology has been successfully expanded to the treatment of deep-seated tumors, such as primary and metastatic liver tumors, colorectal tumors, pancreatic tumors and tumors of the prostate, esophagus, bone, and spinal cord (22–30).

In veterinary medicine, ECT is used for the treatment of cutaneous, subcutaneous and oral tumors in dogs (31–35) and (still mostly) cutaneous tumors in cats (36) and horses (37, 38). In mast cell tumors (MCT) in dogs, up to a 70% complete response rate can be achieved with no major local or systemic side effects (31). In cats with nasal planum squamous cell carcinoma (SCC), the rate of complete responses can be up to 80% (36). The antitumor efficacy is inversely proportional to the tumor size; treating tumors smaller than 2 cm^3 results in a better complete response rate than treating larger tumors (12, 39). In a study comparing ECT and surgery for treating MCT in dogs, treatment with ECT resulted in a 70% complete response rate, while the rate was 50% for surgical treatment. Thus, ECT offers an alternative treatment to surgery, especially in cases of smaller MCTs and those that are non-resectable because of the location (31). In cases of larger tumors, a combination of surgical removal and intraoperative ECT can improve the treatment outcome (40).

FIGURE 1 | Generator of electrical pulses (Cliniporator, Igea s.r.l., Carpi, Italy).

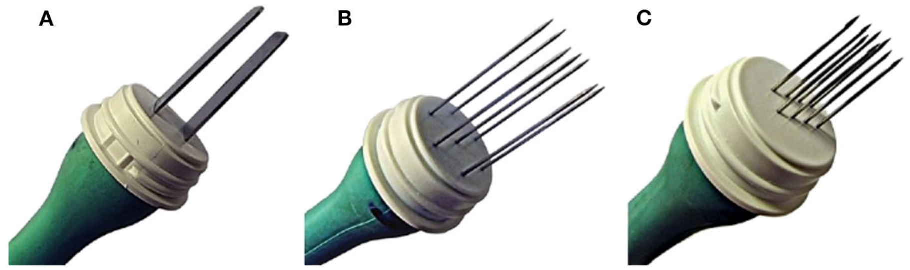

FIGURE 2 | Standard types of electrodes, used for electroporation. **(A)** Non-penetrating plate electrodes, **(B)** penetrating hexagonal electrodes, **(C)** penetrating needle row electrodes (all from Igea s.r.l., Carpi, Italy).

FIGURE 3 | The treatment of a canine oral squamous cell carcinoma with electrochemotherapy with intravenous application of bleomycin, followed by electrical pulse delivery with plate electrodes.

ECT is especially suitable for the treatment of solid tumors in cases where animal owners decline surgical treatment, or when tumors are located in areas where complete removal would be impossible (e.g., tumors near large vessels, tumors of the head and limbs, perianal tumors) (12). In veterinary medicine, the first reports of the use of ECT for the treatment of deep-seated tumors were recently described for the treatment of nasal tumors and colorectal carcinomas in dogs and thymoma in a cat (20, 41–43).

For effective treatment, the electric field should be distributed over the entire tumor and its safety margins while avoiding healthy tissues, which may be especially challenging in the oral cavity due to limited access as well as tissues with different conductivities (i.e., soft tissues, teeth, bone), where treatment pre-planning with numerical modeling providing optimal electrical parameters and electrode positions may increase the chances of treatment success (44–46).

Gene Electrotransfer

Gene electrotransfer (GET) is a method in which plasmid DNA encoding a therapeutic gene is transported into cells by reversible electroporation. In this way, increased production of the desired protein and its release into the extracellular matrix or bloodstream are achieved. The tissues that are most commonly subjected to GET are the skin and muscle. GET can be combined with ECT due to their synergistic action on neoplastic cells; intratumoral ECT directly destroys neoplastic cells, while GET into surrounding tissues transfects healthy cells and, depending on the therapeutic gene, may enhance the antitumor immune response (7, 47).

One of the most widely investigated GET methods in oncology is GET of a plasmid encoding interleukin-12 (IL-12); the safety and efficacy of this treatment has been demonstrated in several pre-clinical studies and translational studies in dogs (48–50). IL-12 has different antitumor effects with direct activation of acquired and innate immunity. It promotes the activation of T cells, enhances T cell survival and the effector functions of T cells and natural killer cells, and promotes interferon gamma (IFN-γ) secretion. IFN-γ acts directly on tumor cells by increasing recognition of major histocompatibility complex 1 (MHC 1), activates M1 macrophages, and enables alteration of the extracellular matrix, resulting in reduced angiogenesis and tumor invasion (50–53). These different actions slow down tumor growth and, finally, destroy the tumor. Additionally, long-term antitumor immunity can be established; in pre-clinical studies in mice with squamous cell carcinoma, the treated animals were resistant to tumor regrowth for 11 months, even when the same tumor cells were administered monthly to the subcutaneous tissues (51). Studies in a murine sarcoma tumor model have shown similar results (54). In addition, in human malignant melanoma and canine squamous cell carcinoma, a systemic "abscopal" effect on untreated tumor and metastases has been observed (55–57).

Following GET of a plasmid encoding human IL-12 (hIL-12), Pavlin et al. (58) evaluated the histological changes in mast cell tumors in dogs. Following intratumoral administration of the plasmid, there was a decrease in the number and degranulation of the tumor cells and extensive infiltration of tumor tissue with lymphocytes and plasma cells (58). Their observations confirmed the findings of previous studies of melanoma in mice, which showed infiltration of helper T cells (CD4+ lymphocytes) and cytotoxic T lymphocytes (CD8+ lymphocytes) after the treatment (59, 60). A similar phenomenon was observed in horses with melanoma treated with intratumoral administration of a plasmid encoding hIL-12 without electrical pulse delivery; in these cases, the infiltration consisted primarily of peritumoral CD4+ lymphocytes (61). Intratumoral infiltration with CD4+ and CD8+ lymphocytes has also been detected in patients with metastatic melanoma treated with GET of a plasmid encoding IL-12 (55).

GET of IL-12 as a monotherapy has demonstrated local and systemic antitumor activity (56, 58, 62), but the success of the treatment can be enhanced if different treatment approaches are combined [e.g., addition of radiotherapy (63) or ECT (7)]. Serša et al. (7) proposed a model using ECT as an *in situ* vaccination, with peritumoral GET of IL-12 to boost the triggered immune response against tumor antigens released from the dying tumor cells due to the action of ECT (7).

The combination of ECT and GET of IL-12 has already been used in the treatment of tumors of different histology in dogs (49, 56, 64, 65). In canine mast cell tumors, the addition of peritumoral GET encoding hIL-12 has been shown to improve the rate of complete response to 72%, compared to 62% for ECT alone (31, 49). Lampreht et al. (66) subsequently developed a plasmid encoding canine IL-12 (cIL-12) with similar or even higher expression capacity than the plasmid encoding hIL-12. This plasmid encoding cIL-12 was subsequently used in the treatment of dogs with oral malignant melanoma (OMM) (65).

Another transgene that was evaluated together with GET was a plasmid encoding chondroitin-sulfate proteoglycan 4 (CSPG4). CSPG4 is a transmembrane glycoprotein that is overexpressed on malignant cells of different tumors and involved in promoting oncogenic transformations, enabling proliferation, motility and metastatic spread of malignant cells via various modes of action. CSPG4 has been recognized as a marker for aggressive, therapy-resistant cancers and simultaneously serves as a target for tumor-selective oncolytic agents [reviewed by Jordaan et al. (67)] and anti-tumor vaccines [reviewed by Rolih et al. (68)]. As CSPG4 displays low expression levels on healthy adult (human and canine) cells and is expressed on the cell surface (Class 1 oncoantigen), it is an ideal target for effective anti-cancer immunotherapy in dogs and humans. A canine model appears to be very useful in translational studies, as CSPG4 expression was found in canine malignant melanoma and osteosarcoma (OSA) (68, 69) and has been related to significantly shorter survival in dogs with appendicular OSA (69). GET of human CSPG4 (hCSPG4) has also been shown to be safe and effective in the treatment of dogs with spontaneous OMM (70, 71); the human sequence of CSPG4 was intentionally used in these studies due to its high homology and similarity to canine CSPG4 and demonstrated ability to induce a specific humoral response against the human and canine protein, which is related to the successful outcome of the treatment (68). CSPG4 immune targeting also appears to be a promising treatment modality for appendicular OSA in dogs, as documented in an *in vitro* model (69).

ELECTROPORATION-BASED TREATMENTS FOR ORAL AND MAXILLOFACIAL TUMORS IN SMALL ANIMALS

Electroporation-based treatments (i.e., ECT, GET, and their combinations) have been shown to be safe and effective in veterinary oncology; however, they are not yet widely accepted as standard treatments, as observed to an even greater extent in veterinary oral and maxillofacial oncology. Currently, ECT and/or GET in veterinary oral oncology are recommended mostly for solid tumors for which clients decline surgery and/or radiotherapy (12).

The aims of this paper are to review the currently available data on the use of electroporation-based treatments in veterinary oral and maxillofacial oncology in dogs and cats. Although electroporation-based treatments, especially ECT, are beneficial for the treatment of nasal planum squamous cell carcinoma (SCC) and cutaneous SCC of the head/face and ears in cats (36, 72, 73) and are even more effective for MCT of the skin on the face and ears, lips and conjunctiva in dogs (40), we do not focus on these tumors. We also do not include tumors of the nasal cavity and frontal sinuses in this review.

Electrochemotherapy for Oral and Maxillofacial Tumors in Small Animals

As early as 2003, Spugnini and Porrello (74) described the principle of potentiation of bleomycin chemotherapy with the application of electroporation in the treatment of different neoplasms without evidence of bone invasion and metastatic disease in different species. Among these patients treated with ECT were also two dogs (one with acanthomatous ameloblastoma (AA) previously addressed with two surgeries and one intralesional chemotherapy, and one with OMM previously addressed with surgery) and three cats (one with oral SCC, one with head fibrosarcoma (FSA) previously addressed with one surgery, and one with an oral anaplastic sarcoma subjected to two previous surgeries). The treatment resulted in a complete response (CR) in the dog with AA (at least 150 days duration) and an initial CR in the cat with anaplastic sarcoma (90 days duration). After an additional ECT treatment upon recurrence of the tumor in this cat, a partial response (PR) lasting 55 days was observed. In the other two cats, a PR of 120 days (SCC) and 14 days (FSA) was reported. The dog with OMM had stable disease (SD) for 40 days. Treatments were generally well-tolerated, although the cat with FSA developed edema at the treated site, and the other two cats developed mild to moderate necrosis at the treated site.

Later, ECT with bleomycin was used by Spugnini et al. (75) in the treatment of 10 dogs with spontaneous OMM without notable metastasis (however, in one dog, metastatic lymph nodes were removed before ECT during surgery) either as a monotherapy ($n = 4$) or after previous surgery resulting in local tumor recurrence ($n = 6$). One week after completion of the treatment (i.e., after four ECT sessions repeated every week), a CR was obtained in seven dogs, and the rest had either SD or a PR. The median survival time (MST) of the dogs was 6 months (mean survival of 16 months). All dogs with either SD or PR eventually developed progressive disease, but four dogs with an initial CR remained in remission for 16–36 months. Moreover, no major local or systemic side effects were noted, except local vitiligo-like discoloration in three dogs, which could potentially indicate recruitment of the immune system by the therapy.

In 2017, Kulbacka et al. (45) described the treatment of one dog with stage IV OMM using a combination of cytoreductive

CO_2 laser surgery and ECT with bleomycin administered intravenously and intratumorally, resulting in the animal's immediate return to function and reduction of the remaining tumor mass within 10 days. However, 14 days after the treatment, enlarged metastatic mandibular lymph nodes caused the dog difficulty eating. At this point, the metastatic lymph nodes as well as the oral tumor were treated with ECT and calcium ions to elicit an additional immune system response. Severe lymphadenitis occurred 5 days post-treatment, but 30 days later no metastases were noted on post-treatment CT. The dog was euthanized 2 months after the first treatment due to seizures.

Suzuki et al. (44) published a case report of an OMM in a dog that was treated with ECT with bleomycin as part of a study to optimize the application of electroporation parameters/electrodes by numerical modeling and measuring oral mucosa conductivity during electroporation. Treatment resulted in clinical CR of the tumor that lasted at least 12 months.

In 2019, Spugnini et al. (76) published results for a study in which 30 dogs with incompletely excised non-metastatic sarcomas not involving bone were treated with ECT combined with bleomycin and cisplatin. Three of these dogs had sarcoma [peripheral nerve sheath tumor (PNST) grade II stage 3, hemangiosarcoma (HSA) stage 2, chondrosarcoma (CSA) stage 2] on the head, although the exact location was not clearly reported in the study. The authors used systemic bleomycin to increase the likelihood of drug distribution in the deeper layers of the tumor bed and local cisplatin to increase the efficacy of the treatment in the superficial layers. In two of the dogs (PNST, CSA) there was no evidence of disease at the end of the observation period [50 months (1,505 days) and 17 months (513 days) post-treatment, respectively], while the dog with HSA was disease-free for 12 months before developing recurrence and metastasis leading to death. Again, all the dogs tolerated the treatment well without major local and systemic side effects.

Torrigiani et al. (34) also treated non-metastatic soft tissue sarcomas in dogs with ECT. Of 52 dogs with 54 spontaneous soft tissue sarcomas, five had a (non-oral) tumor on the head without further specification of the exact location. In contrast to Spugnini et al. (76), the authors only used bleomycin intravenously and compared the efficacy of different treatment approaches–ECT as a monotherapy (performed for macroscopic disease) ($n = 1$), intraoperative ECT (performed immediately after marginal removal of the tumor) ($n = 2$) and adjuvant ECT (for incompletely excised tumors) ($n = 2$). Outcome data specific for head tumors are only available as personal communication with the authors (Torrigiani, personal communication) and hence not included here. Generally, overall response rate for ECT as a monotherapy was 75% and all these dogs were dead at the end of the follow-up period (median 1,113 days). Overall tumor recurrence rate was 23% for dogs treated with intraoperative ECT (median follow-up period 422.5 days) and 25% for dogs treated with adjuvant ECT (median follow-up period 596.5 days). As noted in the previously mentioned studies, treatment was well-tolerated with minor toxicity. In addition, the group compared the treatment outcomes when using two different pulse generators with different ECT parameters. They found no differences in outcomes when comparing the groups treated with different pulse generators, but they observed a higher treatment toxicity score in the group treated with a higher amplitude to electric distance ratio (1,200 vs. 1,000 V/cm) (34).

Recently, two studies on the use of ECT in oral tumors were published for larger cohorts of the patients. Simčič et al. (35) employed ECT with intravenous bleomycin to treat 12 dogs with canine oral non-tonsillar SCC without evidence of distant metastasis at the time of treatment. In that study, only two dogs received two ECT treatments, while the rest were treated only once. The treatment resulted in a calculated response rate for ECT alone of 90.9% and an overall recurrence of 27.3%. This response rate was better than that published for dogs treated with piroxicam alone (response rate of 18%) (77) or the combination of piroxicam and carboplatin (response rate of 57%) (78). However, the recurrence rate was higher compared with that achieved by surgery (8.3–18.2%) (79, 80), but not compared with radiotherapy (39.4%) (81). However, the outcome of the treatment was especially favorable with long-term (median follow-up 1,041 days) CR in dogs ($n = 6$) with tumors smaller than 2 cm compared with larger tumors. Generally, post-operative hypofractionated radiotherapy after incomplete excision of oral SCCs is currently considered to provide the best results (MST of 2,051 days) (82). There was only minor local toxicity (i.e., swelling, necrosis) noted in the majority of cases, and none of the dogs showed signs of systemic toxicity (35).

In the most recent large prospective clinical study using ECT with intravenous bleomycin, Tellado et al. (83) included 67 dogs with OMM of different stages. The animals were rechecked at 14, 30, and 60 days, and dogs with either SD or PD were treated again. Animals were followed-up up to 2 years. The overall objective response (OR) rate was 70.1% with 20.9% CR, 49.3% PR, 16.4% SD and 13.4% PD. The outcome of the treatment was largely dependent on the stage of the tumor; dogs with OMM stages I ($n = 11$) and II ($n = 19$) had a significantly better OR rate (93.3%) than dogs with OMM stages III ($n = 26$) and IV ($n = 11$) with an OR rate 51.4%. Additionally, dogs with OMM stages I (median time to progression 11 months) and II (median time to progression of 7 months) had significantly longer times to disease progression than dogs with OMM stage III or IV (both had a median time to progression of 4 months). The absence of bone invasion was identified as a predictive factor for longer times to progression. Fourteen dogs achieved CR, and the median disease-free survival (DFS) time was 12.5 months (3–30 months). Similarly, the stage was related to the overall survival time, with stage I OMM and absence of bone involvement being predictive of long survival and stage IV being predictive of short survival. The MST for dogs with stage I OMM was 16.5 months, for dogs with stage II OMM 9 months, for dogs with stage III OMM 7.5 months and for dogs with stage IV OMM 4.5 months. Interestingly, none of the dogs treated with stage I OMM developed metastasis, while other dogs that had no metastasis at the time of the first visit subsequently developed metastasis in 23.9% of the cases (83).

Details of the treatments are also summarized in **Table 1**.

TABLE 1 | Studies using electrochemotherapy for the treatment of oral tumors in dogs.

	Additional treatment	Number of treatments	Cytostatic used (dosage, administration)	Electrodes + electrical pulse parameters (number, duration, amplitude to distance ratio, frequency)	Generator of electric pulses	Tumor type	Number of patients	Outcome	References
1	1–2 previous surgeries in 2 dogs and two cats Previous intralesional chemotherapy in one dog	1 in two cats 4 in one cat and 1 dog 6 in 1 dog (1 or 2 weeks apart, repeated until CR or PD)	Bleomycin (intratumorally until saturation)	Caliper electrodes; 8 (biphasic) pulses, 50 + 50 µs, 800 V/cm, frequency not defined	Chemopulse (Center of Bioengineering, Sofia, Bulgaria)	Dogs: AA, OMM Cats: SCC, FSA, anaplastic SA	2 dogs and three cats (22 different animals total with different tumors)	CR 150 days in AA SD 40 days in OMM PR 120 days in SCC PR 14 days in FSA CR 90 days in anaplastic SA	(74)
2	Previous surgery in six dogs	4 (1 week apart)	Bleomycin (intra- and peritumorally, dose/tumor unknown)	Modified caliper and needle electrodes; 8 (biphasic) pulses, 50 + 50 µs, 800 V/cm, 1,000 Hz	Chemopulse (Center of Biomedical Engineering, Sofia, Bulgaria)	OMM	10 dogs	1 week after the 4th ECT: CR 70%, PR 10%, SD 20% MST 6 months, mean ST 16 months	(75)
3	Cytoreductive CO_2 laser surgery	2 (2 weeks apart)	Bleomycin (0.3 mg/kg IV + intratumorally) in the 1st treatment, calcium ions in the 2nd treatment (5 mM, 10 ml intratumorally)	Two-needle array and Petri Pulser electrodes; 8 square wave pulses, 100 µs, 1300 V/cm, 1 Hz	ECM 830 pulse generator, BTX® (Harvard Apparatus, Holliston, MA, USA)	OMM	1 dog	PR at 1 month	(45)
4	NA	1	Bleomycin (15.000 U/m² body surface area IV)	Type II needle electrodes; 8 pulses, 100 µs, 130 kV/m, 1 Hz	ECM 830 pulse generator, BTX® (Harvard Apparatus, Holliston, MA, USA)	OMM	1 dog	CR (follow-up 12 months)	(44)
5	Neoadjuvant surgery	2 (2 weeks apart) starting 10–14 days after surgery	Bleomycin (20 mg/m² IV) + cisplatin 0.5 mg/cm² in the tumor bed	Plate electrodes; 8 (biphasic) pulses, 50+50 µs, 1300 V/cm, frequency unknown	Onkodisruptor	Head (site not specified) PNST, HSA, CSA	Three dogs (30 dogs total included with sarcomas at other locations)	CR in 2 dogs at 1,505 and 513 days, PD in 1 dog at 366 days	(76)
6	Nothing or previous surgery (marginal or resulting in incomplete margins)	1–3 for 54 tumors (with the intervals between dependent on tumor recurrence)*	Bleomycin (15.000 U/m² body surface area IV)	Type II needle electrodes; 8 (monophasic) pulses, 100 µs, 1,200 V/cm* or 1,000 V/cm*, 5,000 Hz*, 1 Hz*	Cytopulse PA4000 or CytopulseOncovet (Cyto Pulse Sciences, Inc., Holliston)	Head soft tissue sarcoma (non-oral, but site not specified)	Five dogs (52 dogs total included with 54 soft tissue sarcomas at other locations)	Overall response rate for ECT alone 75% Overall RR for ECT alone NA, for intraoperative ECT 23% and for adjuvant ECT 25%* (median follow-up 498 days for all dogs*)	(34) *Data specific to head tumors are available as personal communication Torrigiani, personal communication

(Continued)

TABLE 1 | Continued

	Additional treatment	Number of treatments	Cytostatic used (dosage, administration)	Electrodes + electrical pulse parameters (number, duration, amplitude to distance ratio, frequency)	Generator of electric pulses	Tumor type	Number of patients	Outcome	References
7	Surgery before ECT in one case Carboplatin chemotherapy after second ECT in 1 case	1 2 in 2 dogs (1 month apart)	Bleomycin (15.000 U/m² body surface area IV)	type II needle electrodes; 8 (monophasic) pulses, 100 μs, 1,000 V/cm and 1 Hz (PA4000) or 1,200 V/cm and 5 kHz (Oncovet)	Cytopulse PA4000 or CytopulseOncovet (Cyto Pulse Sciences, Inc., Holliston)	SCC	12 dogs	Calculated response rate for ECT alone 90.9%, overall RR 27.3%, DFI and MST for dogs with recurrence 50 days (range 9–83) and 115 days (range 99–1891) Dogs treated with ECT alone with tumors <2 cm obtained CR and showed no recurrence (median follow-up 1041 days)	(35)
8	NA	1 in 41 dogs 2 in 20 dogs 3 in five dogs 4 in 1 dog (with the intervals between dependent on tumor recurrence; usually between 1 and 2 months)	Bleomycin (15.000 U/m² body surface area IV)	6-needle electrodes and Single Needle Electrode® (for nasal duct invasion); 8 (6-needle electrodes) or 32 (single needle electrodes) pulses, 100 μs, 1,000 V/cm, 10 Hz	ECM 830 pulse generator, BTX® (Harvard Apparatus, Holliston, MA, USA)	OMM, stages I–IV	67 dogs	Stage I: CR 72.7%, PR 27.3%; MST 16.5 months Stage II: CR 21.1%, PR 68.4%, PD 10.5%; MST 9 months Stage III: CR 7.7%, PR 50%, SD 26.9%, PD 15.4%; MST 7.5 months Stage IV: PR 36.4%, SD 36.4%, PD 27.3%; MST 4.5 months	(83)

NA, not applicable; ECT, electrochemotherapy; OMM, oral malignant melanoma; AA, acanthomatous ameloblastoma; SCC, squamous cell carcinoma; SA, sarcoma; FSA, fibrosarcoma; PNST, peripheral nerve sheath tumor; HSA, hemangiosarcoma; CSA, chondrosarcoma; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; RR, recurrence rate; MST, median survival time; ST, survival time; DFI, disease-free interval.

Gene Electrotransfer for Oral and Maxillofacial Tumors in Small Animals

After successful implementation of ECT and the combination of ECT and GET as described later in veterinary oncology, GET as a mono-gene therapy was also introduced. The first case in veterinary oral oncology was reported by (84), who, as part of the optimization protocol, also treated one dog with oral amelanotic melanoma with GET of a plasmid encoding hIL-12. The treatment consisted of five cycles; in each cycle, the dog received 1–3 GET therapies as detailed in **Table 2**. The response to each cycle varied, from PR to PD, but finally the local disease was stable after 147 days (further follow-up data are not provided). Moreover, the dog developed metastatic disease (lungs) after the second cycle, but these non-treated metastases stabilized or even regressed with further treatment cycles, although new metastatic lesions developed in the lungs (56).

In the same year, Riccardo et al. (71) introduced GET of a plasmid encoding hCSPG4 as monotherapy to the clinical setting and tested the treatment in 14 dogs with surgically resected (of these three incompletely excised) CSPG4-positive stage II and III OMM. The authors compared this group with the group of 19 dogs with surgically resected stage II and III OMM, of which 13 had CSPG4-positive (4 incomplete excision) and 6 CSPG4-negative (two incomplete excision) OMM. Dogs receiving GET of hCSPG4 had a better survival rate (6- and 12-month survival rates were 100 and 64.3%) than dogs with only surgically resected CSPG4-positive OMM (6- and 12-month survival rates were 69.2 and 15.3%) and then dogs with only surgically resected CSPG4-negative OMM (6- and 12-month survival rates were 83.3 and 33.3%). MST and disease-free interval (DFI) were significantly longer in dogs receiving GET of hCSPG4 (MST 653 days, DFI 477 days) than in dogs with only surgically resected CSPG4-positive OMM (MST 220 days, DFI 180 days), but not longer than in dogs with only surgically resected CSPG4-negative OMM (MST 338 days, DFI 250 days). Apart from transient erythema at the GET site, no other local or systemic side effects were noted. There were no differences in outcome related to the completeness of surgical excision. The treatment resulted in barely detectable circulating T cells reactive to cCSPG4, but there was a marked specific antibody response to hCSPG4 and cCSPG4 in the serum of all dogs, mostly after the second but in all after the third GET treatment. Post-GET sera of most dogs were capable of inhibiting melanoma cell proliferation *in vitro*, although the titer did not correlate with the clinical outcome (71).

In a study by Cicchelero et al. (85), four dogs with (among others) different oral/maxillofacial tumors [FSA (n = 2), OSA (n = 1), SCC (n = 1)], with regional metastatic disease confirmed in the SCC case, were included. Dogs were treated with GET of a plasmid encoding hIL-12. As initial daily treatments resulted in (likely) treatment-related morbidity (immune-mediated anemia) and mortality (fatal thrombocytopenia), further treatments were performed weekly and repeated one or two times. Apart from initial transitory leukopenia, anemia and monocytosis, no other clinically important deviations in hematology and biochemistry were reported. Minor to moderate fatigue, fever, weight loss, anorexia and tumor swelling and pain were also reported in the treated dogs. Although as monotherapy, GET of a plasmid encoding hIL-12 did not result in a clinically relevant suppression of tumor growth, in one dog a significantly increased quality of life was noted. Moreover, the treatment resulted in a local and systemic immune stimulation, decreased blood flow within the tumors and changes suggestive of an anti-angiogenic effect of the treatment (85). The lack of tumor response was probably due to the large size of the treated tumors (one FSA and OSA described as extensive skull invasion).

In the most recent larger cohort study, Piras et al. (70) continued with GET of a plasmid encoding hCSPG4 testing and compared a group of dogs (n = 19) with stage II and III CSPG4-positive OMM treated with curative-intent surgery with a group of dogs (n = 23) receiving GET of a plasmid encoding hCSPG4 3–4 weeks after the surgery. In each group, there were four dogs in which surgery resulted in incomplete tumor removal. Dogs receiving GET of hCSPG4 had DFI and a better survival rate (statistically significantly for dogs <20 kg) compared with dogs treated with surgery alone (for the GET group, 24-month DFI rate was 17.4% and 24-month survival rate was 30.4%, while for the surgery-only group, 24-month DFI rate was 5.3% and 24-month survival rate was 5.3%). Adjuvant GET of a plasmid encoding hCSPG4 also resulted in lower local recurrence (34.5% for the GET group vs. 42.0% for the surgery only group) and a lower metastatic percentage (39.0% for the GET group vs. 79.0% for the surgery only group). Treatment with GET also resulted in the presence of specific anti-hCSPG4 antibodies in sera in all dogs after the fourth treatment, which was more pronounced in dogs <20 kg (70).

Details of the treatments are also summarized in **Table 2**.

Combination of ECT and GET for Oral and Maxillofacial Tumors in Small Animals

The protocol for using a combination of ECT and GET in veterinary oncology described by Cutrera et al. (86) includes a case of a dog with a poorly determined "recurrent papillary tumor with adjacent metastatic bone tumor" on the rostral maxilla, which was treated with a combination of ECT/GET with a plasmid encoding IL-12 (source not mentioned) and bleomycin, both applied intratumorally. Treatment resulted in regression of the visible tumor within 2 weeks after the treatment and complete resolution of the bony lesion at 23 weeks after the treatment (86).

The combination of ECT/GET was also tested on oral SCC (n = 2) and one each AA, OMM and oral FSA by Reed et al. (87), who used intratumoral bleomycin combined with feline IL-12 (fIL-12), which is 91% homologous to cIL-12. The authors adjusted the doses of the fIL-12 and bleomycin to the size of the tumor and administered 0.5 IU of bleomycin and 150 μg fIL-12 for each cm^2 of tumor at the maximum cross-sectional area. There was a good clinical response to the treatment for all tumors—3 dogs had a CR (defined for the study as the disappearance of all measurable tumor at 21 days), and all three dogs were disease-free for at least 9 months (27 months, 56 months). In one dog with SCC, visible tumor disappeared after the second treatment, and the majority of the bone lysis

TABLE 2 | Studies using gene electrotransfer for the treatment of oral tumors in dogs.

	Additional treatment	Number of treatments	Plasmid DNA (dosage, administration)	Electrodes + electrical pulse parameters (number, duration, amplitude to distance ratio, frequency)	Generator of electric pulses	Tumor type	Number of patients	Outcome	References
1	NA	5 cycles of 1–3 treatments with at least 6 days between the treatments	hIL-12 (300–600 ug/treatment, intratumorally)	Needle electrode; 2 pulses, 20 ms, 350 V/cm, 10 Hz	ECM 830 pulse generator, BTX®	Amelanotic melanoma (metastatic)	1 dog (4 dogs total included with tumors at other locations)	SD after 147 days; metastases less opaque, smaller, and difficult to identify	(84)
2	Curative-intent surgery 3–4 weeks before 3 dogs later received additional surgery	2 in 2 weeks, then monthly	hCSPG4 (500 ug, IM)	Electrodes unknown; 9 pulses (1 high voltage, 450 V, 50 ms, 3 HZ; 1 s pause; 8 low-voltage 110 V, 20 ms, pause 300 ms)	Cliniporator™ (Igea, Carpi, Italy)	OMM, stage II and III, CSPG4-positive	14 dogs	6-month survival rate 100%, 12-month survival rate 64.3% DFI 477 days MST 653 days Local recurrence 21.4% Lung metastases 35.7%	(71)
3	NA	2–3 treatments (1 day−1 week interval)	hIL-12 (1 mg/treatment, intratumorally)	Needle electrodes; 2 pulses, 0.05 ms, 750 V/cm, 5 kHz 8 pulses, 10 ms, 183 V/cm, 50 Hz	Agile Pulse generator, BTX®	FSA, OSA, SCC (metastatic)	4 dogs (9 dogs total included with tumors at other locations)	Softening of the tumor, but no effect on tumor growth (observation period up to 270 days)	(85)
4	Curative-intent surgery 3–4 weeks before 8 dogs later received additional surgery or radiotherapy	2 in 2 weeks, then monthly; dogs surviving >2 years re-vaccinated every 6 months	hCSPG4 (500 ug, IM)	9 pulses (1 high voltage, 450 V, 50 ms, 3 HZ; 1 s pause; 8 low-voltage 110 V, 20 ms, pause 300 ms)	Cliniporator™ (Igea, Carpi, Italy)	OMM, stage II and III, CSPG4-positive	23 dogs	24-month DFI 17.4%, 24-month survival rate 30.4%, local recurrence 34.8%, lung metastasis 39%	(70)

NA, not applicable; hCSPG4, human chondroitin sulfate proteoglycan 4; hIL-12, human interleukin-12; OMM, oral malignant melanoma; SCC, squamous cell carcinoma; FSA, fibrosarcoma; OSA, osteosarcoma; SD, stable disease; MST, median survival time; DFI, disease-free interval.

also disappeared after 6 months, with the dog surviving almost 5 years disease-free. According to clinical photos, this is the same dog as described in a protocol by Cutrera et al. (86). Two dogs (OMM, FSA) had a partial response (defined for the study as a >50% reduction in measurable tumor at 21 days) but were soon euthanized due to progressive disease and other medical problems (OMM case) or rapidly recurring local tumor (FSA) without completing the treatment. Apart from transitory leukocytosis, an increase in alkaline phosphatase and diarrhea (the last likely unrelated to the treatment) in the dog with metastatic OMM, and a day of lethargy and decreased appetite in another dog, no major side effects of the treatment were noted (87).

A later report on combined ECT/GET treatment by Cutrera et al. (64) included nine dogs with head and neck AA ($n = 2$), plasmacytoma (PC) ($n = 1$), SCC ($n = 4$), and sarcoma ($n = 2$) (including a subcutaneous sarcoma of the orbital area) with distant metastasis confirmed in the PC case. Four of the dogs were previously treated with surgery or radiotherapy. Intratumoral GET of cIL-12 with or without ECT with either bleomycin or gemcitabine injected intratumorally was performed; these treatments were further repeated based on the tumor response. Bleomycin was the chemotherapeutic agent of choice and was replaced with gemcitabine if the clinical outcome was not favorable. The treatment was divided into cycles composed of one or two treatment sessions 7–28 days apart. The treatments were repeated up to 22 times (12 cycles). The authors concluded that their ECT/GET approach was safe, well-tolerated and similarly effective in reducing the SCC, AA and PC lesion volume within the first 3 weeks after the first treatment, with ECT/GET with bleomycin showing a more rapid effect, especially in SCC cases. However, the treatment was ineffective for sarcomas. Additionally, if only GET was performed, it only temporarily (first 2 weeks with a return to the initial size by the third week) halted SCC growth, but GET alone was more effective for sarcomas than ECT/GET. Interestingly, tumor size was not predictive of the response in cases, that responded to the treatment.

The most recent study on a combination of ECT and GET was published by our group. Nine dogs with histologically confirmed spontaneous OMM stages I to III were treated with cytoreductive surgery (intracapsular excision of the tumors) immediately followed by ECT (intravenous bleomycin) in combination with GET of a plasmid encoding cIL-12 given peritumorally. Treatment was repeated up to five times based on the response to previous treatment(s). The protocol was shown to be safe with no major local or systemic side effects apart from (expected) tumor necrosis and, in some patients, transient systemic leukocytosis. At the end of observation period, all but one animal developed PD with an MST of 6 months, regardless of the tumor stage. We concluded that using a combination of ECT/GET for the treatment of canine OMM is minimally invasive and cost-effective, with a survival comparable to that achieved by radical surgery or radiotherapy, especially for stage II and III tumors (88). Additionally, reduced circulating regulatory T cell numbers were indicative of the systemic antitumor immune response at the end of treatment (65).

Details of the treatments are also summarized in **Table 3**.

CONCLUDING RECOMMENDATIONS ON ELECTROPORATION-BASED TREATMENTS FOR ORAL AND MAXILLOFACIAL TUMORS IN SMALL ANIMALS

There are three major advantages of electroporation-based treatments. First and most important, current treatments offer a safe alternative for the treatment of oral and maxillofacial tumors. Considerable muscle contractions that are consistently encountered during electroporation are expected; hence, ECT/GET treatments must be performed under general anesthesia. Although ECT can be effective as a one-time treatment, several treatments and therefore anesthetic procedures may be needed several weeks apart to increase effectiveness in the case of no response to treatment or only a partial response is achieved (12). Occasional and manageable increases in respiratory and heart rates are also observed during anesthesia. In the post-operative period, transitory mild blood changes may occur, as well as local tumor swelling and necrosis. Generally, ECT/GET treatments are well-tolerated, with most of the dogs in all studies maintaining their normal habits and routines (56, 64, 65, 70, 71, 85, 87). Conversely, Torrigiani et al. (34) reported higher local treatment toxicity if a higher amplitude to electric distance ratio (1,200 vs. 1,000 V/cm) was used with type II needle electrodes. However, the possible post-treatment complications can be considered much less severe than those of surgery for oral tumors (79) or radiotherapy (89, 90). However, it should be noted that data from some other studies point at possible more severe or even fatal complications associated with electroporation-based treatments (85). Local intratumoral application of plasmid encoding IL-12 may cause late focal kidney inflammation without any hematological or biochemical markers of kidney failure in treated animals, as described in mice (91). Although no such reports are available for dogs and cats, monitoring of renal function is recommended in any IL-12 gene-based therapy. In a study including different animals with different types of tumors treated with ECT, acute tumor lysis syndrome and fatal pulmonary thromboembolism were reported in a few cases with large non-oral carcinomas and sarcomas (74).

Further, these treatments, especially ECT, are considered simple and short with no major equipment needed apart from pulse generator, requiring a low dose of chemotherapeutic drugs to produce minimal chemotherapy-related side effects. Therefore, they can be performed on an outpatient basis, further reducing the costs of treatment (12, 20). ECT could therefore be easily offered as an alternative treatment to surgery and radiotherapy, mostly when owners have concerns about the financial burden and/or aesthetic outcome. Conversely, the commercial availability of plasmids for performing GET treatments remains a limitation to the wider application of this procedure.

TABLE 3 | Studies using the combination of electrochemotherapy and gene electrotransfer for the treatment of oral tumors in dogs.

	Additional treatment	Number of treatments	Cytostatic used (dosage, administration)	Plasmid DNA (dosage, administration)	Electrodes + electrical pulse parameters (number, duration, amplitude to distance ratio, frequency)	Generator of electric pulses	Tumor type	Number of patients	Outcome	References
1	NA	1 (unclear from the text)	Bleomycin (0.5 U/cm^2, intratumorally)	IL-12 (150 ug, intratumorally)	Caliper electrode; 2 pulses, 25 ms, 450 V/cm	BTS EC830 pulse generator	"Recurrent papillary tumor with adjacent metastatic bone tumor"	1 dog	CR 23 weeks after the treatment	(86) [see also (87)]
2	Previous surgery in SCC cases	1–3 treatments (at least 10 days interval)	Bleomycin (0.5–2 IU/treatment depending on the tumor size, intratumorally)	fIL-12 (150 ug−400 ug depending on the tumor size, intratumorally)	Hexagonal electrodes (in one case simple caliper electrodes); 2 pulses 20 ms, 400 V/cm, 10 Hz	ECM 830 pulse generator, BTX®	AA (T2N0M0), 2x SCC (T2bN0M0), OMM (T3bN2bM1), FSA (T3N0M0)	5 dogs (6 dogs total included with tumors at other locations)	CR SCC, CAA (observation period 9–56 months) PR OMM, FSA (exact observation period unknown, but both developed PD soon)	(87)
3	Previous surgery or radiotherapy	Multiple treatments with different frequency and combinations	Bleomycin (100 ul) (1 IU)/cm^3, intratumorally or gemcitabin (0.5–10 mg/cm^3, intratumorally)	cIL-12 (2 mg/cm tumor diameter, intratumorally)	Needle electrode; 2 pulses, 20 ms, 350 V/cm, 10 Hz	ECM 830 pulse generator, BTX®	AA, PC, SCC, sarcoma	9 dogs (13 dogs total included with tumors at other locations)	27% volume reduction in, SCC and PC (in 3 weeks); 165% volume increase in sarcomas (exact observation period unknown)	(64)
4	Neoadjuvant marginal surgery	1–5 treatments (2–4 weeks interval)	Bleomycin (0.3 mg/kg once IV)	cIL-12 (2 mg per treatment, peritumorally)	Plate or needle electrodes; ECT (8 pulses, 100 µs, 1,300 V/cm, 5 kHz) Multielectrode array (MEA) electrode; GET (24 pulses, 150 ms, 60 V, 4 Hz)	Cliniporator TM	OMM, stage I, II and III	9 dogs	One month after the treatment: CR 33%, PR 33%, PD 33% End of observation period (2–22 months): CR 11%, PD 89% MST 6 months	(65)

NA, not applicable; ECT, electrochemotherapy; GET, gene electrotransfer; fIL-12, feline interleukin-12; cIL-12, canine interleukin-12; CR, complete response; PR, partial response; PD, progressive disease; MST, median survival time; OMM, oral malignant melanoma; AA, acanthomatous ameloblastoma; SCC, squamous cell carcinoma; FSA, fibrosarcoma; PC, plasmacytoma; MST, median survival time.

Finally, when recommending cancer treatment, clinicians need to successfully manage clients' expectations, especially with regard to their animal's well-being. In this respect, electroporation-based treatments offer a treatment option that mostly results in an increased quality of life of the patients, despite observed tumor necrosis (expected) and transient pain of ∼2 weeks' duration. There are no occurrences of nausea or gastrointestinal problems and mostly no changes in the normal habits of the animal (35, 40, 56, 64, 65, 85). Moreover, most of the owners (86.4%) of the 44 dogs opting for ECT/GET treatment for their dog reported that they would opt for the same treatment modality again as they assessed the health-related quality of their dogs' life improved 1 month after the treatment (as per RECIST criteria the optimal time to evaluate response); additionally, for those owners, dogs receiving the ECT/GET treatment had an OR (92). While this phenomenon may be true for dogs bearing cutaneous or subcutaneous tumors, the owners of six dogs with oral tumors enrolled in the study reported a worsened health-related quality of life; this was expected as most of these cases were treated with a combination of surgery and ECT/GET and as the response rates of oral tumors to ECT/GET are still lower than cutaneous and subcutaneous tumors (92). However, Tellado et al. (83) recently specified further that quality of life of dogs with OMM treated with ECT improved, but only for dogs with tumor stages I–III, and only if CR or PR was achieved.

In terms of the success of electroporation-based treatments for oral and maxillofacial tumors in small animals, recommendations to clients should be given with some precautions. Namely, data obtained from the studies to date are difficult to interpret due to mostly small sample sizes, inconsistencies in staging of the oral tumors, different treatment protocols and lack of data for cats (**Tables 1–3**). Another issue that makes comparisons between outcomes of the studies difficult is the discrepancy among the studies in evaluations of the tumor response; some studies, for example, define CR as the disappearance of all evidence of tumor, PR as a decrease in tumor size by at least 50%, SD as a decrease of <50% or an increase of 25% and PD as an increase of the tumor by 25% (74, 75), while others consider PR as a ≥30% reduction in tumor diameter, SD <30% reduction in tumor diameter or <20% increase in tumor diameter and PD ≥20% increase in tumor diameter (34, 65); in further studies, the distinctions were unclear (83). To improve the quality of reporting clinical studies from this field, recommendations for reporting were published in human oncology (93), which could be extrapolated to veterinary use as well.

Within these limitations, however, it can be safely concluded that the best local response with ECT is reported for small oral tumors (SCC and OMM, **Figure 4**), which is likely related to the nature of the treatment (size of the electrodes) as well as the reduced possibility of necrosis, which affects the distribution of the chemotherapeutic (35, 83). Similarly, tumors not involving the bone are easier to treat due to the easier insertion of electrodes (83) and similar tissue conductivity (44). It must be stressed that ECT is a local ablative treatment without a noticeable/expected effect on distant metastasis and should therefore be combined with other treatments in cases of metastatic disease (12). If ECT is combined with GET or GET performed as a monotherapy, a systemic therapeutic effect is expected (and observed in some studies as systemic immune stimulation), but studies to date show no confirmed effect on distant metastasis (85) or a response that is mixed at best (56). Combination with other treatments, such as surgery, radiotherapy, immune checkpoint inhibitors, antiangiogenic therapy or metronomic cyclophosphamide therapy, have also been suggested by several authors (40, 64, 65, 70, 71, 76, 85), but further studies are needed combining different treatment modalities. Additionally, ECT should be used with caution in previously irradiated fields due to the possibility of the animals developing radiation recall (94). However, in human oncology, several studies have evaluated tumor treatment in previously irradiated fields. The major conclusion from these studies is that the antitumor effectiveness of ECT is lower in previously irradiated tumors than naïve tumors, also resulting in more severe necrosis and inflammation. However, no studies have reported major radiation recall; thus, it can be expected that this will not develop in veterinary patients (95, 96).

FUTURE TRENDS OF ECT/GET IN VETERINARY ORAL AND MAXILLOFACIAL ONCOLOGY

Although ECT, GET and their combination are already used for the treatment of client-owned dogs and cats with cancers of different origins and at different locations (32, 49, 65), several open questions remain.

It has been well-established that ECT can be effective as a one-time treatment, and that if it is not, further ECT treatments can be performed, but there is currently no consensus on when is the best time for retreatment (12, 83). Similarly, electroporation condition optimization for oral tumors is needed (44, 85).

Candidate genes for GET, the location of delivery and the dose of the plasmids encoding candidate genes as well as the dose of chemotherapeutics also require further investigation. While GET with peritumoral delivery of a plasmid encoding IL-12 has already shown promising results in the treatment of different tumor types in dogs (49, 65, 97), the effect of intratumoral application of the plasmid must be established. Similarly, bleomycin pharmacokinetics has to be better understood; in elderly human patients treated with ECT, a lower bleomycin dose was recommended based on pharmacokinetic studies (98), as ECT with a lower bleomycin dose showed comparable antitumor efficacy to using a standard dose (99, 100).

Finally, the reasons for clinically observed individually different responses to treatment remain to be elucidated with the identification of biomarkers that will enable the better selection of patients that will benefit from the treatment and prediction of response and recurrence (**Figure 5**). Specific T cell populations in whole blood can be potentially used as biomarkers for early recurrence, showing that additional treatments should

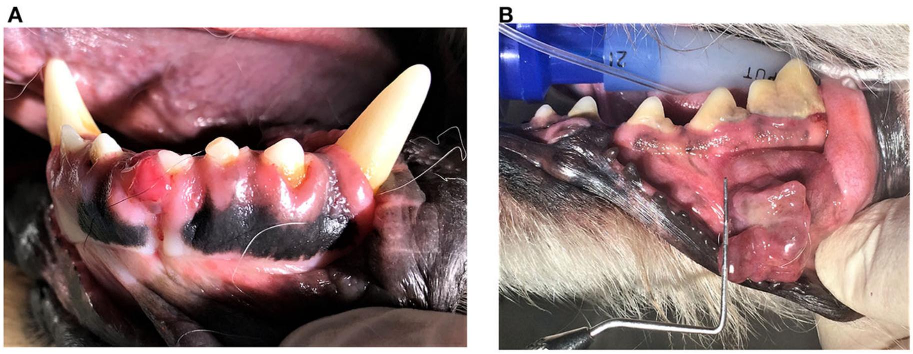

FIGURE 4 | Clinical example of a small squamous cell carcinoma (SCC) affecting the gingiva at the right mandibular first incisor tooth **(A)** and malignant melanoma of the buccal mucosa **(B)** in a dog. Appropriate staging of the disease (biopsy of the lesion, evaluation of the local disease extent and regional lymph nodes and distant organs metastasis employing advanced imaging techniques) is needed before any treatment and prognosis are discussed.

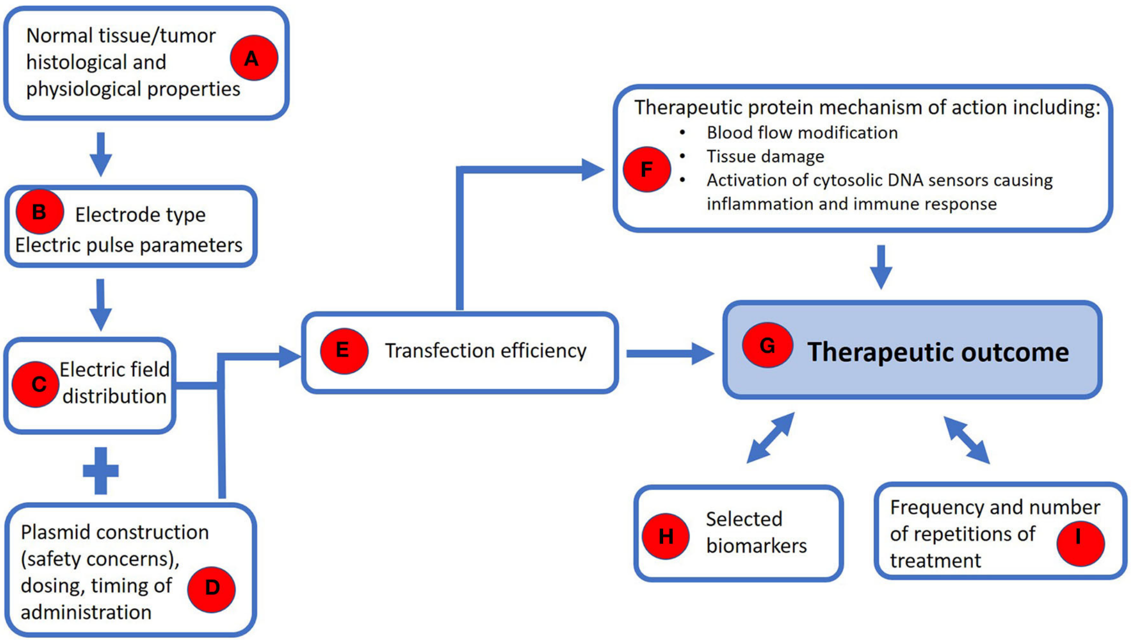

FIGURE 5 | A model for safe and effective use of the GET with further guidance for its wider clinical use. The histological and physiological properties of the tumor and its surrounding normal tissue potentially involved in the neoplastic process (e.g., bone) **(A)** dictate selection of the type of electrode and the parameters of electric pulses **(B)** in order to achieve an appropriate distribution of the electric field **(C)**. A safe plasmid should be used at the appropriate dose and time window prior to the application of electrical pulses **(D)** to ensure sufficient transfection **(E)** that would lead to the production of therapeutic protein **(F)**. The mechanism of action of the therapeutic protein takes place on several levels–through blood flow modification and tissue damage, thereby activating DNA sensors in the cytosol, leading to an inflammatory and immune response **(F)**. To achieve a therapeutic outcome **(G)**, we need to monitor selected biomarkers **(H)**, based on which we could determine the appropriate frequency and number of repetitions of treatment **(I)**.

be applied (101, 102). High expression of immune checkpoint inhibitors, such as PD-1 and PD-L1, as well as their binding and consecutive immune evasion of the tumor, could also be used as predictive factors for the response to immunotherapy (103, 104). Moreover, the response to immunotherapy of different tumors seems to be specific to the individual, and in particular, the intestinal microbiota is considered an important immune response modulator in human oncologic patients (105). Research has shown that in human melanoma patients, the success of immunotherapy correlates/varies with the composition of the intestinal microbiota (106, 107). This phenomenon could be of a great importance in implementing personalized therapeutic protocols (108). In dogs, a difference in intestinal microbiota composition has been reported between those with colorectal epithelial tumors (109) or lymphoma (110) and healthy individuals. However, there is currently no information on peripheral solid tumors and the role played by the gastrointestinal microbiota in the response to immunotherapy in dogs.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work and approved it for publication.

FUNDING

This work was supported by Slovenian Research Agency grants P4-0053, P3-0003, and J3-6796.

ACKNOWLEDGMENTS

The manuscript was edited for language by American Journal Experts.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Veterinary Science

ORIGINAL RESEARCH
published: 05 November 2020
doi: 10.3389/fvets.2020.588945

Ligation of the Maxillary Artery Prior to Caudal Maxillectomy in the Dog—A Description of the Technique, Retrospective Evaluation of Blood Loss, and Cadaveric Evaluation of Maxillary Artery Anatomy

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OPEN ACCESS

Edited by:
*Boaz Arzi,
University of California, Davis,
United States*

Reviewed by:
*Santiago Peralta,
Cornell University, United States
Carsten Staszyk,
University of Giessen, Germany*

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Specialty section:
This article was submitted to Veterinary Dentistry and Oromaxillofacial Surgery, a section of the journal Frontiers in Veterinary Science

Received: *29 July 2020*
Accepted: *08 October 2020*
Published: *05 November 2020*

Citation:
Carroll KA and Mathews KG (2020) Ligation of the Maxillary Artery Prior to Caudal Maxillectomy in the Dog—A Description of the Technique, Retrospective Evaluation of Blood Loss, and Cadaveric Evaluation of Maxillary Artery Anatomy. Front. Vet. Sci. 7:588945. doi: 10.3389/fvets.2020.588945

Two different surgical techniques have been described for performing caudal maxillectomies in dogs including the intraoral (IO) and combined dorsolateral and intraoral (DL-IO) approach. Hemorrhage is the most common intraoperative complication reported during these procedures as maxillary arterial ligation is not performed until after all osteotomies and mobilization of tumor-bearing bone. The objectives of this study were to describe a modified approach for caudal maxillectomy in the dog involving preligation of the maxillary artery, to retrospectively evaluate the ability of this modified approach to limit hemorrhage in a cohort of 22 dogs, and to clarify the vascular anatomy of the maxillary artery and its branches in relation to associated nerves. Medical records were retrospectively reviewed for cases that had caudal maxillectomy via a combined approach (with or without preligation of the maxillary artery) from January 1, 2004 to December 31, 2019. Twenty-two cases were identified, six without, and 16 with arterial preligation, respectively. Osteotomies were completed with a high-speed handpiece and rotary bur ($n = 18$), or oscillating bone saw ($n = 4$). All six (100%) dogs in the traditional DL-IO group developed hypotension under general anesthesia. Four (67%) of these required intraoperative blood transfusions, one of which required an additional postoperative blood transfusion. In contrast, only one of 16 (6%) dogs in the modified DL-IO group required an intraoperative blood transfusion, and only three (19%) developed hypotension. Moreover, a significant association was detected between postoperative PCV and the two different surgical approaches ($P = 0.021$). These results demonstrate the effectiveness of preligation of the maxillary artery in preventing hemorrhage in caudal maxillectomies in dogs and this represents an improvement in outcome over previously reported studies. Decreased intraoperative hemorrhage may improve surgical exposure and decrease overall patient morbidity.

Keywords: caudal maxillectomy, preligation, hemorrhage, blood loss, maxillary artery ligation

INTRODUCTION

The oral cavity is a common location for the development of neoplasia in small animals (1, 2). Caudal maxillectomy is often part of the treatment plan for caudally located oral neoplasms that have not yet crossed the midline of the hard palate (3, 4). It is common for these tumors to be large in size, as it can be difficult for owners to recognize tumor growth in this location (5).

Two different surgical techniques have been described for performing a caudal maxillectomy in dogs including intraoral (IO) and combined dorsolateral and intraoral (DL-IO) approaches. The IO approach was first described in 1985 (6, 7) and is recommended for unilateral benign and malignant tumors located along the alveolar margins of the mid-to-caudal maxilla (8). While this technique is effective for tumors adjacent to the dental arch, visualization becomes difficult for larger tumors in this area, especially for more dorsal or caudally located neoplasms (9). The combined DL-IO approach was described in 2003 as a modification of the existing procedure (9). This technique is recommended for tumors of the mid-to-caudal maxilla that arise or extend dorsolaterally and/or caudally into the inferior orbit, and provides improved exposure and thus increased ability to resect the mass to microscopic disease and potentially achieve clean surgical margins (8, 9).

The maxillary nerve is a branch of the trigeminal nerve and as such contains sensory fibers (10). After exiting the rostral alar foramen into the caudal aspect of the orbit, the maxillary nerve gives off the pterygopalatine nerve ventromedially which in turn gives rise to the minor palatine, major palatine and caudal nasal nerves [(10–12); **Figure 1**]. Both the maxillary and pterygopalatine nerves run rostrally on the dorsolateral surface of the medial pterygoid muscle along the medial aspect of the orbit. Further rostrally, after giving off the caudal superior alveolar and caudal nasal nerve branches ventrally, the maxillary nerve becomes the infraorbital nerve caudal to the maxillary foramen (entrance to the infraorbital canal). This area just caudal to the maxillary foramen is where a maxillary nerve block would occur (10).

Blood supply to the maxilla is provided by four branches of the maxillary artery: the major and minor palatine, sphenopalatine, and infraorbital arteries (11). The maxillary artery is the main continuation of the external carotid artery (11). In the rostral aspect of the orbit, the maxillary artery gives off the minor palatine artery ventrally, and this then divides into the similarly sized infraorbital and descending palatine arteries [(11, 12); **Figure 1**]. The descending palatine artery subsequently divides into the major palatine and sphenopalatine arteries ventrally, while the infraorbital artery continues rostrally through the maxillary foramen to enter the infraorbital canal.

Because of the maxillary artery's location, it is typically not ligated until after the majority of tumor resection has been completed during caudal maxillectomies (3, 8, 9). Regardless of bone cutting device (e.g., osteotome and mallet, oscillating bone saw, high speed rotary bur, or piezoelectric oral surgery unit), hemorrhage is the most common intraoperative complication reported during caudal maxillectomy (4). Intraoperative hemorrhage requiring transfusion was noted in six of 20 (30%) dogs in one study (9), 44 of 103 (42.7%) dogs in a second study (4), and one of two (50%) dogs in another study (5). Additionally, one dog died of hemorrhage and hypovolemic shock following caudal maxillectomy in another study (13). In each of these studies, an oscillating bone saw was the predominant bone cutting device used.

According to the initial description of the DL-IO approach, if bleeding is encountered, this is to be addressed at the end of the osteotomies, following mobilization of the free segment of bone via ligation or vascular clips (9). These patients will inevitably hemorrhage, so speed is the major factor in preventing excessive blood loss, especially when using an oscillating bone saw (9). While temporary occlusion of the ipsilateral carotid artery can be performed (14), no studies have examined the specific effectiveness of preligation of the maxillary artery in preventing blood loss in caudal maxillectomy patients. Additionally, although the anatomy of the maxillary and infraorbital arteries and nerves has been well-described, the relationship of these structures to each other is only briefly mentioned (10, 12). In one "anatomy of the dog" textbook, the maxillary artery and its branches are drawn lateral to the maxillary and infraorbital nerves (10). An understanding of the spatial relationship of these structures caudal to the infraorbital canal could be important to surgeons performing arterial ligation prior to completion of large caudal maxillectomies and to anyone performing maxillary nerve blocks. The objectives of this study were therefore, (1) to describe a modified DL-IO approach for caudal maxillectomy in dogs involving preligation of the maxillary artery, (2) to retrospectively evaluate the ability of this modified approach to limit hemorrhage, and (3) to clarify the vascular anatomy of the maxillary artery and its branches in relation to associated nerves in this area by evaluating latex injected canine cadavers. We hypothesized that ligation of the maxillary artery at the start of caudal maxillectomy would result in less hemorrhage and subsequent need for blood transfusion.

MATERIALS AND METHODS

Part I: Retrospective Evaluation

Retrospective Inclusion Criteria

Medical records of dogs undergoing maxillectomy ± orbitectomy at the North Carolina State University Companion Animal Veterinary Medical Center between January 1, 2004 to December 31, 2019 were reviewed. Intraoral maxillectomies ($n = 69$) were not included in this study. Cases without a complete surgical or anesthetic report were excluded from the study. All caudal maxillectomy cases performed via a combined approach with or without preligation of the maxillary artery ($n = 22$) were evaluated for tumor location, regional lymph node involvement and pulmonary metastases via computed tomography (CT) of the head, aspiration of affected lymph nodes and thoracic radiographs. A minimum of 4 weeks postoperative follow-up was necessary for study inclusion.

Data Collection

For each dog included in the study, data was collected from the medical record including signalment, weight

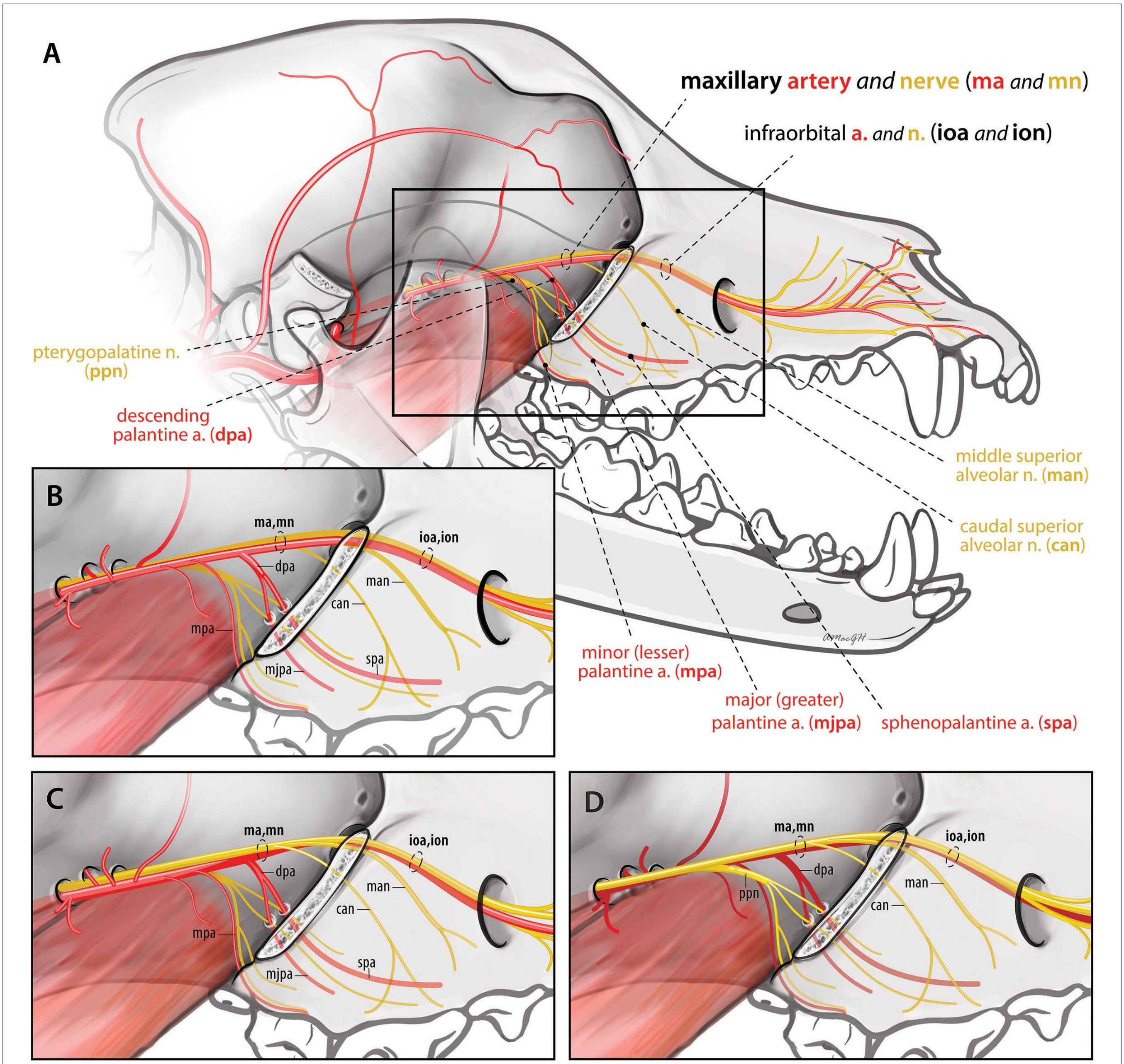

FIGURE 1 | Spatial relationship of the maxillary nerve and artery branches caudal to the infraorbital canal. **(A,B)** (inset) Version 1: Arterial branches remain lateral to nerve branches throughout ($n = 7$). Maxillary artery (ma), minor palatine artery (mpa), descending palatine artery (dpa), infraorbital artery (ioa), major palatine artery (mjpa), sphenopalatine artery (spa), maxillary nerve (mn), caudal superior alveolar nerve (can), middle superior alveolar nerve (man), infraorbital nerve (ion). **(C)** Version 2: The infraorbital artery rostral to the descending palatine artery runs ventral and then medial to the nerve prior to entering the infraorbital canal ($n = 12$). **(D)** Version 3: All arterial branches lay medial to the maxillary, caudal superior alveolar, middle superior alveolar and infraorbital nerves ($n = 1$).

(kg), clinical signs, date of surgery, tumor size (cm) as measured by CT, tumor location, extent of surgical resection, type of surgical approach (DL-IO vs. modified DL-IO ± orbitectomy and enucleation), duration of surgery (minutes), preoperative PCV (%), intraoperative PCV (%), postoperative PCV (%), requirement of a blood transfusion (including amount), intraoperative complications, immediate postoperative complications (<48 h postoperatively), short-term complications (48 h to 4 weeks postoperatively), histopathological diagnosis (with margins), and adjunctive therapy. Wound dehiscence was defined as the breakdown or opening of an oral wound or suture line, and oronasal fistula formation was defined as an abnormal opening between the oral and nasal cavity.

Surgical Procedure

All procedures were performed by a board-certified surgeon assisted by a surgical resident. Surgical approach (IO vs. DL-IO vs. modified DL-IO) was ultimately chosen at the discretion of the attending surgeon. Likewise, anesthetic protocols were determined by the attending anesthesiologist and included a combination of pre-, intra- and postoperative opioids, as well as regional nerve blocks to provide an appropriate level of analgesia throughout the procedure. The DL-IO surgical technique was performed routinely as per previous recommendations (9). However, the modified DL-IO approach included preligation of the maxillary artery at the start of caudal maxillectomy.

Modified DL-IO Approach (Preligation of the Maxillary Artery)

All animals were placed in lateral recumbency with the affected side up and prepared for surgery in a routine manner as outlined by the original DL-IO approach (9). Enucleation, if required was performed first. Following enucleation, the maxillary artery and associated nerve were identified caudal to their entrance into the infraorbital canal (maxillary foramen) within the orbit and were occluded with at least two vascular clips (Weck Hemoclip, Teleflex Medical, Research Triangle Park, NC, USA). The remaining procedure was performed as per the original DL-IO description (9). For dogs that did not require enucleation, the skin incision made for the combined approach was extended caudally over the zygomatic arch. Dissection through the subcutaneous tissues and paired levator nasolabialis muscles provided exposure of the zygomatic arch. Angularis oculi and fascial veins were double ligated and transected as they were encountered. The masseter muscle (ventrally) and periosteum were cleared from the zygomatic arch. Rather than performing a single zygomatic osteotomy as previously described (9), two osteotomies were performed and a section of zygomatic arch was removed just caudal to the tumor. Osteotomies were performed using either a high-speed handpiece and rotary bur (Surgairtome Micro100^{TM} Pneumatic, Conmed, Utica, NY, USA), or an oscillating bone saw, and irrigation was provided with continuous lavage of sterile saline. Regional vasculature was approached and cleaned of surrounding soft tissues, primarily periorbital fat, by retracting the eye dorsocaudally with a small malleable retractor. The maxillary artery and nerve were then exposed in the rostral aspect of the orbit using blunt dissection with cotton-tip applicators and right-angled forceps. Vital structures running into the maxillary foramen had vascular clips placed across them. No attempt was made to separate artery from nerve or to locate/identify ventral branches of the maxillary artery (e.g., minor or descending palatine arteries). The maxillary artery was occluded with at least two vascular clips in the rostral aspect of the orbit. The section of the zygomatic arch was submitted for caudal margin histopathological analysis in all 16 cases.

The remainder of the procedure was performed through a combined approach as previously described (9). In brief, the initial dorsal skin incision was extended rostrally to the desired level, and dissection was continued through the subcutaneous tissue, between the paired levator nasolabialis muscles, and down to bone. Periosteum and associated soft tissues were reflected with a periosteal elevator. A second incision was made in the buccal mucosa immediately dorsal to the gingiva. Dissection was continued until connection was made with the skin incision, thereby creating a bipedicle flap of skin, buccal mucosa and associated soft tissues. Retraction of this flap allowed adequate visualization of the lateral aspect of the maxilla and identification of osteotomy locations. Rostral, dorsal and caudal osteotomies were then performed after all soft tissues had been dissected, and residual bleeding was controlled with digital pressure, electrocautery, suture ligation, or hemoclip application. Once all bleeding was controlled, various IO and extraoral reconstructive techniques were used at the discretion of the attending surgeon to achieve a tension-free closure. Further information regarding closure is available in the study describing the original DL-IO approach (9).

Upon recovery, all dogs were given a non-steroidal anti-inflammatory drug such as carprofen (Rimadyl, Zoetis, Parsippany, NJ, USA; 4.4 mg/kg SQ). Postoperatively, they were maintained on a combination of intravenous fluids with lactated Ringer's solution (Baxter Healthcare, Deerfield, IL, USA; 45–60 mL/kg/day), analgesic agents including fentanyl (Generic, Hospira, Lake Forrest, IL, USA; 2–4 μg/kg/h IV) and ketamine (Ketaset, Zoetis, Parsippany, NJ, USA; 0.2–0.5 mg/kg/h IV), and anti-nausea medications such as ondansetron (Mylan, Canonsburg, PA, USA; 0.5 mg/kg IV q8h). PCV, total protein and blood glucose was checked 2 h postoperatively, and overnight, these dogs were monitored closely for any signs of hypotension, facial edema, epistaxis and/or respiratory compromise. Most animals were transitioned onto a transdermal fentanyl patch (Generic, LTS Lohmann, West Caldwell, NJ, USA; 2 μg/kg/h), as well as oral pain medications including carprofen (2.2 mg/kg PO q12h 7 days) and gabapentin (Generic, Strides Pharma, East Brunswick, NJ, USA; 5–10 mg/kg PO q8–12h 14 days), and discharged from the hospital at a minimum of 2 days postoperatively.

Part II: Evaluation of Cadaveric Maxillary Artery Anatomy

To better define the spatial relationship of the maxillary artery and its branches to the maxillary nerve and thus to assist surgeons with vascular clip placement, cadaveric evaluation was performed after collection of retrospective data. Twenty medium to large breed formalin fixed latex injected canine cadaver heads were evaluated. Heads had been sagittally sectioned prior to fixation (Sargeants Wholesale Biologicals, CA, USA). Ten left and 10 right sided specimens were dissected to expose the maxillary artery and nerve branches along the medial wall of the orbit. The globe, zygomatic arch, lateral wall of the infraorbital canal and orbital soft tissues were removed to improve exposure. The maxillary artery, minor (lesser) palatine artery, major (greater) palatine artery, sphenopalatine artery and infraorbital arteries caudal to the maxillary foramen and within the infraorbital canal were identified. The relationship of these vessels to the maxillary nerve branches was recorded, drawn and photographed (**Figure 1**).

Statistical Analyses

Clinical data was summarized using descriptive statistics (median with range; mean ± SD). Duration of surgery (minutes), preoperative PCV (%), intraoperative PCV (%), postoperative PCV (%), requirement of a blood transfusion (including amount) were compared between the two groups (combined approach ± preligation) using a two-tailed student's *t*-test and boxplot graphs. Immediate postoperative complications (<48 h), short-term complications (48 h to 4 weeks), and clean histopathological margins were compared between the two groups (combined approach ± preligation) using a one-way ANOVA test. Pearson correlation analyses were performed between dog size and tumor size, in relation to duration of surgery and requirement of an intraoperative blood transfusion. All analyses were performed with commercially available software (GraphPad Prism8, San Diego, CA, USA), and values of $P < 0.05$ were considered statistically significant.

RESULTS

Retrospective Case Series

Ninety-one maxillectomies were identified. Sixty-nine (76%) dogs had IO resections and these maxillectomies were not included in this study. Twenty-two (24%) animals had undergone caudal maxillectomy via a combined approach. Of these 22 dogs, six (27%) were performed via a traditional DL-IO procedure between 2004 and 2007 without preligation of the maxillary artery prior to osteotomies, and 16 (73%) were performed via a modified DL-IO approach between 2004 and 2018 with preligation of the maxillary artery prior to maxillectomy.

For the combined approach ($n = 22$), the most commonly reported breeds included the Labrador Retriever ($n = 5$), English Bulldog ($n = 2$), Golden Retriever ($n = 2$), Husky ($n = 2$) and German Shepherd ($n = 2$). Twelve dogs were female (one intact and 11 spayed) and 10 were male (one intact and nine neutered). Body weight and age ranged from 5.2 to 42.1 kg (median, 28.1 kg; mean, 26.5 ± 11.3 kg) and 1.5 to 14 years (median, 8.1 years; mean, 8 ± 3.5 years), respectively.

The right caudal maxilla ($n = 19$; 86%) was the most common location for the combined approach, followed by the left caudal maxilla ($n = 3$; 14%). These tumors often encompassed the premolar teeth and extended caudally to include the last molar tooth, as well as the rostral zygomatic arch and ventral orbit ($n = 18$; 81%). The total number of teeth removed ranged from three to nine (median, five teeth). Tumor types included acanthomatous ameloblastoma ($n = 6$; 27%), osteosarcoma ($n = 4$; 18%), fibrosarcoma ($n = 4$; 18%), and 1 (5%) each of the following tumor types: malignant melanoma, peripheral odontogenic fibroma, keratinizing ameloblastoma, multilobular tumor of bone, amyloid-producing odontogenic tumor, myxosarcoma, rhabdomyosarcoma, and squamous cell carcinoma. Based upon CT measurements, maximum tumor diameter ranged from 1.5 to 9 cm (median, 4 cm; mean, 4.2 ± 1.9 cm) by 1 to 7 cm (median, 4 cm; mean, 3.8 ± 1.4 cm) in the dorsoventral and mediolateral directions, respectively.

Six (27%) dogs had concurrent enucleation performed at the time of combined approach, and 16 (73%) did not. Of the six dogs that required enucleation, four had a traditional DL-IO approach without preligation of the maxillary artery and these were performed early in the study period between 2004 and 2006. The other two enucleations were performed in conjunction with the modified DL-IO approach between 2010 and 2018. The mean surgical time for the DL-IO and modified DL-IO approaches were 245 ± 69 and 196 ± 56 min, respectively. There was no significant difference in surgical times between the two types of procedures ($P = 0.107$), and there was no correlation in surgical duration when compared to dog size ($r = 0.24$, $P = 0.287$) or tumor size ($r = 0.04$, $P = 0.856$). Finally, there was no difference in surgical duration for cases that did (mean, 200.5 ± 55.3 min) and did not (mean, 212.1 ± 65.7 min) have an enucleation performed ($P = 0.705$).

In the traditional DL-IO group, four of the six (67%) animals required intraoperative blood transfusions. Two of these dogs required multiple units of intraoperative packed red blood cells (pRBCs), and one required an additional postoperative transfusion. Osteotomies were completed with a high-speed handpiece and rotary bur in five animals, and an oscillating bone saw in one animal. There was no association between bone cutting instrument and requirement for an intraoperative blood transfusion ($P = 0.294$). In this group, preoperative and postoperative PCV ranged from 40 to 54% (median, 43%; mean, 44.5 ± 5.1%) and 23 to 44% (median, 32.5%; mean, 32.7 ± 7.1%), respectively. The mean drop in PCV was 11.8 ± 9.8%. All six (100%) dogs in this group developed hypotension under anesthesia with a mean blood pressure <70 mmHg for >5 min.

In contrast, only one of the 16 (6%) dogs in the modified DL-IO group required an intraoperative blood transfusion. The preoperative PCV for this patient was 50%. Moderate hemorrhage was observed intraoperatively and the PCV dropped to 32%, necessitating the requirement of two units of pRBCs. Osteotomies were completed with a high-speed handpiece and rotary bur in 13 animals, and an oscillating bone saw in three animals. There was no association between bone cutting instrument and requirement for an intraoperative blood transfusion ($P = 0.395$). Of the 16 dogs in this group, preoperative and postoperative PCV ranged from 38 to 52% (median, 45.5%; mean, 46 ± 3.7%) and 39 to 48% (median, 39%; mean, 39.2 ± 4.9%), respectively. The mean drop in PCV was 6.8 ± 5.3%. Only three of 16 (19%) dogs in this group developed hypotension with a mean blood pressure <70 mmHg for >5 min. When compared, no significant differences were found between surgical approach (DL-IO vs. modified DL-IO) and preoperative PCV ($P = 0.456$) or drop in PCV ($P = 0.135$). However, a significant association was detected between postoperative PCV between the two groups ($P = 0.021$) with the postoperative PCV being higher in the maxillary artery preligation group, as compared to dog's treated with the traditional DL-IO approach (**Figure 2**). There was no correlation in terms of the requirement for an intraoperative blood transfusion when compared to dog size ($r = 0.05$, $P = 0.838$) or tumor size ($r = 0.09$, $P = 0.700$).

The most common immediate postoperative (<48 h) complications included facial swelling ($n = 17$; 11 with, and six without preligation), epistaxis ($n = 14$; eight with, and six without preligation), and facial pawing ($n = 2$; 0 with, and two

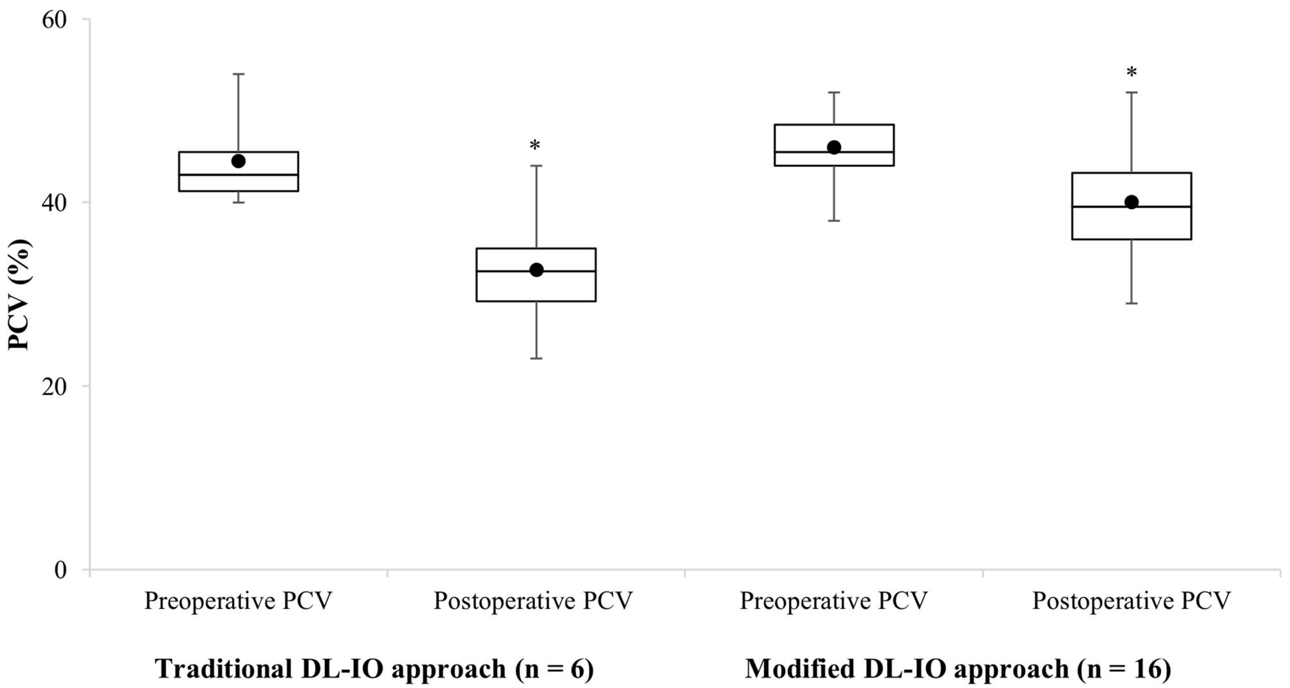

FIGURE 2 | Boxplot representation of the preoperative and postoperative changes in PCV in 22 dogs undergoing caudal maxillary via a combined approach (traditional DL-IO approach, $n = 6$, four animals received intraoperative packed red blood cell transfusions; modified DL-IO approach with preligation of the maxillary artery, $n = 16$, one animal received an intraoperative packed red blood cell transfusion). The horizontal line denotes the median, the boxes represent cases within the 25–75th percentile, and the upper and lower bars represent the highest and lowest observed values that are not outliers. Asterisk (*) denotes significant difference.

without preligation). While facial swelling was most common (77%), this complication was self-limiting and resolved with supportive care. The most common short-term (48 h to 4 weeks) complications included oronasal fistula formation ($n = 5$; three with, and two without preligation), wound dehiscence ($n = 4$; two with, and two without preligation), lip ulceration ($n = 3$; one with, and two without preligation), and orbital swelling ($n = 2$; two with, and 0 without preligation). All five dogs with oronasal fistulas required revision surgery involving a buccal mucosal flap. Clean histopathological margins were obtained in 16 of 22 (73%) dogs as a result of the initial procedure. All six patients with incomplete surgical margins were treated with adjunct therapy including a combination of radiation and chemotherapy. When compared, no significant differences were found between surgical approach (DL-IO vs. modified DL-IO) and immediate postoperative complications ($P = 0.224$), short-term complications ($P = 0.341$), or clean histopathological margins ($P = 0.124$). Of the 22 dogs in the study, five had died by 6 months postoperatively. Each of these dogs had incomplete surgical margins and were euthanized for local disease recurrence ($n = 2$), pulmonary metastases ($n = 2$), or other medical conditions ($n = 1$; gastric dilatation and volvulus).

Cadaveric Evaluation

In the 20 latex injected canine cadaver heads, three different spatial relationships of the arteries to the nerves were identified:

Version 1

All arteries remained immediately lateral to ventrolateral, to the nerves (maxillary, caudal superior alveolar, infraorbital and middle superior alveolar nerves) throughout their course (**Figures 1A,B**). Seven out of 20 sides (35%) (five left and two right) exhibited this neurovascular relationship. The infraorbital artery remained ventrolateral to the infraorbital nerve and its branches throughout the infraorbital canal.

Version 2

The arterio-neural spatial relationship was similar to version 1 except that the infraorbital artery rostral to the descending palatine artery, laid ventral and then medial to the ventral half of the infraorbital nerve as they entered the maxillary foramen. Within the infraorbital canal after the infraorbital nerve gave off the middle superior alveolar branches ventrally, the infraorbital artery laid ventrolateral to the infraorbital nerve. Additionally, the maxillary artery prior to giving off the minor palatine artery was just ventral to (contacting) the maxillary nerve, rather than lateral or ventrolateral to it (**Figure 1C**). Twelve out of 20 sides (60%) (four left and eight right) exhibited this neurovascular relationship.

Version 3

In one sample (5%) (left sided) the previously noted shift of the artery to a medial position occurred even earlier in its course (**Figure 1D**). The dorsal aspect of the maxillary artery laid medial to the maxillary nerve caudally. All arterial branches including the minor, descending, and major palatine, sphenopalatine and infraorbital arteries laid medial to the corresponding nerves. The infraorbital artery remained medial to the infraorbital nerve and its branches within the infraorbital canal.

In all specimens, the infraorbital and descending palatine arteries were of similar diameter and were the two largest branches of the maxillary artery.

DISCUSSION

Preligation of the maxillary artery resulted in less hemorrhage and subsequent need for blood transfusion when compared to historical data (4, 5, 9, 13) and when compared to caudal maxillectomy at this institution without preligation. Additionally, the spatial relationship of the maxillary artery and nerve branches was clarified and should aid surgeons performing this technique. Our overall findings support the safety and efficacy of this technique in caudal maxillectomy in dogs.

In six dogs, the maxillary artery was not preligated prior to caudal maxillectomy. Intraoperative hemorrhage was considered excessive in four of the six (67%) animals in this group who required intraoperative blood transfusions. Two of these dogs required multiple intraoperative transfusions, and one required an additional blood transfusion postoperatively. All six (100%) animals developed hypotension under anesthesia. These findings correspond with previous reports in the literature (4, 5, 9, 13). In contrast, results were more favorable for dogs that had preligation of their maxillary artery performed prior to caudal maxillectomy. Specifically, one out of 16 (6%) dogs required an intraoperative blood transfusion, and only three (19%) animals developed hypotension. A difference was identified between the two surgical approaches and postoperative PCV ($P = 0.021$; **Figure 2**). As four of the six dogs without preligation required an intraoperative transfusion, it is likely that the postoperative PCV difference between the two techniques would have actually been greater. These results suggest that preligation of the maxillary artery should be performed whenever possible, either through an enucleation prior to maxillectomy or via zygomatic ostectomy.

Zygomatic ostectomy allows access to the maxillary artery and nerve in the rostral aspect of the orbit. The rostral osteotomy can be performed close to the tumor to improve identification of these structures as long as the saw blade or bur used to make this cut is changed prior to other osteotomies to decrease the risk of tumor seeding. The zygomatic ostectomy is completed by making a second osteotomy caudal to the first and the section of the zygomatic bone is submitted for caudal margin histopathological analysis. Removal of this section of bone allows the surgeon improved exposure and facilitates retraction of the globe. Given the previous limited descriptions of the relationship of the maxillary artery to the nerve, the vascular clips were placed across all structures running horizontally into the maxillary foramen. All ligated structures including the maxillary nerve were transected at the end of the procedure, so having a clip that crosses both the artery and nerve was not an issue and we saw no complications postoperatively. The loose areolar connective tissue around the maxillary artery and nerve were easily separated in the cadaveric specimens using blunt dissection. Now that we have better defined the neurovascular anatomy of this area, it may be possible for surgeons to dissect the artery away from the nerve for more focused clip placement if desired. It is not known if preoperative assessment of the spatial relationship between the maxillary artery and nerve is possible, or which imaging modality would be most useful.

Regardless of maxillary artery preligation, the risk of hemorrhage during caudal maxillectomy may also vary depending on several other factors including surgeon experience (e.g., surgical resident, board-certified surgeon or dentist, oral and maxillofacial surgery fellow, years in practice, number caudal maxillectomies performed), preoperative diagnostic imaging and surgical planning, size of the tumor, size of the patient and breed, and availability and selection of surgical instrumentation. For this current study, all procedures were performed by a board-certified surgeon and a preoperative CT was performed prior to all caudal maxillectomies. Likewise, in similar studies where hemorrhage was also an important factor, a primary boarded surgeon and faculty member were consistently listed for each procedure (4, 5, 9, 13) and CT imaging was performed prior to all surgeries (4, 5, 9). However, it is acknowledged that the risk of hemorrhage may increase with less surgical training and experience, as well as lack of preoperative imaging. The majority of breeds in this study were mesocephalic, with the Labrador Retriever being most common ($n = 3$). Only three (14%) brachycephalic breeds were included in the study, and while head conformation may influence the risk of hemorrhage, the authors cannot comment further on this. Finally, it is possible that both dog size and tumor size may influence the risk of hemorrhage during caudal maxillectomy. However, in this study no statistically significant correlations in dog size or tumor size were detected in relation to surgical duration ($r = 0.24$, $P = 0.287$; $r = 0.04$, $P = 0.856$) or requirement for an intraoperative blood transfusion ($r = 0.05$, $P = 0.838$; $r = 0.09$, $P = 0.700$).

Two different types of bone cutting instruments were used in the present study including a high-speed handpiece and rotary bur ($n = 18$), and an oscillating bone saw ($n = 4$). Of the four caudal maxillectomies performed with an oscillating bone saw, two required an intraoperative blood transfusion. When further evaluated, there was no association between osteotomy instrument and the need for an intraoperative blood transfusion (traditional DL-IO group, $P = 0.294$; modified DL-IO group, $P = 0.395$). In terms of the veterinary literature, an oscillating saw is the predominant bone cutting device reported in caudal maxillectomy patients (4, 5, 9, 13). While the original DL-IO technique was described using an oscillating bone saw (9), it can be difficult to control fine movements with this instrument, and it has the potential to cause inadvertent and catastrophic damage to the neurovascular bundle (4, 15). The majority of caudal maxillectomies in the present study were performed using a high-speed handpiece and rotary bur. Irrigation was provided with sterile saline throughout the procedure, and these instruments allow for one cortex to be cut at a time and more precise

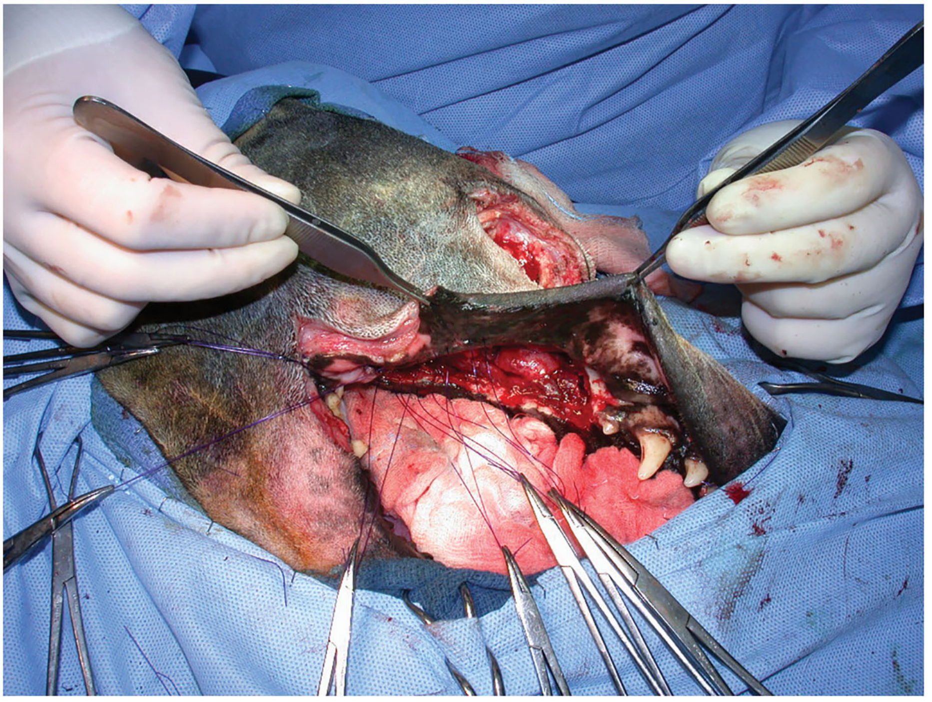

FIGURE 3 | Intraoperative photograph following arterial preligation and caudal maxillectomy via a combined approach. Note the lack of hemorrhage on the surgical drape.

osteotomies than an oscillating bone saw (4, 15). In human oral surgery, high speed handpieces are often contraindicated as they can generate inadvertent heat and can cause tissue emphysema and thermal necrosis (15). However, this complication has not been documented in animals, and was not seen in any of the dogs in the present study (16).

An alternative to these bone cutting devices is the piezoelectric oral surgical unit (17). Piezoelectric bone surgery is a recent and innovative technology in veterinary medicine where incisions are achieved through microvibrations (4). This instrument selectively cuts mineralized tissue at frequencies of 25–35 kHz, and prevents the user from inadvertently transecting nerves, blood vessels and soft tissue (17, 18). This thereby allows for precise osteotomies, excellent visibility within the surgical field, and significant reduction of soft-tissue trauma (17, 18). This technology was first developed for human dental and maxillofacial surgery (17), and its clinical application in people has since expanded to include neurosurgery, orthopedic surgery, as well as ear, nose and throat surgery (18, 19). However, piezoelectric bone surgery is less commonly documented in the veterinary literature (20–22), and while it would have been advantageous for cases in the present study, it is not currently available at the author's veterinary institution.

Knowledge of the spatial relationship of the maxillary artery and its branches to the maxillary nerve just caudal to the infraorbital canal may be important to surgeons performing caudal maxillectomy and to anyone performing maxillary nerve blocks prior to oral surgery. To our knowledge, this relationship has not been previously described in dogs, although it is illustrated in one canine text (10). This illustration, and a photograph from a single cadaver in another text (23) show the second most common relationship noted in the current study (Version 1; **Figures 1A,B**). Because cadaveric hemisections were used in this study, we were not able to evaluate potential asymmetry in anatomy between sides. Images from feline and equine anatomy articles and text books demonstrate the maxillary artery lateral to the maxillary nerve just caudal to the maxillary foramen, with no discussion regarding their spatial relationship or description of possible variations (24–26).

Relative diameters of the maxillary artery and its branches in this area are reported to be approximately 4 mm (maxillary artery), 0.5 mm (minor palatine artery), 1 mm (major palatine artery), and 2 mm (sphenopalatine artery), respectively in a medium sized dog (11). The infraorbital and descending palatine arteries are reportedly of similar diameter (11). Given the similar diameter of the infraorbital and descending palatine arteries, diminution of blood flow to the maxilla would be greatest if both branches were occluded, or if the maxillary artery was occluded caudal to their origins. The location of hemoclip application was not recorded for the retrospective cases reported. Regardless, blood loss was found to be diminished compared to previous reports given that only three dogs with maxillary artery preligation developed intraoperative hypotension and one dog required an intraoperative blood transfusion (**Figure 3**). This would appear to be an improvement over the 30–50% of dogs requiring intraoperative transfusions as reported in the literature (4, 5, 9, 13). In the one dog that required an intraoperative blood transfusion in the modified DL-IO group, a large degree of hemorrhage was reported following osteotomy with an oscillating bone saw. After discussing the case with the surgeon, they felt that the vascular clips may not have been fully across the maxillary artery or its branches, as additional clips were necessary at that location following tumor resection. Therefore, this hemorrhage was likely the result of inadequate hemoclip placement across the maxillary artery.

Preemptive arterial ligation to decrease the risk of intraoperative hemorrhage in maxillofacial surgery has a precedent in the veterinary literature, including ligation of the carotid artery when local measures or direct ligation of the bleeding vessel is not possible. Unilateral or bilateral common carotid artery ligations have been demonstrated to decrease lingual arterial pressure in the dog (14). However, no studies have evaluated the effectiveness of carotid artery ligation in reducing hemorrhage during maxillectomies. Carotid ligation requires a separate surgical approach to the cervical region and can be performed with no notable adverse effects due to the well-developed collateral circulation system in the dog originating from the vertebral arteries (27–32). There are at least five described sites of anastomoses between the internal and external carotid arteries in the dog, allowing them to survive with no residual effect following long-term ligation of one or both carotid arteries and other major vessels supplying the head (27–32). Likewise, multiple studies have demonstrated that ligation of the maxillary, infraorbital and/or major palantine vessels to control intraoperative hemorrhage does not result in adverse effects (6, 13). Circulation to the face and oral tissues is maintained by facial artery branches and contralateral maxillary and ethmoidal vasculature (9, 11). It is not known if maxillary artery ligation would eventually compromise structures especially the teeth, rostral to the surgical site. Although this retrospective study focused on intraoperative and short-term complications (and lacked long-term follow-up >1 year from the time of surgery), this complication was not identified in any of the animals during the study period.

As a retrospective study at a single academic referral hospital, several limitations are associated with the study presented here. The study was constrained by a limited sample size. Of the 22 dogs that met the inclusion data, only six and 16 were treated via a DL-IO and modified DL-IO approach, respectively. This variation in case numbers between the two procedures was likely due to different surgeon preferences over the long study period. Surgeon experience and overall comfort levels may have also affected decision making in terms of surgical approach. Enucleations described herein tended to be earlier in the study, reflecting the shift from two-dimensional and three-dimensional conformal radiotherapy to modern intensity-modulated radiotherapy, which is capable of super ocular-sparing (33). Concerns over including the eye in a potential radiation field likely had an influence on whether or not enucleation was performed.

In conclusion, this study demonstrates the effectiveness of preligation of the maxillary artery in limiting hemorrhage and the subsequent need for blood transfusions during caudal maxillectomy procedures in dogs. To the authors' knowledge, this is the first study to describe a modification of the existing DL-IO combined approach, and to clarify the regional vascular anatomy of the maxillary artery and nerve. Further prospective studies are indicated to evaluate the feasibility and safety of this approach in a larger cohort of clinical animals.

DATA AVAILABILITY STATEMENT

The original contributions generated for this study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

Ethical review and approval was not required for the animal study because, the data was collected retrospectively from medical records. Written informed consent for participation was not obtained from the owners because, there was no change in procedure other than the timing of arterial ligation.

AUTHOR CONTRIBUTIONS

KC: data collection, data analysis, manuscript composition, and final approval of the version to be published. KM: concept generation and design, data collection, data analysis, manuscript composition, and final approval of the version to be published. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

The authors would like to thank Alice Harvey for providing the illustrations and images used in this study, and to the surgeons who performed some of these procedures including Drs. Christopher Adin, Elizabeth Hardie, Kieri Jermyn, Duncan Lascelles, Milan Milovancev, Marije Risselada and Valery Scharf.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2020.588945/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Veterinary Science

METHODS
published: 11 December 2020
doi: 10.3389/fvets.2020.569747

Excision of Extensive Orbitozygomaticomaxillary Complex Tumors Combining an Intra- and Extraoral Approach With Transpalpebral Orbital Exenteration

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OPEN ACCESS

Edited by:
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University of California, Davis, United States

Reviewed by:
Kyle G. Mathews,
North Carolina State University, United States
Peter Southerden,
Eastcott Referrals, United Kingdom

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Specialty section:
This article was submitted to Veterinary Dentistry and Oromaxillofacial Surgery, a section of the journal Frontiers in Veterinary Science

Received: *05 June 2020*
Accepted: *25 November 2020*
Published: *11 December 2020*

Citation:
Thomson AE, Rigby BE, Geddes AT and Soukup JW (2020) Excision of Extensive Orbitozygomaticomaxillary Complex Tumors Combining an Intra- and Extraoral Approach With Transpalpebral Orbital Exenteration. Front. Vet. Sci. 7:569747. doi: 10.3389/fvets.2020.569747

The junction of the bones of the orbit, caudal maxilla and zygoma intersect to form an anatomically intricate region known as the orbitozygomaticomaxillary complex (OZMC). Given the critical role of the OZMC in the structure, function and esthetics of the skull and midface, tumors in this region present unique challenges to the oromaxillofacial surgeon. Attempts to achieve histologically clean tumor margins in a cosmetically pleasing manner requires excellent intra-operative visualization. Additionally, minimized intra-operative and post-opertive complications is of paramount importance. In this manuscript we describe a combined intra- and extraoral approach to extensive tumors of the OZMC that incorporates orbital exenteration as a technique, which allows for excellent intra-operative visualization and mitigate intra- and post-operative complications. In addition, we describe our experience utilizing the technique in five clinical cases.

Keywords: maxillectomy, zygomectomy, zygomatic tumors, zygomaticomaxillary tumors, orbitozygomaticomaxillary complex tumors, oral tumors, maxillary tumors, orbitectomy

INTRODUCTION

It is generally accepted that the primary goal of any tumor excision is to obtain tumor-free margins. Maintaining proper function, managing local pain, and achieving good to excellent cosmesis are additional goals. The degree to which one may achieve these goals is dependent on location and biological behavior of the tumor as well as the complexity of the resultant reconstruction procedure.

The orbitozygomaticomaxillary complex (OZMC) is the region of the skull where the zygomatic, and maxilla bones as well as bones that contribute to the medial orbital wall (lacrimal, frontal, and palatine bones) unite (**Figure 1**). The OZMC plays a critical role in the structure, function and esthetics of the skull and orbit contributing to the strength, stability and normal anatomical contours of the facial skeleton. Given the unique intersection of structurally and cosmetically important structures; the proximity of the orbit to the calvarium; and the challenges of access, the surgical excision of OZMC tumors may present unique challenges that make primary surgical goals difficult to achieve.

Excision of OZMC tumors often involves the medial orbital wall, which requires excellent visualization to prevent inadvertent penetration of the rostral cranial fossa. Additionally, significant ophthalmic side-effects, such as enophthalmos, diplopia and entropion, are expected if orbital

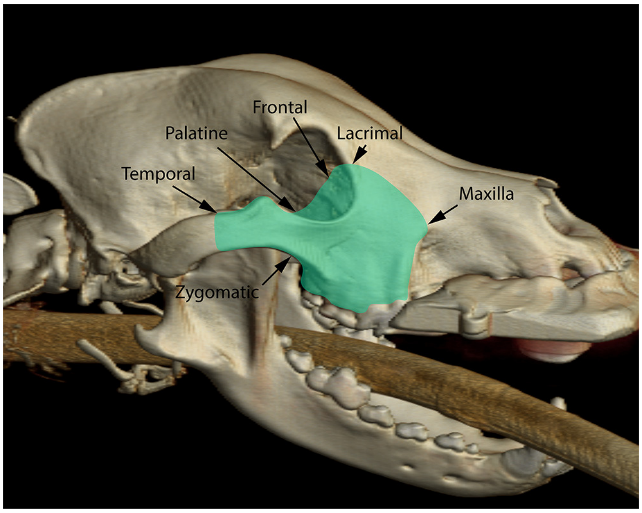

FIGURE 1 | Three-dimensional rendering of a dog skull highlighting (green overlay) the various osseous components of the orbitozygomaticomaxillary complex.

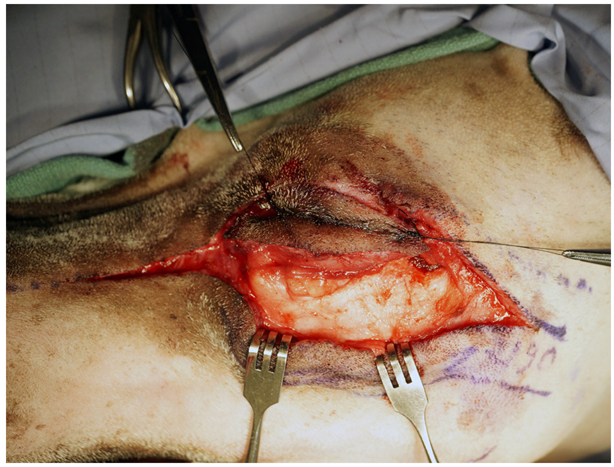

FIGURE 2 | Intraoperative photograph depicting the extraoral approach combining a horizontal incision from the dorsolateral maxilla into the zygoma with a transpalpebral approach to orbital exenteration.

reconstruction is not pursued (1–5). The degree to which a tumor of the OZMC has invaded orbital and periorbital structures may be uncertain. In such situations, a surgical approach to OZMC tumors that combines an intra- and extraoral approach with transpalpebral exenteration may be utilized. Transpalpebral exenteration is a modification to a previously reported technique (6, 7) that facilitates surgical excision of tumors significantly involving the medial orbit. Here we describe this approach to OZMC tumors and report our clinical experience in five dogs. To the authors' knowledge, such an approach has not previously been described in the peer-reviewed English language literature.

SURGICAL TECHNIQUE

Prior to surgical planning all patients received a head computed tomography (CT) study with and without contrast (GE Lighspeed Ultra, GE Healthcare, Milwaukee, WI), mandibular lymph node aspiration as well as aspiration of any parotid or medial retropharyngeal nodes that showed deviation from normal size or echogenicity. The airway was secured by standard orotracheal intubation immediately following induction of general anesthesia. Due to the nature of an academic anesthesia and pain management service, anesthetic protocols were not standardized and were determined by the attending anesthesiologist. Where indicated, extraction of occluding mandibular teeth was preformed. In order to minimize oral bacterial load and contamination, an oral antiseptic (0.12% chlorhexidine) rinse was applied and the dentition in the region of the surgery were scaled and polished. Intra-operative regional anesthesia using 0.5% bupivicaine was provided with an ultrasound-guided trigeminal nerve block as described by Viscasillas et al. (8) Dogs were then positioned in a modified dorsal recumbency position with the head positioned slightly lateral. The entire ipsilateral maxilla and skull was clipped and prepared for aseptic surgery from the nasal planum caudally to the level of the vertical ear canal and from ventral midline of the lower jaw to at least 2 cm beyond the dorsal midline of the head and muzzle. A sterile surgical pen was used to outline the extraoral approach and the intraoral surgical margins of 1–2 cm based on the combination of tumor type, size and location; biological behavior of the tumor (as determined from history and pre-operative computed tomographic studies); client goals; and expected post-operative patient function and cosmesis. A temporary tarsorrhaphy was performed prior to beginning the surgical approach.

An extraoral incision was begun ∼3 cm rostral to the medial canthus of the eye along the dorsolateral muzzle and extending caudally toward the medial canthus of the eye. The incision was continued caudally, incorporating a transpalpebral incision, and extending along the dorsal aspect of the zygoma (**Figure 2**). Exenteration of the globe was performed as described elsewhere (9) (**Figure 3**). Following exenteration, a commissurotomy was preformed to allow for improved visualization and access to the caudal oral cavity (10).

The intraoral approach was commenced by incising the gingiva, vestibular and hard palatal mucosa along the intraoral surgical markings. A combination of sharp and blunt dissection was used to incise the submucosal tissue and muscles along the maxilla and zygoma to approach the underlying OZMC. The levator nasolabialis, orbicularis oris, rector anguli oculi, zygomaticus, sphincter coli pars intermedia and frontalis muscles were incised followed by the levator labii maxillaris and caninus, masseter and temporalis muscles. Vessels crossing the surgical margins were double ligated with a 4-0 synthetic monofilament absorbable suture (Monocryl, Ethicon) and transected. A periosteal elevator was used to reflect the periosteum and associated soft tissues off the underlying bones. By joining the

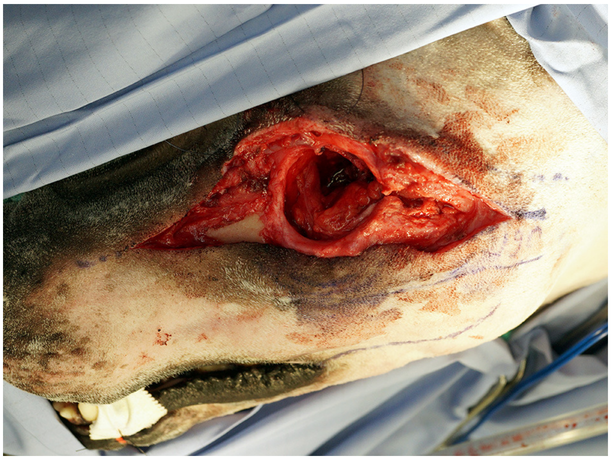

FIGURE 3 | Intraoperative photograph depicting completion of the extraoral approach with orbital exenteration.

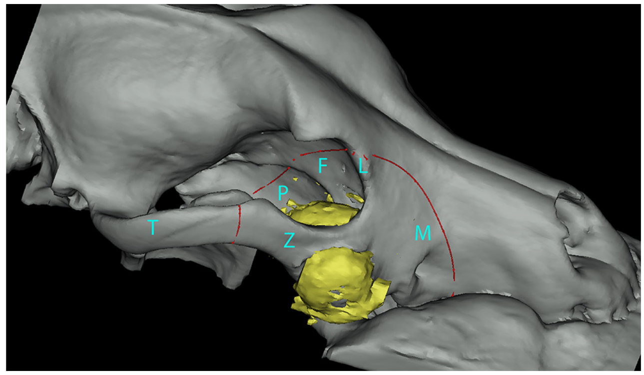

FIGURE 5 | Segmented three-dimensional rendering of a dog maxilla representing an OZMC tumor (yellow) and the area of excision (red line). Bones of the OZMC are labeled (M, maxilla; L, lacrimal; F, frontal; P, palatine; Z, zygomatic; T, temporal).

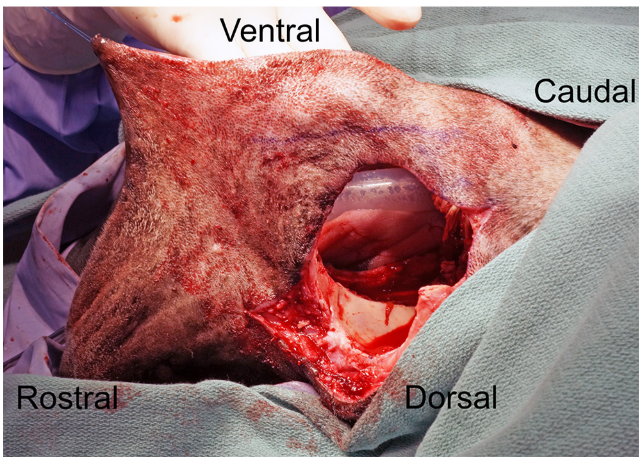

FIGURE 4 | Intraoperative photograph depicting the communication of the intra- and extraoral approaches; in effect, creating a bipedicle flap of dermis and oral mucosa.

intraoral approach with the extraoral approach, this step, in effect, created a composite bipedicle flap (**Figure 4**).

Utilizing a piezoelectric surgical unit, excision of the OZMC was performed by making osteotomies through the maxilla, palatine, lacrimal and zygomatic bones and the orbital part of the frontal bone and the zygomatic process of the temporal bones as well as the ethmoidal crest and sphenoethmodal plate, as dictated by the intended surgical margins (**Figure 5**). A winged dental elevator was then used to elevate the resected segment. The maxillary artery was then isolated and ligated with 4-0 synthetic monofilament absorbable suture (Monocryl, Ethicon). Nasal mucosa, ectoturbinates and endoturbinates were then incised with Metzenbaum scissors to complete the *en bloc* excision, which included the suborbital tissues and the zygomatic salivary gland when indicated (**Figure 6**). Prior to closure, a single injection of *Nocita*, a bupivacaine liposome injectable suspension, (0.4 ml/kg or 5.3 mg/kg) was infused into the muscular and subcutaneous tissues surrounding the excision to provide post-operative analgesia. The remaining bipedicle flap of the cheek/superior labia was then bluntly dissected to separate the buccal/labial mucosa from the dermis and muscles. An advancement flap was utilized to suture the buccal/labial mucosa to the palatal mucoperiosteum with 4-0 synthetic monofilament absorbable suture (Biosyn, Covidien) in a simple interrupted pattern (**Figure 7**). Extraorally, the muscles were apposed starting rostrally with the levator nasolabialis. Continuing with musculature closure caudally the orbicularis oris was apposed to the sphincter coli pars intermedia, followed by the rector anguli oculi and frontalis muscles apposed to the zygomaticus with 4-0 synthetic monofilament absorbable suture (Biosyn, Covidien) in a simple interrupted pattern. The dermis of the bipedicle flap was advanced dorsally and secured with 4-0 synthetic monofilament non-absorbable suture (Dermilon, Covidien) in a simple interrupted pattern (**Figure 8**). All patients remained in the critical care unit (CCU) following anesthesia to ensure adequate post-operative analgesia with hourly pain scoring. Instructions to feed only canned or softened kibble and to avoid toys or mouth play were given to the owners.

CASE REPORTS

Case 1

A 10.5-year-old neutered male vizsla was referred to the Medical Oncology service for evaluation of a mass located on the palatal aspect of the right maxillary fourth premolar tooth. On presentation, the visible mass measured 1 cm in diameter, was erythematous with a smooth contour. Mandibular & medial retropharyngeal lymph node aspiration and thoracic radiography revealed no indication of metastasis. Computed tomography (CT) of the head revealed a lobulated, soft tissue attenuating, non-uniformly enhancing 1.6 cm diameter mass with numerous areas of patchy, coalescing mineralization along the palatal aspect of maxillary fourth premolar and molars. The tumor primarily

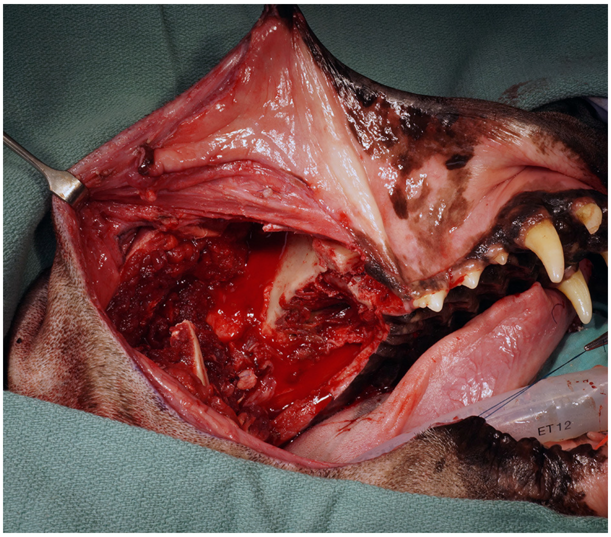

FIGURE 6 | Intraoperative photograph depicting the intraoral view of the caudal maxilla after completion of the OZMC excision. Note a commissurotomy has been performed in this case to improve exposure.

FIGURE 7 | Intraoperative photograph depicting an intraoral view of the mucosal advancement flap for the vestibular reconstruction.

involved the caudal maxilla and palatine bones; advancing dorsally into the pterygopalatine fossa. Previously perform histopathology revealed a diagnosis of osteosarcoma.

The case was referred to the Dentistry and Oromaxillofacial Surgery service for surgical excision. The tumor was excised as an en bloc excision of the OZMC and associated musculature,

FIGURE 8 | Photograph depicting an extraoral view of the dermal advancement flap and closure of a commissurotomy **(A)** and photograph of case #3 at the 14-day post-operative recheck examination **(B)**.

oral mucosa, gingiva and dentition utilizing a combined intra- and extraoral approach with a transpalpebral exenteration. The osseous excision extended from the mesial aspect of the right maxillary third premolar tooth to the mid-zygoma. The medial extent of the excision was the median palatine raphe and the inferior aspect of the medial orbit. Following excision, the wound was closed as described above.

Histological evaluation of the resected tissue confirmed the diagnosis of osteosarcoma and surgical margins were free of tumor with a narrow margin (16 mm) at the distal aspect of the excision. The dog recovered uneventfully from general anesthesia despite hypotension that responded to a transfusion of packed red blood cells. The patient remained on a continuous rate infusion (CRI) of fentanyl (3 μg/kg/hr), and ketamine (3 μg/min/hr) overnight. Transdermal fentanyl patches (100 mcg/hr + 25 mcg/hr) and ampicillin/sulbactam (20 mg/kg IV q 8 h) was also administered. The dog was discharged the following day with oral carprofen (2 mg/kg q 12 h), tramadol (5 mg/kg q 8–12 h) and amoxicillin/clavulanic acid (13.75 mg/kg q 12 h). Instructions to feed only canned or softened kibble and to avoid toys or mouth play were given to the owners.

At the time of recheck and suture removal 14 days later the owners reported the dog was eating and drinking well. The skin and oral incisions had healed well and no discharge, redness, swelling or dehiscence was noted. The owners elected to pursue definitive radiation therapy over 20 fractions of 2.7Gy to a total of 54Gy. This intensity-modulated radiation therapy was delivered using 6 MV helical tomotherapy (TomoTherapy HiArt Treatment System®, Accuray Inc., Sunnyvale, CA, USA) and planned with inverse planning software (TomoTherapy HiArt® v3-5)]. Following radiation therapy, vinorelbine chemotherapy was initiated. Pulmonary metastasis was identified 21 months after the surgical excision, radiation therapy and chemotherapy was initiated. Two weeks later, the patient was euthanized due to poor quality of life secondary to pulmonary disease.

Case 2

A 7.5-year-old neutered male Standard Poodle was referred to the Dentistry and Oromaxillofacial Surgery service for evaluation and biopsy of an oral mass affecting the right caudal maxilla. An approximately 3.5 cm diameter, firm mass was present on the palatal aspect of the right maxillary fourth premolar tooth and first molar tooth. Thoracic CT, mandibular and medial retropharyngeal lymph node aspirations revealed no evidence of metastasis. Head CT revealed a large lobulated, heterogenous, mineral attenuating 5.1 × 3.8 × 2.9 cm mass centered at the right maxillary fourth premolar tooth and first molar tooth. The mass was invading and destroying the palatine, zygomatic and frontal bone as well as the rostroventral ethmoid turbinates, maxillary recess, infraorbital canal and nasopharyngeal meatus (**Figure 9**). Histopathology of the biopsied tissue revealed a diagnosis of multilobular tumor of bone.

FIGURE 9 | Axial CT image of a multilobular tumor of bone seen in case #2, which revealed a large lobulated, heterogenous, mineral attenuating 5.1 × 3.8 × 2.9 cm mass centered at the right maxillary fourth premolar tooth and first molar tooth.

The tumor was excised as an en bloc excision of the OZMC and associated musculature, oral mucosa, gingiva and dentition utilizing a combined intra- and extraoral approach with a transpalpebral exenteration. The osseous excision extended from the mesial aspect of the right maxillary third premolar tooth to the mid-zygoma. The medial extent was 7 mm toward the contralateral side from the median palatine raphe and included portions of the maxilla, lacrimal, palatine, pterygoid and frontal bones. Following excision, the wound was closed as described above.

Histological evaluation of the resected tissue confirmed the diagnosis of multilobular tumor of bone and tumor-free margins. All margins were reported as clean with at least 1 cm tumor-free tissue, except for the palatal margin, which was reported as narrow (3 mm). The dog recovered uneventfully from general anesthesia with minimal blood loss and was managed overnight on a CRI of sufentanil (0.3 µg/kg/hr), lidocaine (10 µg/min/hr) and ketamine (3 µg/min/hr). Additionally, a subcutaneous dose of carprofen (4.4 mg/kg) was administered. The dog was discharged the following day with a 150 mcg transdermal fentanyl patch, oral tramadol (4 mg/kg q 8-12 h) and carprofen (2.2 mg/kg q 12 h). The owners were instructed to feed only canned or softened kibble and to avoid toys or mouth play.

At seven days post-operatively there was significant halitosis noted by owners and evidence of a 3 cm x 12 cm dehiscence at the caudal aspect of the intraoral closure site. The remaining closure sites were healing well and a good cosmetic appearance was apparent. An island axial pattern flap based on the contralateral major palatine artery was used to close the caudal dehiscence and healing was uncomplicated. At 4 months post-operatively recurrence of the neoplasia was noted on CT and owners elected against further surgical intervention. The patient was palliatively managed with the referring veterinarian and euthanized 11 months after the original surgery date.

Case 3

An 8.5-year-old neutered male Siberian husky was referred to the Dentistry and Oromaxillofacial Surgery service for treatment of a left maxillary papillary squamous cell carcinoma. On presentation, a 0.5 cm diameter, round and firm mass was visible in the buccal gingiva of the left maxillary first molar tooth. The patient was placed under general anesthesia for thoracic CT and regional lymph node aspiration, which revealed reactive lymphoid tissue and no evidence of pulmonary metastasis. A head CT was performed and revealed a 1.9 × 3.0 × 2.6 cm soft tissue attenuating, inhomogeneous contrast enhancing nodular mass centered at the left maxillary first molar tooth.

The tumor was resected as an en bloc excision of the OZMC and associated musculature, oral mucosa, gingiva and dentition utilizing a combined intra- and extraoral approach with a transpalpebral exenteration. The excision extended from the mesial aspect of the left maxillary fourth premolar tooth distally to the mid-zygoma. The palatal extent of the excision was the median palatine raphe. The excision extended from the inferior orbital crest of the lacrimal bone and maxilla at its dorsomedial extent to the dentition at its ventrolateral extent including the maxilla and dorsal zygoma. Following excision, the wound was closed as described above.

Histological evaluation of the resected tissue confirmed the diagnosis of papillary squamous cell carcinoma and surgical margins were free of tumor with a narrow margin (4 mm) at the distal aspect of the excision. While this patient received a whole blood transfusion due to hypotension in the face of only moderate blood loss, the dog recovered uneventfully from general anesthesia. The patient was managed overnight on a CRI of fentanyl (3 μg/kg/hr) and ketamine (3 μg/min/hr). Carprofen (2.2 mg/kg SQ) and cefazolin (25 mg/kg IV q 8 h) was also administered. The dog was discharged the following day with a 75 mcg transdermal fentanyl patch and oral carprofen (2.2 mg/kg q 12 h), tramadol (5 mg/kg q 8–12 h) and amoxicillin/clavulanic acid (13.75 mg/kg q 12 h). Instructions to feed only canned or softened kibble and to avoid toys or mouth play were given to the owners.

At a 14-day post-operative recheck examination the patient was doing well with occasional sneezing. The extraoral closure had healed well. The intraoral incision was mostly healed with only a pin-point (∼0.5 mm) dehiscence seen at the rostral extent. At the next recheck under general anesthesia another 14 days later, persistence of the pin-point oronasal fistula previously noted was identified. Surgical correction was declined by the owners, as the patient was asymptomatic. Two weeks later, the patient presented for left nasal discharge with suspected rhinitis suspected to be due to the persistent pin-point oronasal fistula. Medical management was initiated with azithromycin (10 mg/kg q 24 PO x 2 weeks, then 10 mg/kg q48 PO × 2 weeks). At the last contact with owners, five months post-operative, the patient was doing well with no sneezing or nasal discharge and no gross evidence of recurrence.

Case 4

A 13-year-old neutered male Labrador retriever was referred to the Radiation Oncology service for evaluation and treatment of a suborbital spindle cell sarcoma. On presentation, a 1.5 cm diameter, multilobulated mass could be appreciated intraorally at the palatal aspect of the right maxillary second molar tooth. Thoracic radiography and regional mandibular lymph node aspiration was performed prior to referral, which revealed reactive lymphoid tissue and no evidence of pulmonary metastasis or intrathoracic lymphadenopathy. Head CT revealed a 3 cm x 2 cm soft tissue attenuating mass along the right caudal maxilla extending from the distal aspect of the right maxillary second molar tooth and into the suborbital adipose tissues with enlarged and attenuating zygomatic salivary gland (**Figure 10**).

The patient was referred to the Dentistry and Oromaxillofacial Surgery service for excision of the OZMC and associated musculature, oral mucosa, gingiva and dentition utilizing a combined intra- and extraoral approach with a transpalpebral exenteration. The excision extended from the furcation of the right maxillary fourth premolar tooth and extended caudally to the mid-zygoma. From dorsal to ventral, the excision extended from the inferior orbital crest of the lacrimal bone and extended into the mandibular ramus and body just ventral to the mandibular second and third molar teeth. The excision including portions of the caudal maxilla, lacrimal, frontal, palatine, zygomatic, temporal, pterygoid and mandible bones as well as the zygomatic salivary gland. The palatal extent of the excision was the median palatine raphe. Following excision, the mesial and palatal roots of the maxillary fourth premolar tooth were extracted and the wound was closed as described above.

Histological evaluation of the resected tissue confirmed the diagnosis of spindle cell sarcoma, but could not differentiate the tumor between fibrosarcoma and nerve sheath sarcoma. Greater than 1 cm tumor free histologic margins were reported. The dog recovered uneventfully from general anesthesia without any need for blood products and was managed overnight in the CCU on hydromorphone (0.5 mg/kg q 6 h IV). Meloxicam (0.2 mg/kg SQ) and ampicillin (22 mg/kg IV) were also administered. The dog was discharged 48 h post-operatively with oral acetaminophen (11 mg/kg q 8 h), gabapentin (10 mg/kg q 8 h PO) and meloxicam (0.1 mg/kg q 24 h). Instructions to feed only canned or softened kibble and to avoid toys or mouth play were given to the owners.

The patient returned 2 days post-operatively and a small amount of mucoid discharge from the rostral skin incision was noted. The client was instructed to give oral amoxicillin/clavulanic acid (13.75 mg/kg q 12 h). At the time of oral surgery recheck and skin suture removal 14 days post-operatively the owners reported the dog was doing very well at home, eating and drinking well and was not experiencing difficulty breathing. However, occasional “hacking” had been observed. The extraoral closure site and the rostral extent of the oral incision was healed. However, an ∼2 cm diameter area of dehiscence was noted at the caudal most extent of the intraoral closure site. The owners were advised that a definitive repair of the resultant oronasal fistula would be delayed until inflammation in all tissues had resolved. At 13 weeks post-operatively the client reported the development of a purulent draining tract on the face at the previous surgical site. Physical examination of the patient at 14 weeks post-operatively confirmed a purulent draining tract at the level of the medial canthus of the exenteration site. At 16 weeks post-operatively the patient was anesthetized for examination, which revealed communication of the facial draining tract with the previous surgery site and a concurrent oronasal fistula. Samples were taken from the draining tract for culture and sensitivity prior to thorough lavage and debridement. The patient was discharged with instructions to continue the previously prescribed amoxicillin/clavulanic acid and meloxicam pending culture and sensitivity results, which yielded moderate growth of methicillin resistant *Staphylococcus pseudointermedius* (MRSP). The patient was instructed to stop the prescribed amoxicillin/clavulanic acid and was provided a 2-week course of oral doxycycline (6 mg/kg q12 h) and metronidazole (10 mg/kg q12 h) as per the results of sensitivity testing. The patient presented at 23 weeks post-operatively for definitive surgery to close the oronasal fistula. An island axial pattern flap of the left hard palate was

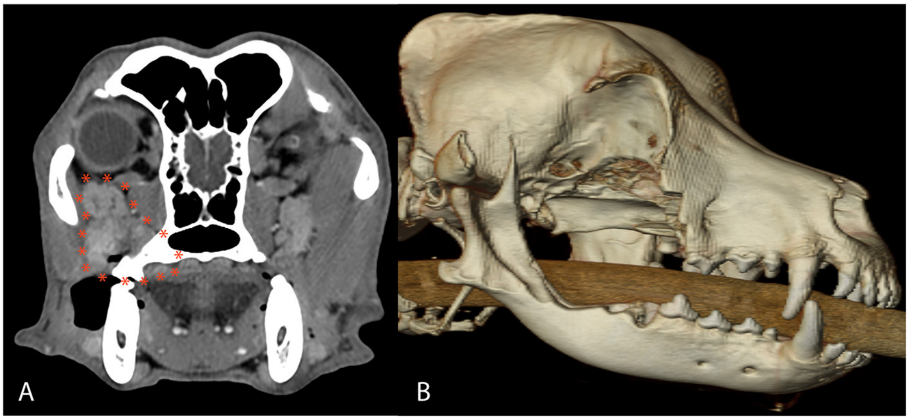

FIGURE 10 | Axial CT image **(A)** and postoperative 3D CT image **(B)** in case #4. The axial CT reveals a 3 cm × 2 cm soft tissue attenuating mass along the right caudal maxilla extending from the distal aspect of the right maxillary second molar tooth and into the suborbital adipose tissues with enlarged and attenuating zygomatic salivary gland diagnosed as a spindle cell sarcoma. The 3D CT image depicts the extent of the OZMC excision, which included portions of the caudal maxilla, lacrimal, frontal, palatine, zygomatic, temporal, pterygoid, and mandible bones.

utilized to close the previously described defect. The patient was discharged with oral meloxicam (0.1 mg/kg q24 h) and gabapentin (13 mg/kg q8 h) for 14 days. The patient presented 4 weeks after revision surgery reportedly doing well at home. Oral examination confirmed healing of the previously repaired oronasal fistula site. Exposed palatal bone that was left to heal by second intention showed minor signs of granulation. Concern for inhibition of healing from the previously diagnosed MRSP was communicated to the client and the patient was restarted on the previous antibiotic regimen. The patient was reassessed by his primary care practitioner 5 weeks after revision surgery and photographs confirmed a near complete granulation bed at the open site. Follow up email contact with the client 13 weeks after revision surgery confirmed the patient was doing well at home with complete healing of the surgery site.

Case 5

An 8-year-old spayed female vizsla was referred to the Medical Oncology service for evaluation of an osteosarcoma located inferior to the right eye. The referring veterinarian had previously extracted the right maxillary fourth premolar tooth and the right maxillary first molar tooth at the time of biopsy. On presentation, the visible mass measured 2.7 cm in length and was firm and non-mobile. Fine needle aspirates of the mandibular lymph nodes revealed no evidence of local metastasis. Thoracic CT revealed a partially mineralized 0.4 cm nodule in the left cranial lung lobe of unknown significance. Head CT revealed a 3.2 cm × 3.2 cm × 3.5 cm osteolytic and osteoproliferative soft tissue and mineral attenuating, moderately contrast enhancing mass within the right caudal maxilla. The mass extended caudally into the right pterygopalatine recess and orbital space causing dorsolateral displacement of the right globe and caudoventral displacement of the right zygomatic salivary gland. The mass extended rostrally and ventrally to include the alveolar bone associated with the previously extracted right maxillary fourth premolar tooth.

The case was referred to the Dentistry and Oromaxillofacial Surgery service for surgical treatment. The tumor was resected as an en bloc excision of the OZMC and associated musculature, oral mucosa, gingiva and dentition utilizing a combined intra- and extraoral approach with a transpalpebral exenteration. The osseous excision extended from the furcation of the right maxillary second premolar tooth to the mid-zygoma. The medial extent of the excision was the orbital part of the frontal bone and 2 mm to the contralateral side of the median palatine raphe. The excision including portions of the caudal maxilla, lacrimal, frontal, palatine, zygomatic, temporal, and pterygoid bones. Following excision, the wound was closed as described above.

The dog recovered uneventfully from general anesthesia and was managed overnight on CRI of fentanyl (2–5 μg/kg/hr) and ketamine (2–4 μg/kg/min) and an injection of carprofen (4.4 mg/kg SQ). A transdermal fentanyl patch (75 μg/hr) and piperacillin/tazobactam (45 mg/kg IV q 8 h) was also administered. The dog was discharged the following day with oral gabapentin (13 mg/kg q 8 h) and carprofen (4.4 mg/kg q 24 h). A nylon stockinette, and a loosely fitted tape muzzle was also applied to limit oral range of motion and tension on the intraoral surgery site. Instructions to feed only canned food and to avoid mouth play were given to the owners.

Histological examination confirmed the diagnosis of osteosarcoma with a narrow margin (500 μm) at the caudal aspect of the excision. Four days post-operatively, the patient presented for evaluation of malodorous smell from the oral cavity and hyporexia. Examination revealed a 6 cm dehiscence at the most caudal aspect of the intraoral closure. There was also purulent discharge present at the extraoral closure. The patient was discharged with instructions to continue medications as previously prescribed. The client reported unwillingness to administer gabapentin due to the level of sedation of the patient. Amoxicillin/clavulanic acid (17 mg/kg PO q 12 h) and metronidazole (10 mg/kg PO q 12 h) was prescribed, empirically. Mirtazapine (0.7 mg/kg PO q 24 h) was also prescribed as an appetite stimulant. Oronasal fistula repair was planned to occur when local inflammation and infection had resolved.

The patient was assessed by a veterinary dental specialist at a separate institution 7 days post operatively where physical examination revealed dehiscence of the extraoral closure and complete dehiscence of the intraoral closure resulting in a maxillo-oronasal fistula. Given the nature of the dehiscence and the likelihood of pursuing radiation therapy, surgical repair was recommended ahead of the previously planned schedule.

Fourteen days post-operatively the patient presented for repair of the maxillo-oronasal fistula with the Dentistry and Oromaxillofacial Surgery service. Under general anesthesia, a right inferior alveolar nerve block was administered with 1.5 mg bupivacaine and 15 μg buprenorphine. The right mandibular molar teeth were routinely extracted to prevent occlusal trauma to the maxillary soft tissue. Samples from the fistula were collected and submitted for culture and sensitivity. The maxillo-oronasal fistula was thoroughly lavaged. A myofascial axial pattern flap based on the temporalis muscle was utilized for repair of the oronasal fistula as described by Cavanaugh et al. (11) The patient recovered from anesthesia uneventfully and was monitored in the critical care unit with a CRI of fentanyl (2–5 μg/kg/hr) and ketamine (2–4 μg/kg/min). A subcutaneous injection of carprofen (4.4 mg/kg q24 h) was also administered. Intravenous piperacillin/tazobactam (45 mg/kg q 6 h) and metronidazole (10 mg/kg q 12 h) were also administered and the surgical site was iced every 2–4 h while hospitalized. Culture and sensitivity reported a multi-drug resistant Enterococcus infection and antibiotics were adjusted accordingly (discontinued metronidazole administration, added 5 mg/kg marbofloxacin PO q 24 h).

Significant facial edema developed post-operatively, which resolved by the fourth post-operative day. However, the patient was intermittently anorexic and the owners were unable to provide medications orally. The patient was re-admitted to the hospital 8 days following the revision surgery. An area of eschar overlying the rostral aspect of the extra-oral incision was debrided under sedation revealing a 3 cm diameter cutaneous dehiscence with exposure of the underlying temporalis muscle. The wound was thoroughly lavaged and cleansed with dilute chlorhexidine and sterile saline. Nylon (Dermilon, Covidien) sutures were placed around the periphery of the wound to allow for a tie-over bandage to be placed. An absorbable, antimicrobial polymeric wound matrix was used for direct wound dressing followed by sterile gauze and a moisture-protective barrier superficially. The bandage was held in place with umbilical tape. An esophagostomy tube was routinely placed during a short general anesthetic event the day prior to discharge to allow for administration of all oral medications. The patient was discharged with a new transdermal fentanyl patch (75 μg /hr) and oral carprofen (2.2 mg/kg q 12 h), acetaminophen (10 mg/kg q 12 h), amoxicillin/clavulanic acid (17 mg/kg q 12 h), marbofloxacin (5 mg/kg q 24 h) and ondansetron (2 mg/kg q 12 h). All medications were instructed to be administered via the esophagostomy tube, but the patient was to be allowed and encouraged to eat canned food. The owner was instructed to change the tie-over bandaging materials every 3 days.

The patient re-presented to the Dentistry and Oromaxillofacial Surgery service 20 days after the revision surgery. Orofacial examination revealed a layer of healthy granulation tissue over the dermal dehiscence site. Additionally, there was approximately 80% epithelialization of the intra-oral myofascial flap and no evidence of a persistent oral nasal fistula on conscious oral exam. The esophagostomy tube was removed and the sutures previously placed for the tie-over bandage were also removed. Surgical closure of the extra-oral dehiscence was discussed with the owner, but healing by second intention was elected. A soft food diet was recommended for an additional 3 weeks.

Six weeks following revision surgery, the patient presented to the medical oncology service to discuss adjunctive therapy options. Moderate restricted range of motion was identified when opening the patient's mouth. The patient was placed under general anesthesia for thorough oral examination and head CT which revealed a communication between the oropharynx and nasopharynx measuring ∼1 cm in diameter, which was located at the junction of the myofascial flap and the soft palate. The client reported no clinical signs associated with the fistulation. Given the non-clinical nature of the fistula, surgical repair was not pursued. With narrow histologic tumor margins and the pulmonary nodule identified on thoracic CT, radiation therapy and chemotherapy were recommended, but not pursued by the owner.

DISCUSSION

In the report conveyed here, we have described a surgical technique to resect extensive OZMC tumors in which an intraoral approach is combined with a horizontal extraoral incision along the dorsolateral muzzle and zygoma that also incorporates a transpalpebral approach to orbital exenteration. This approach is a modification of a previously described combined intra- and extra-oral approach in order to access tumors of the caudal maxilla, in which the extraoral incision is made ventral to the globe (6).

Excision of the extensive OZMC tumors in this series included the entire inferior orbit and significant portions of the medial orbit. The medial orbit osteotomy was generally along a line beginning rostrally in the orbital crest of the lacrimal bone, just superior to the fossa of the lacrimal sac; coursing caudally along a line just inferior to the inferior orbital crest and optic canal; terminating just caudal to the hamulus of the pterygoid bone. The angle of approach (∼40° from the median plane) for the medial orbitectomy must also be exact to ensure communication with the intraoral palatal approach and the caudal nasopharynx rather than penetrating into the rostral cranial fossa. An alteration of as little as 15° would direct the saw blade into the cerebrum, which could lead to significant neurological complications (12–14) (**Figure 11**).

In addition, maintaining the globe without orbital reconstruction may result in enophthalmos and resultant significant ocular complications (1). Diplopia (i.e. double vision) can occur secondary to malposition of a globe that leads to

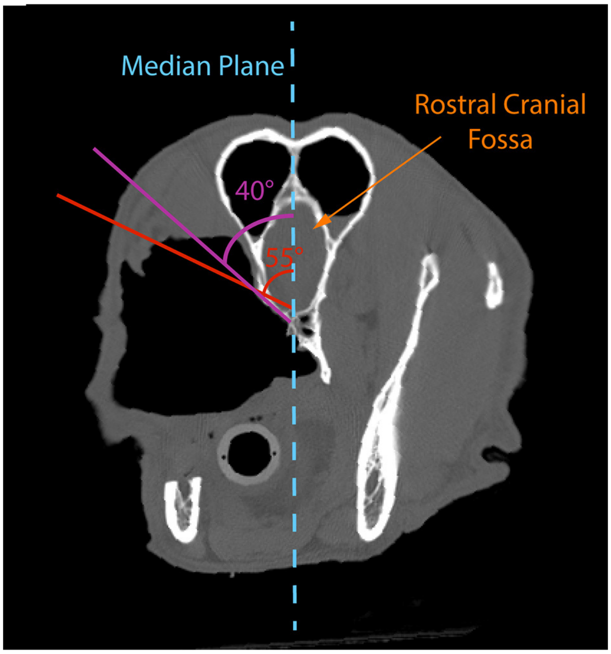

FIGURE 11 | Postoperative axial CT image of case #5 depicting the need to make the medial orbitotomy at an angle of ∼40° to the median plane. An alteration of as little as 15° (55° to the median plane) would direct the saw blade into the rostral olfactory bulb, which could lead to significant neurological complications.

failure to fuse visual images (1). In humans, globe malposition has been reported to stimulate vagal activity from activation of the oculocardiac reflex resulting in significant headache, vertigo and severe nausea (2, 3). Entropion occurs secondary to an increase in orbital volume, as may be expected in facial fractures and orbitectomy and can lead to blepharospasm, conjunctival hyperemia, epiphora, mucoid ocular discharge, corneal ulceration, corneal scarring and vision loss (4, 5).

Visualization is vital to successful complete excision of OZMC tumors. Previous literature indicates that invasive orbital and periorbital tumors are often inadequately excised with local recurrence or persistence occurring in 36.7% of cases (15). In order to avoid the aforementioned ocular complications and achieve excellent visualization, we elected to perform orbital exenteration. By incorporating the extraoral skin incision with the transpalpebral approach to exenteration, we were able to accomplish the exenteration and OZMC excision with a singular extraoral incision, which may improve cosmetic outcomes and operative time.

Exenteration as part of the surgical plan for these patients was further supported by the possible need for post-operative radiation therapy. Commonly reported ocular-related radiation side effects are conjunctivitis, alopecia, keratoconjunctivitis sicca, erythema, uveitis and ulceration (16–18). Keratoconjunctivitis has been reported in more than a quarter of dogs undergoing radiation therapy of this site (18). Acute ocular side effects have a higher incidence with the use of curative radiation protocols and are less prevalent with the use of palliative radiation protocols (19). However, over 80% of patients in one study had ocular anomalies following radiation therapy and half of the canine patients had three or more ocular anomalies (19). Additionally, even with lower doses, histological evidence of irreversible long-term retinal changes has been reported (18). Given the risk of both acute and chronic ocular disease in these patients should post-surgical radiation be required, exenteration as part of a definitive surgery was elected.

The decision to exenterate the globe may be debatable. Highly conformal intensity modulated and image guided forms of radiotherapy have been successfully utilized to spare the globe from severe radiation toxicity in the setting of full-course definitive-intent radiotherapy in dogs with maxillofacial tumors. On the other hand, at our institution, stereotactic radiation therapy is selected on a case by case basis and utilized in the gross disease setting *only*, not to treat microscopic disease after surgery. Stereotactic radiation therapy is not recommended following surgery due to the concern of high doses of radiation to normal tissues especially to nerves, bone, and ocular structures. In the population of patients discussed here, definitive radiation therapy would be recommended following surgery.

In the future, we may consider sparing the globe in the few cases in which orbital reconstruction is pursued and exenteration is not necessary to achieve a medial orbitectomy. Orbital reconstruction would be necessary to avoid complications related to diplopia. Orbital reconstruction showed promising results in one case series (20). Short-term side effects consisted primarily of periocular swelling. Short and long-term follow-up revealed all eyes remained visual, with normal position, alignment and movement. However, detecting the effects of diplopia in dogs is challenging. Additionally, in our cases, surgical oncology tenets and need for precision mostly dictated orbital exenteration.

The major complication seen in these patients was significant intraoral incision line tension, which led to dehiscence and persistent oronasal fistula in three out of the five cases. The tension seen in these patients was not unexpected given that a significant amount of alveolar and palatal mucosa was excised as part of the excision. Tension was also related to the medial extent of removed tissue, often to or across the median palatine raphe. Taken with limited buccal mucosa, the lack of palatal mucoperiosteum made the reconstruction of an oral vestibule quite challenging. The dehiscence rate in this small case series (60%) is higher than previous reports in maxillectomies of 5–33% (7), likely due to the reasons described above. It is also reasonable to assume the location of these tumors played a role in dehiscence. Previous reports state that the rate of dehiscence is directly proportional to the caudal extent of the tumors, with up to 80% of reported post-operative dehiscence located caudal to the canine tooth (7). Additionally, the excision volumes of the cases presented here were large and likely represent the upper end of a normal distribution. Thus, the inherent risk of tension in this case series was considered pre-operatively and, in most patients, the occluding mandibular molar teeth were prophylactically extracted to reduce tension on the intraoral incisions. Surgical sites were closed with subdermal plexus advancement flaps. Other axial pattern flaps such as the angularis oris (21) or the superficial cervical axial pattern (22) flaps may have been considered. However, these flaps inherently possess concurrent complications that should not be dismissed. Ultimately, we elected to use the simplest flap design to achieve the objective. This principal is based on the concept that more advanced flaps should be reserved for the uncertain event of a dehiscence. In cases 2 and 4, an island axial pattern flap based on the contralateral major palatine artery was used to close the defect (23). In case 5, a myofascial axial pattern flap based on the ipsilateral temporalis artery was used (11). In all cases, the revision surgeries were successful at closing the dehiscence and resultant oronasal and maxillo-oronasal fistulas.

In order to improve visualization and access to the caudal oral cavity, a commissurotomy was also performed. It is conceivable that the proximity of the commissurotomy to the intra-oral mucosal incisions may have compromised the mucosal vascular supply and contributed to the risk of dehiscence. It was determined that the ability to achieve clean margins through optimal visualization was of paramount importance. As no patient experienced visible evidence of post-operative flap necrosis, the author's do not believe vascular supply was compromised. However, should one wish to use an angularis oris mucosal axial pattern flap to aid in intraoral closure, a commissurotomy would not be possible as it would likely damage the angularis oris artery. A plausible alternative would be to make a full-thickness, vertically oriented releasing incision through the superior labia to connect with the most rostral extent of the extra-oral incision; in effect, creating a full thickness labial flap that could be reflected caudally. This approach has been used for visualization of the OZMC in human oromaxillofacial surgery (24) and has been used to close wounds of the lip with a labial advancement flap in dogs (25). Such an approach should provide excellent visualization with the added benefit of preservation of the angularis oris artery should a mucosal axial pattern flap based on that artery become necessary for palatal reconstruction. To the author's knowledge this type of full-thickness labial flap used solely for improved access to the caudal oral cavity has not been described or evaluated in dogs.

While all our patients generally had minimal intra-operative hemorrhage, two patients did require a transfusion. Previous reports describe significant hemorrhage as the most common intra-operative complication seen in maxillofacial surgery (7). Hemorrhage was reported to be as high as 53% in other case series, with the majority of cases described as excessive to the point of needing transfusions (7). Hemorrhage has also been found to be significantly associated with larger and more caudally located tumors (7). Excessive hemorrhage could be expected given the location and size of the OZMC tumors of this case series, in which several large arteries are encountered during excision. It is the authors' opinion that the visualization achieved with the combined intra- and extra-oral approach coupled with exenteration and commissurotomy, as well as the use of piezosurgery for ostectomy, limited the amount of hemorrhage. Piezosurgery units have a higher frequency of vibration, 25–35 kHz, which allow cutting of mineralized tissue while sparing soft tissue; thereby, decreasing the likelihood of an inadvertent transection of a major vessel (26). Additional benefits of piezosurgery include decreased neurotrauma, increased visualization and decreased thermal injury to bone (26).

The surgical approach described here in which an orbital exenteration is used in conjunction with the dermal incision of a combined intra-and extraoral approach provides significant benefits for the excision of extensive OZMC tumors. Excellent visualization is obtained, common intra-operative complications are minimized and post-operative ocular complications are eliminated. The major complication, primarily caused by the size of the excision rather than the approach, is intra-oral wound dehiscence. This complication may be mitigated with the addition of axial pattern flaps (e.g., angularis oris mucosal or temporal myofascial axial pattern flaps) to avoid intra-oral wound tension.

DATA AVAILABILITY STATEMENT

The original contributions generated for the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

Ethical review and approval was not required for the animal study because the surgical procedures reported within this manuscript were performed in the course of providing a medically necessary procedure. The results are retrospective in nature and included clinical cases. Therefore, ethical review and approval was not required and is exempt from IACUC requirements. Written informed consent was obtained from the owners for all procedures performed.

AUTHOR CONTRIBUTIONS

AT, BR, and AG: drafting of case descriptions and discussion and final approval of version to be published. AT and JS: overall drafting of entire manuscript. JS: surgical concept and design, graphic development, interpretation, manuscript drafting, and final approval of version to be published. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Veterinary Science

METHODS
published: 29 March 2021
doi: 10.3389/fvets.2021.648766

Management of Septic Arthritis of the Temporomandibular Joint in Dogs

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Septic arthritis of the temporomandibular joint (TMJ) in dogs and other mammals is a rare condition. It is typically associated with notable pain, swelling, and difficulty in opening the mouth. Unlike degenerative TMJ disease, septic arthritis requires urgent intervention. The etiology of the condition may include penetrating trauma, an extension of local infection, such as otitis media, or the hematogenous spread of a pathogen. However, the precise cause may not always be identified. Diagnostic imaging with Computed Tomography (CT), cone-beam CT (CBCT), and/or Magnetic Resonance Imaging (MRI) are helpful for honing the definitive diagnosis and formulating a treatment plan. Subsequently, exploratory surgery may be required to obtain samples for culture and sensitivity and histology and to lavage the joint. In this "methods" article, we provide a detailed description of our approach to diagnosis and management of septic TMJ arthritis in four dogs.

Keywords: computed tomography, septic, arthritis, bacteria, temporomandibular joint

OPEN ACCESS

Edited by:
Margherita Gracis, Istituto Veterinario di Novara, Italy

Reviewed by:
Christopher John Snyder, University of Wisconsin-Madison, United States
Carlos Alberto Antunes Viegas, Universidade de Trás-os-Montes e Alto, Portugal

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Specialty section:
This article was submitted to Veterinary Dentistry and Oromaxillofacial Surgery, a section of the journal Frontiers in Veterinary Science

Received: *01 January 2021*
Accepted: *10 February 2021*
Published: *29 March 2021*

Citation:
Arzi B, Vapniarsky N, Fulton A and Verstraete FJM (2021) Management of Septic Arthritis of the Temporomandibular Joint in Dogs. Front. Vet. Sci. 8:648766. doi: 10.3389/fvets.2021.648766

INTRODUCTION

Septic arthritis of the temporomandibular joint (TMJ) is a rare condition that occurs by seeding pathogens, typically bacteria, by hematogenous route or by direct spread into the joint (1–6). Unlike osteoarthritis of the joint that is considered "low inflammatory" arthritis, septic TMJ arthritis is considered a "high-inflammatory" arthritis and exhibits distinct biological and clinical behavior (6, 7). The development of TMJ septic arthritis is dependent on various factors, such as the pathogen (i.e., bacteria, fungi, or parasite) and its ability to colonize the joint and host-related factors (6).

Affected individuals typically exhibit periauricular pain, pain when opening and closing the mouth, swelling, redness, and occasionally purulent discharge (6, 8–10). Diagnosis of TMJ septic arthritis requires a physical examination and advanced diagnostic imaging, namely CT, cone-beam CT (CBCT), and, occasionally, MRI. Importantly, laboratory analysis of joint fluid obtained by arthrocentesis, or exploratory arthrotomy may be required to optimize the treatment of TMJ septic arthritis (4, 11–13). Although no gold standard exists in the management of TMJ septic arthritis, initiating therapy early, preferably based on precise information obtained from diagnostic imaging, joint decompression, and sampling (i.e., culture and sensitivity), can optimize patient outcomes (7, 14–17).

Although the management of TMJ septic arthritis has been described in human, in many reports, and in animals, in some reports, to the knowledge of the authors, a detailed description of the management of this condition in dogs has not been described in the English peer-reviewed literature in the past 10 years (1, 9, 12, 18, 19). Therefore, we sought to report our experience in the diagnosis and management of TMJ septic arthritis and report the outcome in four dogs.

Diagnostic Techniques

Dogs that were presented with pain when opening the mouth, swelling of the TMJ area, or pain of unknown origin were screened by conventional CT (HiSpeed FX/i or LightSpeed16, GE Healthcare, Waukesha, WI) and/or CBCT (NewTom 5G CBCT Scanner, NewTom, Verona, Italy or VetCAT, Xoran Technologies, Michigan, Unites States) scans at their initial visit. In most cases, contrast CT studies were acquired after intravenous administration of iopamidol (Isovue 370, Bracco Diagnostics, Monroe Township, NJ, USA).

Images from MRI were acquired as needed using a 1.5T MRI system (GE Healthcare Signa, Waukesha, WI). MRI and conventional CT images were viewed using eFilmWorkstation® 3.3 (Merge Healthcare, Hartland, WI, USA). All CBCT DICOM files were viewed using a specialized software (*In vivo* 5 or *In vivo* 6, Anatomage, San Jose, CA, USA). Each case was evaluated on medical-grade monitors (ASUS PB278Q 27-inch, ASUSTeK Computer Inc., Taipei, Taiwan).

Surgical Technique

In order to obtain samples for culture and sensitivity testing and for histopathology, a surgical approach to the joint was performed. Furthermore, surgical access to the dorsal and ventral compartments of the TMJ was performed to allow for copious lavage and reduction of bacterial load and gross evaluation of the integrity of the TMJ tissues (**Figure 1**). The affected side of the head was clipped and aseptically prepared for surgery. The dogs were placed in lateral recumbency with the head and cervical region slightly elevated.

A lateral approach to the TMJ was performed as described in an earlier study (20, 21). A full-thickness skin incision along the caudoventral aspect of the zygomatic arch was performed. Blunt dissection through the platysma muscles and the zygomatic and sphincter colli muscles was performed to expose the masseter muscles. The auriculopalpebral and buccal branches of the facial nerve, as well as the parotid gland and duct, were avoided. The masseter muscular attachment at the ventral aspect of the caudal zygoma was elevated by blunt and sharp dissection using a periosteal elevator. Once the lateral aspect of the TMJ capsule and lateral ligament were identified, a careful horizontal incision using a #15 scalpel blade was performed to expose the joint. Then, the TMJ disc was identified and released from its dorsal and ventral attachments to allow full access to both the dorsal and ventral compartments. If foreign material was identified, it was removed. At this juncture, tissue samples were obtained from the joint capsule and from other grossly affected tissues and submitted for culture and sensitivity testing as well as for histopathological evaluations. Then, antibiotic therapy (ampicillin 20 mg/kg) was administered systemically. After the collection of samples, a sterile IV catheter (18G or larger) was inserted into each of the joint compartments and the joint was copiously irrigated with 0.9% sterile saline (**Figure 1**). Debridement of soft tissues and bones was performed as needed to remove gross debris. Finally, a routine three-layer closure of the surgical site was performed without drain placement.

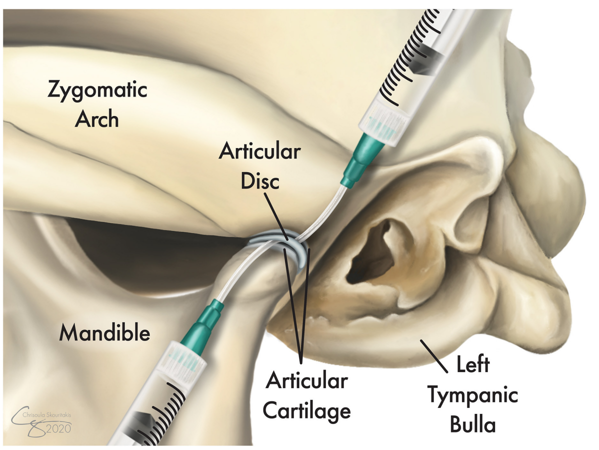

FIGURE 1 | Illustration of the topographic bone and cartilage anatomy of the left temporomandibular joint (TMJ) and its proximity to the tympanic bulla. Following exposure of the lateral aspect of the joint, the TMJ disc is identified and released from its dorsal and ventral attachments to allow full access to both the dorsal and ventral compartments. Tissue samples were obtained from the joint capsule and other grossly affected tissues and were submitted for culture and sensitivity testing as well as for histopathological evaluations. A sterile IV catheter (18G or larger) is inserted into each of the joint compartments and the joint is copiously irrigated with 0.9% sterile saline.

CASE REPORTS

Case 1

A 5-year-old, intact, male Australian cattle dog weighing 23.2 kg was presented for evaluation of oral pain and swelling in the left caudal maxillary and zygomatic regions, which was noticed ∼1 month earlier. The dog had a history of a stick injury in the mouth about 1 year earlier. The owner reported that removing the stick, which was followed by some bleeding, self-resolved the issue. At that time, the owner did not seek further medical attention for the dog. Three weeks before the presentation, the referring veterinarian evaluated the dog and obtained pre- and post-contrast CT images. These scans revealed a region of cellulitis and myositis in the proximity of the left TMJ, osteophytes on the medial aspect of the left condylar process, narrowing of the joint space, and periosteal reaction at the lateral side of the left condylar process. Notably, a foreign body was identified lateral to the TMJ (**Figure 2A**).

An oral and maxillofacial examination revealed swelling on the left maxilla up to the base of the ear, with the apex of the swelling over the TMJ (**Figure 2B**). The dog exhibited substantial pain in its attempt to open the mouth and was reluctant to allow a fully awake oral examination. Complete blood count and urinalysis were within the normal limits, and the serum biochemistry analysis revealed very mild hyperproteinemia and hyperglobulinemia.

A surgical approach to the TMJ was performed as described above. Upon approaching the TMJ, purulent material was found in the TMJ, and the periarticular area included two small foreign bodies that were grossly suspected to be plant materials. Incisional biopsies of the joint capsule and surrounding tissues were obtained (i.e., intracapsular and extracapsular)

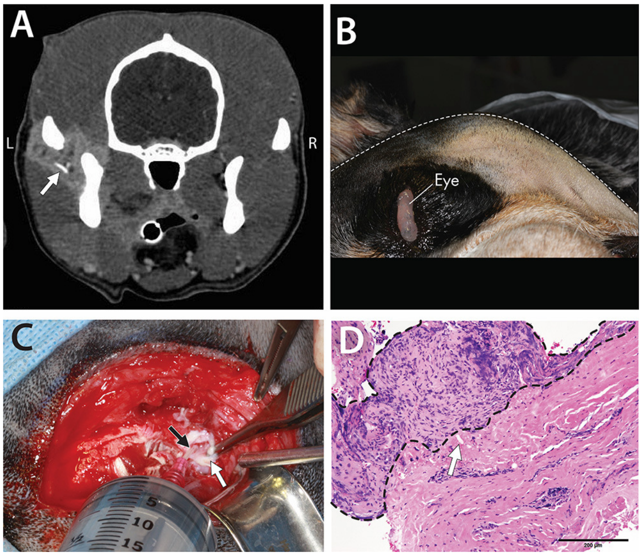

FIGURE 2 | A 5-year-old dog affected by septic arthritis of the left TMJ described in Case 1. **(A)** Contrast-enhanced CT demonstrated inflamed soft tissues as well as a foreign body (arrow) surrounding the TMJ. **(B)** Image obtained with the dog in the right lateral recumbency. Note the substantial swelling on the left side of the face. **(C)** The exposed lateral aspect of the left TMJ with the disc (white arrow) retracted ventrally to allow the 18G catheter (black arrow) to enter the dorsal compartment of the joint for irrigation. **(D)** Histopathological findings demonstrated chronic suppurative inflammation with profound hyperplasia of the joint synovium and extensive fibrosis. Also noted is a foreign body embedded in the tissues (white arrow) [H&E staining and Bar = 200 µm].

and submitted for culture and sensitivity testing and for histopathology. Following copious lavage (**Figure 2C**), closure was performed routinely. The dog was discharged with the following medications: amoxicillin/clavulanic acid (20 mg/kg, q 12 h for 14 days), enrofloxacin (10 mg/kg, q 24 h for 12 days), tramadol (3 mg/kg q 8–12 h for 8 days), and carprofen (2.2 mg/kg q 12 h for 14 days). The owner was informed that the medical therapy might need to be modified based on the culture and sensitivity results.

Histopathological evaluation revealed severe, chronic, multifocal cellulitis, and steatitis with extensive fibrosis. In addition, small fragments of foreign material were noted (**Figure 2D**). Culture and sensitivity analysis reported a large number of *Pasteurella canis*, a moderate number of *Streptococcus viridans*, and a small number of *Corynebacterium* species. Anaerobic culture identified mixed growth of beta-lactamase negative *Porphyromonas* species, *Propionibacterium* species, *Bacterioides* species, and *Fusobacterium* species. Given the culture and sensitivity results and after a consultation with a medical pharmacologist, administration of amoxicillin/clavulanic acid continued for 4 additional weeks, and metronidazole at a dose of 21 mg/kg q 12 h for 4 weeks was added. At the 2-week follow-up, the dog was doing well, and the swelling had resolved. A phone interview with the owner revealed that the dog was free of clinical symptoms for the next 7.5 years with no sign of recurrence.

Case 2

A 7-year-old male, neutered bullmastiff dog, weighing 65.5 kg, was presented for evaluation of right-sided severe masticatory muscle atrophy and pain. The dog was diagnosed with atrophy of the masticatory muscles on the right side and ear infection ∼2 months earlier by the referring veterinarian and was treated with topical otic medications to resolve the infection (Posatex®, Merck Animal Health). Antibody testing for masticatory muscle myositis was negative. One month later, the dog exhibited an increase in pain on the right side of the face, was hesitant to eat, and was administered amoxicillin (1,500 mg q 8 h) for an unknown duration. The owner also reported a decrease in the energy level but reported that the dog was still exhibiting a good appetite.

Neurologic examination revealed right-sided atrophy of the temporal and masseter muscles, right-sided facial drooping, and pain on palpation of the right TMJ region. In addition, there were proprioceptive deficits in the pelvic limbs. Complete blood count and serum biochemistry were within the normal limits. Standard thoracic radiographic series were obtained and were within the normal limits; however, an abdominal ultrasound revealed a mural mass at the gastric fundus with mineralization. Standard MRI sequences of the head were obtained followed by a conventional CT scan as described earlier. Severe erosive septic arthritis of the right TMJ with concurrent osteomyelitis was diagnosed, including involvement of the mandibular branch of the trigeminal nerve and associated neuritis (**Figure 3**). Other changes of the affected joint included a subchondral bone cyst, periosteal reaction at the right condylar process, and osteophytes. Secondary right-sided atrophy, which was likely neurogenic, was also observed. In addition, degenerative changes, such as irregular joint space and joint surfaces and subchondral bone sclerosis, at the left TMJ were also apparent. Cerebrospinal fluid was obtained, and it revealed mild mononuclear reactivity and possible marginal suppurative inflammation. No infectious organisms or neoplastic cells were observed. The owner declined the exploratory approach to the TMJ to obtain samples and an empirical therapy was initiated which included amoxicillin/clavulanic acid (17 mg/kg, q 12 h for 8 weeks), enrofloxacin (10.5 mg/kg, q 24 h for 8 weeks), tramadol (3 mg/kg q 8–12 h), and meloxicam (0.11 m g/kg q 24 h). The dog was lost to short-term follow-up and was presented again 1 year later to emergency service for evaluation of a mass on the chest and for limb edema. The owner reported that the dog exhibited progressive inability to open the mouth since the diagnosis of septic arthritis 1 year earlier. At that time, the dog was diagnosed with undifferentiated round cell neoplasia with local lymphadenomegaly and pitting edema at the right forelimb and was humanely euthanized as per the request of the owner.

Case 3

An ∼3-year-old female, spayed mixed breed dog, weighing 18.5 kg, was presented for a draining tract in the left TMJ area. The draining tract was associated with extensive scar tissue, reduced mandibular range of motion, progressive oral pain, and frequent head-shaking. The dog was adopted 4 months before presentation after being rescued. Since the adoption, the dog was

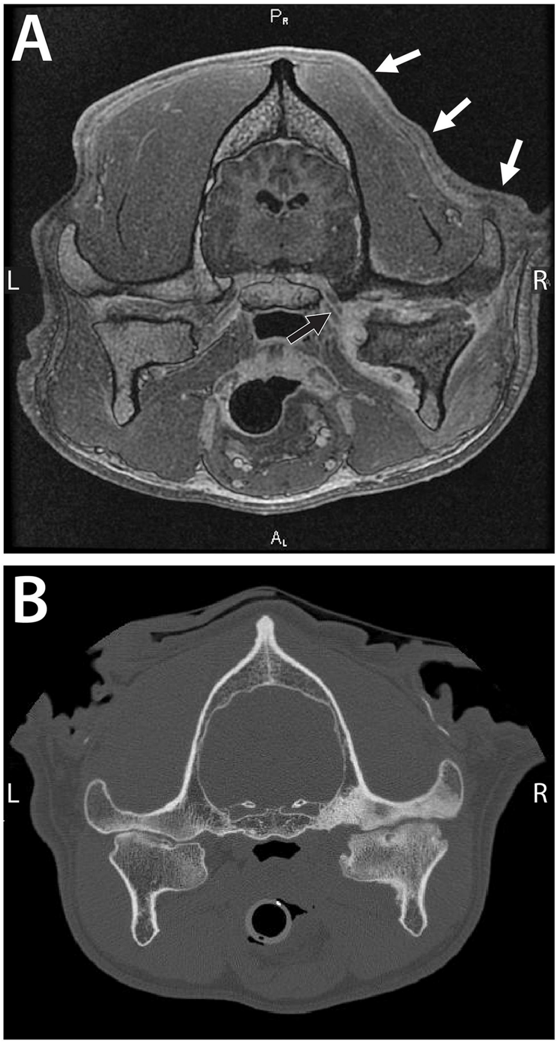

FIGURE 3 | A 7-year-old dog with severe and chronic septic arthritis of the right TMJ described in Case 2. **(A)** Images from MRI demonstrating severe erosive septic arthritis of the right TMJ with concurrent osteomyelitis including involvement of the mandibular branch of the cranial nerve V (black arrow) and associated neuritis. Note the secondary right-sided atrophy of the masticatory muscles and the temporal muscles, in particular (white arrows). **(B)** A conventional CT image demonstrating severe bone sclerosis of the condylar process, mandibular head, and mandibular fossa as well as irregular joint space. In addition, lateral and medial osteophytes were noted as well as a periosteal reaction at the medial aspect of the condylar process and subchondral bone cyst at the mandibular head. Note that the contralateral TMJ exhibits features consistent with advanced degenerative changes (Cissell et al., Diagnostic imaging in oral and maxillofacial surgery. In: Verstraete FJM, Lommer MJ, Arzi B, Editors. Oraland Maxillofacial Surgery in Dogs and Cats, 2nd ed St. Louis, MO: Elsevier; 2020. p. 56-64. Reproduced with permission, copyright Elsevier).

treated for the draining tract with multiple antibiotics with no apparent resolution of the condition.

Oral and maxillofacial examination revealed a moderately atrophied temporal and masseter musculature, substantial scar tissue, and contraction of the skin on the left side of the face. In addition, a draining tract with hemorrhagic purulent discharge at the left TMJ area was noted (**Figure 4**). Multiple tooth fractures were also noted as well as mild generalized dental plaque and calculus accumulation. It was estimated that mouth opening was ∼75% of normal opening on both an awake examination and an examination under general anesthesia. Complete blood count, serum biochemistry, and urinalysis were within the normal limits. Ultrasound of the left TMJ area revealed potential osteomyelitis of the zygomatic arch with suspected sequestrum and regional cellulitis with possible TMJ involvement.

Pre- and post-contrast CT images of the skull were obtained as described earlier. Severe chronic septic arthritis of the left TMJ, characterized by full destruction of the articular surfaces as well as osteomyelitis of the zygoma and sequestrum formation, was noted. In addition, secondary cellulitis, abscessation, and draining tract (**Figure 4**), as well as enlarged, likely reactive, left mandibular and medial retropharyngeal lymph nodes and chronic otitis at the left ear, were noted.

A lateral approach to the left TMJ was performed as described earlier. In addition to the procedure described above, zygomectomy of the affected bone was performed with piezosurgery (Piezotome® Cube, Acteon, Mérignac, France) from caudal to the orbital process (and just rostral to the lytic bone defect) and to the rostral aspect of the mandibular fossa (not penetrating the joint). Once the infected and grossly necrotic zygomatic arch was removed, the approach to the TMJ was continued. The removed tissues were submitted for culture and sensitivity testing and histopathology. The dog was discharged with the following medications: fentanyl transdermal patch (50 mcg/h), clindamycin (23 mg/kg, q 12 h until sensitivity results were available), gabapentin (10–20 mg/kg q 8–12 h), and carprofen (2.2 mg/kg q 12 h for 14 days). The owner was informed that the medical therapy might need to be modified based on the culture and sensitivity results.

Histopathological evaluation revealed lymphoplasmacytic, histiocytic, neutrophilic fasciitis, osteomyelitis, and osteonecrosis (**Figure 5**). Culture and sensitivity analysis yielded a small number of *P. canis*. Anaerobic culture identified a moderate number of mixed growths, including beta-lactamase negative *Bacterioides/prevotella species*, and a small number of *Peptostreptococcus anaerobius* species. Given the culture and sensitivity results, and in consultation with a medical pharmacologist, administration of clindamycin was discontinued, and administration of amoxicillin/clavulanic acid was started for 6 weeks (14 mg/kg, q 12 h), along with metronidazole at a dose of 14 mg/kg q 12 h for 6 weeks. The dog was evaluated at 6 days and 3 weeks postoperatively, and was found to have substantial improvement and resolution of the draining tract. No pain was noted on palpation and the dog was noted yawning multiple times without perceived pain.

An oral and maxillofacial examination was performed at the 4-month recheck appointment, and CBCT images of the skull were obtained (**Figures 4D–F**). The dog exhibited complete resolution of clinical signs, with no recurrence of the draining tract, and increased mouth opening as visually compared to pretreatment (gape angle 49.6 degrees, interincisive measurement 75 mm). CBCT demonstrated static to slightly improved articular surfaces of the left TMJ with persistent irregular articular margins of both the mandibular head of the condylar process and the mandibular fossa.

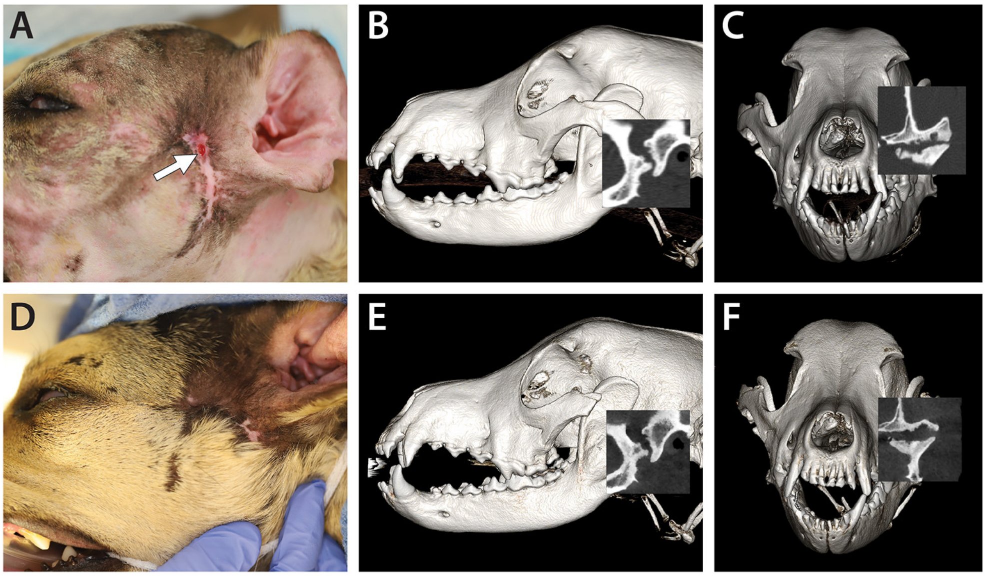

FIGURE 4 | A 3-year-old dog affected by septic arthritis of the left TMJ is described in Case 3. **(A)** Preoperative image demonstrating the extensive scar and the presence of a draining tract in the area of the TMJ (white arrow). **(B)** A lateral three-dimensional image and **(C)** transverse preoperative CBCT image demonstrating severe destruction of the mandibular head and the condylar process as well as erosive destruction of the articular surface of the mandibular fossa. **(D)** A 4-month follow-up image demonstrating the resolution of the draining tract. **(E)** A lateral three-dimensional image and **(F)** a transverse CBCT image at the 4-month follow-up demonstrating static to slightly improved features of the left TMJ.

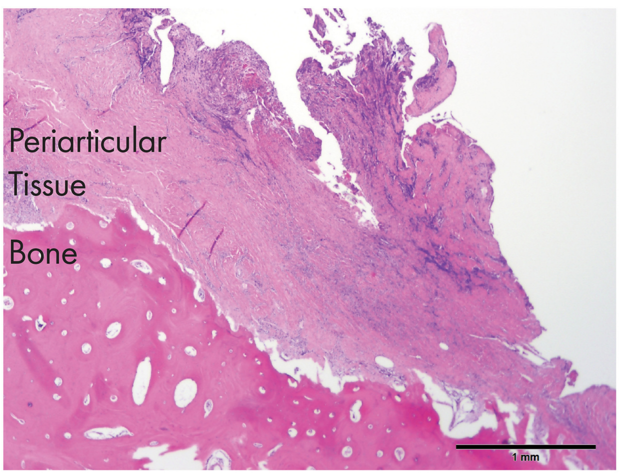

FIGURE 5 | An histological section of the tissues obtained from dog 3. Note the severely inflamed periarticular tissues and the necrotic bone (zygomatic arch) in the proximity of the affected TMJ [H&E staining and Bar = 1 mm].

Case 4

An 11-year-old, male, neutered pug, weighing 8.7 kg, was presented for profound pain when opening the mouth. The dog has had, over a year, history of chronic bilateral middle ear disease treated with two ventral bulla osteotomy surgeries. Despite surgery and medical management, the dog continued to experience profound pain. The dog also had concurrent atypical Addison's disease, moderate to severe periodontal disease, and brachycephalic airway syndrome. The dog was receiving a daily dose of prednisone for the management of Addison's disease.

Oral and maxillofacial examination revealed that the dog experienced substantial pain on its attempt to open the mouth which had developed and worsened over the last year and a half. The dog had previously been seen for periodontitis and had had multiple extractions. In the interim between the previous extractions and the current presentation, the dog had had several ear infections that had been difficult to control and had been treated by a specialty group combining veterinary dermatology, surgery, and neurology, given the myriad needs of the dog. After a first bulla osteotomy, the dog developed further ear infection on the right side, and a second bulla osteotomy found a cholesteatoma on the right side. After the second surgery, the dog developed worsening signs of pain, difficulty in opening the mouth, and eating. On oral examination, apart from the previously extracted teeth and moderate dental calculus, no obvious oral abnormalities were detected. The dog was reluctant to allow an open-mouth examination or palpation of the ears and TMJ. Complete blood count and urinalysis were within the normal limits and the serum biochemistry analysis revealed very mild hyperproteinemia and hyperglobulinemia.

Cone-beam CT images of the skull were obtained. Severe erosive arthritis of the right TMJ was observed, which was characterized by a pronounced periosteal reaction of the condylar process, osteophytes present on the medial aspect of the joint, and subchondral bone cyst at the lateral aspect of the condylar process. There was also increased density of the bone of the condylar process as compared to the left side, suggesting a septic process. Importantly, the bulla on the right side was deformed,

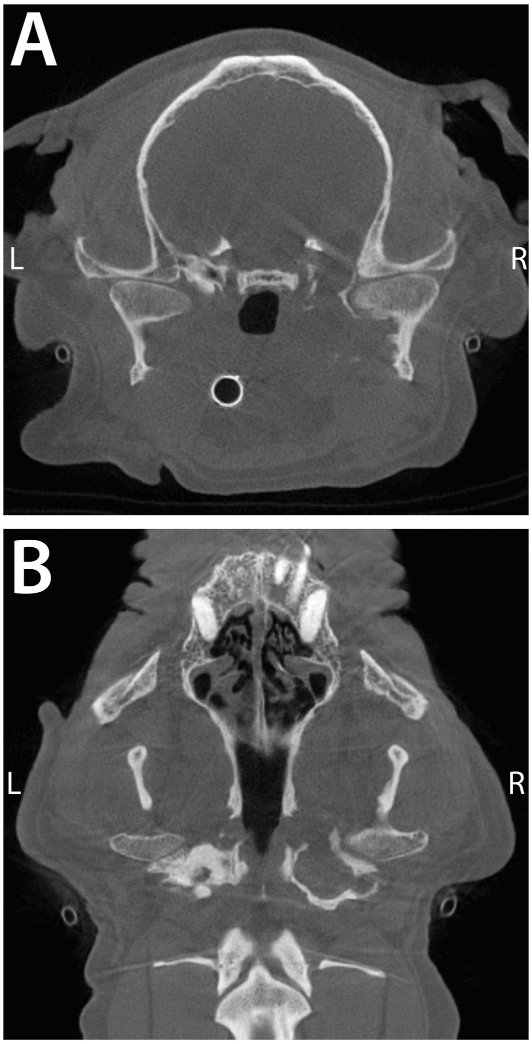

FIGURE 6 | Transverse **(A)** and dorsal **(B)** CBCT images of a 11-year-old, dog described in Case 4. Note the severe periosteal reaction at the medial aspect of the right condylar process and the engulfment of the medial aspect of the TMJ by the deformed bulla. The intimate proximity of the middle ear to the TMJ provides a readily available source for infection to the TMJ.

with bony expansion, and it engulfed the medial aspect of the condylar process (**Figure 6**). A surgical approach to the TMJ area was performed as described earlier. Fluid and samples were obtained from the joint capsule and the surrounding tissues and were submitted for culture and sensitivity testing. An enlarged Lymph node in the proximity of the joint was removed and submitted for histopathological analysis.

The dog was discharged with the following medications: amoxicillin/clavulanic acid (20 mg/kg, q 12 h for 14 days), enrofloxacin (10 mg/kg, q 24 h for 12 days), tramadol (3 mg/kg q 8–12 h for 8 days), and carprofen (2.2 mg/kg q 12 h for 14 days). The owner was informed that medical therapy may need to be modified based on the culture and sensitivity results.

Histopathological evaluation of the lymph node revealed a reactive process with no evidence of neoplasia. Culture and sensitivity analysis yielded a low number of non-enteric gram-negative rods, *Pseudomonas putida*, and *Chryseobacterium indologenes.* The organisms isolated were uniformly resistant to common antibiotics, such as cephalosporins, amoxicillin/clavulanic acid, doxycycline, chloramphenicol, and trimethoprim/sulfa. Based on the culture and sensitivity results, marbofloxacin (50 mg q 24 h) was prescribed for 2 months. The day following the procedure, the client updated that the dog exhibited an immediate remarkable improvement in his level of comfort. At the 2-week recheck, the dog had more energy, allowed a full oral examination, and had a good range of motion of the TMJ. Unfortunately, about 2 weeks after this recheck appointment, the dog exhibited a sudden severe decrease in the energy level and appetite that did not respond to hospitalized supportive care for 24 h and was humanely euthanized.

DISCUSSION

In this report, we describe our approach to the diagnosis and management of suspected septic arthritis of the TMJ in dogs and exemplify it with reports from our experience with four dogs. Overall, septic arthritis is a rare clinical entity, but when presented, it provides a diagnostic and therapeutic challenge. Affected dogs typically exhibit profound pain and dysfunction necessitating urgent and precise diagnosis in order to identify the correct targeted therapy. In that context, a surgical approach to the TMJ, in order to (1) obtain diagnostic samples for culture and sensitivity, (2) decompress the joint and the associated abscess (if present), (3) remove joint debris, and (4) copiously irrigate the joint with sterile saline to reduce the inflammatory burden, was demonstrated to be beneficial in managing the disease.

A previous study demonstrated osteoarthritis of the TMJ (TMJ-OA) to be the most common disorder of the TMJ in dogs, followed by trauma (22, 23). From the standpoint of biological behavior, osteoarthritis or degenerative joint disease is considered a "low-inflammatory" arthritis (7, 23, 24). We have found that TMJ-OA is symptomatic and is associated with mild to moderate pain or merely discomfort in only ∼25% of the cases (22). In contrast, septic arthritis of the TMJ is considered a "high inflammatory" arthritis and is presented with a pronounced pain and dysfunction (i.e., limitation in the range of motion), stiffness, swelling, and potentially the presence of a draining tract. Septic TMJ is typically caused by bacteria, fungi, viruses, or parasites (6, 7, 25, 26).

While it may be difficult to assert the precise initiating cause of septic arthritis of the TMJ, we observed and reported, from a literature point of view, the following four potential scenarios: local dissemination of infection from adjacent anatomical structures, such as the inner ear/tympanic bulla that acts as a continuous focus of infection; trauma (i.e., direct inoculation by a dog bite); penetrating stick injury to the pharyngeal area; or migration of microorganisms from a distant site *via* hematogenous spread (1–3, 18, 19, 27–29). Regardless of the source and cause, once infection of the joint is established, articular destruction ensues and typically progresses rapidly (6, 30). The rapid proliferation of bacteria within the synovial fluid and membrane-induced activation of the inflammatory cascade result in the influx of macrophages, neutrophils, and synoviocytes, as well as in the accumulation of various high levels of pro-inflammatory cytokines and chemotactic molecules (6, 31, 32). In addition, there is the release of proteases and antigen–antibody complex formation within the joint. This profound inflammatory insult on the joint results in catabolic enzymes, causing damage and degradation to the articular surfaces and the disc of the joint (2, 12, 33, 34).

Advanced diagnostic imaging with CT, CBCT, and/or MRI is essential to confirm the clinical suspicion of septic arthritis of the TMJ (6, 11, 14, 15, 22, 35, 36). In that context, contrast-enhanced CT studies have been reported to be the recommended imaging modality in the assessment of septic TMJ arthritis as they allow visualization of the inflamed soft tissues, presence of fluids (i.e., purulent), and permit evaluation of adjacent clinically relevant structures to determine the presence of concurrent myositis and osteomyelitis (11, 17). If the initial diagnosis by CT is not rewarding and the clinical suspicion remains high, MRI is recommended. The MRI displays good resolution in the detection of joint effusion, purulent material accumulation, evaluation of the articular joint surfaces, and reasonable resolution of the articular disc and adjacent structures, such as the cranial nerves V and VII (14, 35). This was proven to be highly beneficial in Case 2 in the present report. Conventional CT and CBCT display better diagnostic performance in identifying osseous structures and diagnosing bone changes (i.e., erosion and subchondral cysts), in identifying spatial locations, and have less interference with metal implants, if present (11, 15, 37).

In order to confirm the clinical and imaging suspicion of a septic process and provide appropriate antimicrobial therapy, it is valuable to approach the joint surgically and obtain several samples of the tissues and fluids for culture and sensitivity and histopathology (1, 6, 13, 38). Given the small size and tight confinement of the TMJ in dogs, attempting fine needle aspiration of the area may result in negative or insufficient findings (39). As noted in this report, laboratory tests, such as complete blood count and serum biochemistry, may be non-rewarding and/or non-specific and should not be used as a sole method to diagnose or rule out septic TMJ arthritis. A blood culture may be useful in identifying causative organism(s) if culture results are negative (40). Finally, given the clinical examples in this study, obtaining culture and sensitivity samples was essential due to the presence of anaerobic or widely resistant bacteria. Only in Case 2, where the owner declined the sampling of the TMJ, an empirical treatment was given using a broad-spectrum antibiotic. In the remaining three cases, empirical treatment was given after surgery and was modified based on the culture and sensitivity findings.

A surgical approach to the TMJ allowed draining of the purulent content of the joint and its surrounding, removing necrotic bone (i.e., sequestrum), debris, and foreign material. Moreover, the surgical approach allowed the irrigation of the joint compartments and the surrounding area to reduce the inflammatory burden (1, 4, 6, 41, 42). Condylectomy should be considered as the last resort and should be reserved for non-responsive osteomyelitis of the condylar process or in cases that develop ankylosis (1, 12, 43). Unfortunately, given the dramatic insult to the joint and the innate inability of cartilage to repair (44), therapy may not result in a long-term positive outcome, and dysfunction in the form of decreased range of motion may remain.

CONCLUSION

Septic arthritis of the TMJ is a rare, painful, and morbid form of arthritis in the dog. Understanding the fundamental aspects of this disease and how the joint may become infected is imperative to identify, diagnose, and treat the condition correctly. Diagnosis requires obtaining a good history, a thorough clinical examination, and the application of advanced diagnostic imaging. Furthermore, obtaining diagnostic samples of the joint *via* an open surgical approach proved beneficial in applying the targeted antimicrobial therapy and allowing for a rapid return to normal function. Finally, delayed diagnosis and treatment and non-specific (and ineffective) empirical therapy may result in loss of function, continuous pain, and overall poor outcome.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Ethical review and approval were not required for the animal study because the study is retrospective in nature and included clinical cases, hence, it is exempt from IACUC requirements. The standard written informed consent required for all procedures performed at the William R. Pritchard Veterinary Medical Teaching Hospital of the University of California, Davis was obtained from the owners.

AUTHOR CONTRIBUTIONS

BA: study concept and design, provision of study material or cases, manuscript writing, data analysis and interpretation, and review of the manuscript for important intellectual input. NV: provision of the study material, manuscript writing, data analysis and interpretation, and review of the manuscript for important intellectual input. AF: provision of study material or cases, manuscript writing, and review of the manuscript for important intellectual input. FV: provision of study material and review of the manuscript for important intellectual input. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

The authors thank Dr. Chrisoula Toupadakis Skouritakis for assistance with the figures.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Veterinary Science

REVIEW
published: 21 May 2021
doi: 10.3389/fvets.2021.685036

Locoregional Flap Reconstruction Following Oromaxillofacial Oncologic Surgery in Dogs and Cats: A Review and Decisional Algorithm

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OPEN ACCESS

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Specialty section:
This article was submitted to Veterinary Dentistry and Oromaxillofacial Surgery, a section of the journal Frontiers in Veterinary Science

Received: *24 March 2021*
Accepted: *21 April 2021*
Published: *21 May 2021*

Citation:
Guzu M, Rossetti D and Hennet PR (2021) Locoregional Flap Reconstruction Following Oromaxillofacial Oncologic Surgery in Dogs and Cats: A Review and Decisional Algorithm. Front. Vet. Sci. 8:685036. doi: 10.3389/fvets.2021.685036

Primary treatment of most oromaxillofacial tumors in dogs and cats is resective surgery. Management of malignant tumors may be very challenging as wide/radical free-margin surgical removal must be achieved while preserving vital functions. Removal of orofacial tumors may result in large defects exposing the oral cavity or creating a communication with the nasal, pharyngeal, or orbital cavities. Such defects require orofacial reconstruction in order to restore respiratory and manducatory functions. The veterinary surgeon must be familiar with reconstructive techniques in order to prevent the inability of closing the defect, which could lead to an insufficient resection. Small oral defects exposing the nasal cavity are best closed with local random mucosal flaps. Closure of large oral defects may be better achieved with a facial or major palatine-based axial-pattern flap. Small to moderate facial defects can be closed with local advancement or transposition skin flaps. Reconstruction of large facial defects often requires the use of locoregional axial pattern flaps such as the caudal auricular, the superficial temporal, or the facial (angularis oris) myocutaneous axial pattern flaps. Recent publications have shown that the facial (angularis oris) flap is a very versatile and reliable flap in orofacial reconstructive surgery. A surgical decision algorithm based on the size, nature, and location of the defect is proposed.

Keywords: oral, maxillofacial, flap, surgery, reconstruction, oncology, dogs, cats

INTRODUCTION

Oral tumors are common in dogs and cats, representing 1.2–10% of all tumors (1, 2). The incidence of oral tumours has been calculated to be 4.9 per 1,000 dogs (0.5%) and 4.9 per 1,000 cats (0.5%) and malignant tumours represented 53.6% of the canine oral tumours and 58.1% of the feline oral tumours identified in a recent demographic study (3). Malignant melanoma, squamous cell carcinoma, and fibrosarcoma in dogs, and squamous cell carcinoma in cats are the most common oral malignancies (4, 5).

Though a lot of progress has been made in adjunct therapy, surgery is still the primary treatment for most orofacial tumours in humans as well as in dogs and cats (6–12). Modern diagnostic imaging technology such as CT scan, MRI and, more recently, ^{18}FDG-PET CT has contributed to better evaluation of local, regional, and distant extent of disease and better surgical and radiation appraisal (13–29). Nevertheless, in our experience, oral tumours in dogs and cats are often first discovered, or referred, late. Studies in humans have shown that the greater diagnostic delay for oral tumours, the more advanced the disease is at staging. Advanced disease requires more radical surgery and results in a poorer outcome (9, 30, 31). Despite the progress made in adjunct therapy (radiation therapy, chemotherapy, immunotherapy, targeted therapy), wide/radical resection of bone and soft tissue is often required (9, 11). Curative-intent maxillofacial ablative (oncologic) surgery is aimed at achieving an en-bloc free-margin resection (R0 resection). Insufficient resection, either microscopically positive margins (R1 resection) or macroscopically cutting through the tumour (R2 resection), constitutes high-risk situations leading to relapse and potential neoplastic spread (6, 7, 11, 12, 32–38). While achieving clean margins (R0 resection) is mandatory, avoiding the unnecessary sacrifice of normal tissue and preserving respiratory and oral functions are also of fundamental importance (37, 39). Cosmesis (preservation or restoration of form) seems less of a concern in dogs and cats compared to that in humans, though in our experience acceptance of the surgery by owners may be strongly influenced by the post-operative appearance. The major risk for the surgeon is the fear of being unable to close the surgical defect in a functional and/or cosmetic manner. Having this concern in mind while performing the surgery will negatively affect the extent of the ablation and increase the risk of relapse. As the veterinary surgeon is most of the time both the maxillofacial surgeon performing the resective surgery and the plastic surgeon performing the reconstructive surgery, knowledge of surgical techniques enabling the closure of large hard and soft tissue defects is mandatory in order to achieve the best results. Ideally, the options for reconstruction are thoroughly discussed and decided prior to performing the ablative surgery. Improvement in median survival and progression-free survival in recent publications may be due to earlier detection of the disease, better evaluation of its extent but also to a more aggressive surgery and adjunct treatments (11).

Orofacial reconstructive techniques used in veterinary medicine include local and regional random flaps, tension relieving techniques, local and regional pedicle flaps, free partial/total skin grafts, and vascularized free flaps (40–44). Various decisional algorithms have been published over time in human head and neck reconstructive surgery, with main criteria based on the location and the size of the defect. However, no consensus has been made or generally accepted in the veterinary field to date (45–51). Vascularized free flaps, either simple or composite, constitute the gold standard treatment for the reconstruction of most large defects in humans, with good clinical outcome achieved in up to 98% of the cases. Specific contraindications may include some medical conditions (e.g., atherosclerosis, on-going tobacco consumption) or elderly patients (52, 53). Despite recipient vessels of the head and neck having been studied in dogs, only very few clinical cases of orofacial reconstruction with a free flap have been reported in the veterinary literature (54–57). Slow development of these techniques in the veterinary field may be due to the prolonged time and cost inherent to such surgeries, the need for highly trained surgeons with microsurgical skills, specific instrumentation and monitoring, and appropriate facilities equipped with intensive care units (44, 58, 59). Nevertheless, recent trends in orofacial plastic and reconstructive surgery are reconsidering locoregional flaps in terms of functional outcomes, complication rate, cost, and prognosis in some clinical situations in humans (53, 60).

Abbreviations: AOMC, Angularis Oris MyoCutaneous; CAMC, Caudal Auricular MyoCutaneous; FAMM, Facial Artery MyoMucosal; FTL, Full-Thickness Local; FV, Free (vascularized); LR, Local Random; PAMP, Palatine Artery MucoPeriosteal; SCMC, Superficial Cervical MyoCutaneous; STMC, Superficial Temporalis MyoCutaneous; TMF, Temporal MyoFascial.

CUTANEOUS AND MUCOSAL VASCULARIZATION OF THE OROMAXILLOFACIAL REGION

The skin (cutis) consists of a superficial epidermis of stratified squamous epithelium and an underlying connective tissue, the dermis (corium) separated by the basement membrane. The dermis contains blood vessels, lymphatics, muscles, and nerve endings. A subcutis or hypodermis, composed of fat and loose collagenous trabecular and elastic fibers, connects the dermis with the fascia and acts as a moveable support allowing the skin to glide over the underlying tissue. Cutaneous or panniculus muscles are attached to the dermis and are anchored to the subcutaneous fascia rather than to the bone. In the head and neck area, they comprise the superficial craniofacial muscles, the platysma, the sphincter colli superficialis, and the sphincter colli profundus (61, 62). Main regional arteries give off perforator branches traversing the skeletal muscles to supply the subdermal plexus. The arteries to the skin include simple direct cutaneous arteries running between muscles toward the skin and musculocutaneous (mixed cutaneous) arteries supplying both muscles and skin (63–65). In mucocutaneous regions, such as the lip and the cheek, there is a transition from the integument to the mucous membrane, which is a stratified epithelium lining the oral cavity. The cutaneous portion is typically supplied by three arterial and venous plexuses: (1). the deep or subcutaneous plexus providing the adipose and areolar tissue on the deep face of the dermis as well as the superficial muscles of the head, (2). the middle plexus layer located below or at the level of the sebaceous glands, (3). The superficial plexus layer lying in the outer layers of the dermis. In the mucous membrane, the vessels form a superficial plexus supplied by a meshwork in which there may be layers suggestive of a middle and deep plexuses; large vessels lie on the oral surface of the orbicularis oris muscle (65). Arteries supplying the orofacial region originate from the external carotid artery. Main branches are the ascending pharyngeal, lingual, facial, caudal auricular, superficial temporal, and maxillary arteries and its terminal branches (66) (**Figure 1**).

Upper Face and Neck

The ***caudal auricular artery*** arises from the base of the annular cartilage. Among its branches, the sternocleidomastoideus supplies the cleidocephalic and sternocephalic muscles and also

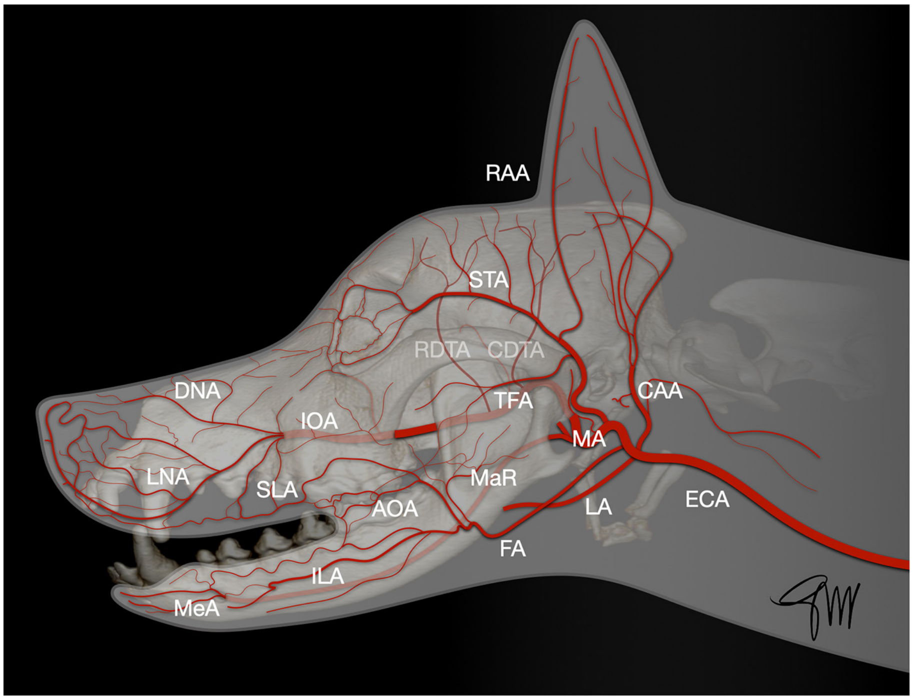

FIGURE 1 | Main arterial blood supply to the head in the dog. AOA, angularis oris artery; CAA, caudal auricular artery; CDTA, caudal deep temporal artery; DNA, rostral dorsal nasal artery; ECA, external carotid artery; FA, facial artery; ILA, inferior labial artery; IOA, infraorbital artery; LA, lingual artery; LNA, lateral nasal artery; MA, maxillary artery; MaR, masseteric ramus; MeA, mental artery; RAA, rostral auricular artery; RDTA, rostral deep temporal artery; LA, superior labial artery; STA, superficial temporal artery; TFA, transverse facial artery.

supplies the cranial part of the cervical skin, the platysma and subcutaneous fat (66, 67). This cutaneous branch originates in the area between the lateral aspect of the wing of the atlas and the vertical ear canal. Then it runs ventrocaudally toward the scapula, and eventually anastomoses with some cutaneous branches originating from the omocervical artery (67).

The ***superficial temporal artery*** provides blood supply to the skin and frontalis muscle in the temporal region in dogs and cats. It arises rostral to the base of the auricular cartilage and extends dorsally toward the zygomatic arch. From the base of the zygomatic arch, it extends rostrally along the zygomatic arch toward the upper eyelid (66, 68). A direct cutaneous branch of the superficial temporal artery is observed where the artery and its accompanying vein enter the temporal fascia (69).

Middle and Lower Face

The main arteries supplying the middle and lower face, are the facial artery and the infraorbital artery (70). They originate from the external carotid artery (71). Veins follow a similar path, nearby the arterial web, eventually draining in the maxillary and linguofacial veins, themselves merging in the external jugular vein, that constitutes the main collecting vessel of the head (65, 66, 70–72). After sending off the inferior labial artery, just rostral to the masseter muscle and medial to the digastric muscle, at the level of the of the mandibular lymph nodes, the facial artery runs caudo-rostrally between the platysma and the buccinator muscle into the cheek opposite to the last molar teeth and divides into the angularis oris and superior labial arteries; the angularis oris is usually thicker at its origin than the superior labial artery (66, 70, 71). The ***angularis oris artery*** runs rostrally and divides at the labial commissure into a superficial and a deep ramus. It supplies in part the buccinator and orbicularis oris muscles as well as the skin and mucosa of the commissure and caudal part of the lips (66). The superficial (cutaneous) ramus is thinner than the deep one and divides into a superior and inferior branch, which anastomoses with the superior and inferior labial arteries, supplying the skin and the orbicularis oris of the caudal part of the superior and inferior lips. The deep (mucosal) ramus of the angularis oris has a course similar to the superficial ramus and has a broader distributing territory on the oral mucosa than on the skin. It also anastomoses with the superior labial artery, forming an arterial arch, and to the inferior labial artery (70). The ***superior labial artery*** is the termination of the facial artery. It runs rostrally in the superior lip and anastomoses at the level of the second and third maxillary premolar teeth with the superior ramus of the infraorbital artery forming an arterial arch (70). It also sends branches caudally to the eyelid and to the dorso-rostral part of the masseter, one of these branches anastomosing with the transverse facial artery, and ventrally to the superior branches of the angularis oris (70). The caudal half of the superior lip is vascularized by the superior labial artery. This vascular territory is connected through anastomoses to the rostral half supplied by the infraorbital artery (72). The ***inferior labial artery*** runs rostrally along the orbicularis oris muscle from the ventral border of the mandible just rostral to the masseter muscle. It supplies ∼60% of the lower lip (72). In the rostral part of the lower lip, the inferior labial artery anastomoses with the caudal mental branch of the inferior alveolar artery at the caudal mental foramen (66). The ***infraorbital artery*** supplies the rostral half of the superior (maxillary) lip. It leaves the maxillary bone through the infraorbital foramen. In the infraorbital canal or near the foramen it branches into the dorsal nasal artery. About 1–2 cm rostral to the foramen, the artery changes direction and enters the superior lip passing in the mucosa at the level of the first maxillary premolar tooth. At that level, it releases one or two lateral nasal arteries and continues as the dorsal anterior nasal artery. Numerous anastomoses occur between these branches forming a dense arterial network in the rostral part of the nose (70).

PRINCIPLES OF FLAPS

A flap (pedicle graft) is a transfer of tissue that has its own blood supply. In contrast with a non-vascularized graft, a flap is less dependent on the recipient site supply as it brings its own. It allows more tissue bulk and predictable healing and viability. Based on the main pattern of their blood supply, two types of flaps are recognized: random and axial pattern flaps (64, 73).

Random Pattern Flap

A random pattern flap is a local flap relying on general subdermal (submucosal) blood vessels. Because there is no specific artery supplying the flap, risk of necrosis at the distal end of the flap is increased and narrow flaps need to be avoided. To prevent this, the general guideline is a ratio 2:1 for base to length of the flap. Owing to the very good vascularisation of orofacial structures, random pattern flaps on the face can sustain a 3:1 length to width ratio (74). To compensate for excessive shrinkage after harvest, the soft tissue flap should be designated to be 10–20% larger than the defect to cover (75). Because they only rely on the subdermal plexus for survival, they should not be fenestrated, tacked down with walking sutures, or stretched excessively under tension. Excess of tissue (dog's ear) at the base of the flap should not be removed during initial closure of the wound (41).

The type of primary movement or motion of the flap can further subdivide random pattern flaps into three categories: advancement, rotation, and transposition. Advancement and rotation flaps, also called sliding flaps, recruit adjacent lax tissue and move in, respectively, either a linear or arced motion to fill in the primary defect. Transposition flaps, also called lifting flaps, recruit non-contiguous donor tissue incised, and lifted over intact tissue and placed into the primary defect (76, 77).

In a recent retrospective study, the complication rate for random flaps in the face and head area was 17%, with no major complications (>50% flap failure or requiring a second surgery) reported. This was significantly less than for skin deficits of the torso or limbs (78). The most common complication was dehiscence of the distal wound edge. Advancement and transposition flaps had lower complication rates than rotation flaps. The mean time (±SD) to onset of clinical signs of complications was 6.9 days (±4.0) (78).

Axial Pattern Flap

The axial pattern flap is a pedicle graft that incorporates a direct cutaneous artery and vein into its base. The vessels extend up the length of the flap to a variable degree, the terminal branches of which supply blood to the subdermal/submucosal plexus. All the skin or mucosa supplied by this vascular territory, or angiosome, can be lifted from the donor site and will survive provided these vessels remain intact and patent (79). As a result of this, axial pattern flaps have better perfusion (80). Nevertheless, in a recent retrospective study reporting the outcome and complications of axial pattern skin flaps in dogs and cats, postoperative complications occurred in 83% of the cats and 92% of the dogs with 38% of the cats and 34% of the dogs requiring one or more additional surgeries. Wound dehiscence was the most common complication with 58 % of dogs and cats affected (81). Though the percentage of complications was high, most of them were minor complications. The outcome of the flap surgery was considered good to excellent in 58% of the cats and 66% of the dogs. Overall, 93% of the wounds were successfully reconstructed. As only a few surgeries involved facial/head flaps, unfortunately no specific conclusion can be drawn for maxillofacial reconstructive surgery (81).

Flaps may also be described in terms of proximity to the recipient site (local, regional, distant). Local flaps involve transfer of tissue from the immediate adjacent anatomic subsite. Regional flaps use tissue from a neighbouring site. Flaps where the bulk of tissue is limited to the head and neck region are classified as loco-regional flaps; they may be random or axial pattern flaps and may involve only one tissue component (e.g., oral mucosa, skin) or several tissue components such as mucosa, muscle, skin or bone harvested from the same angiosome (composite flaps).

RECONSTRUCTION TECHNIQUES

Oral Defects

Oral defects may involve bone and oral mucosa. Most of the time, bone reconstruction is not performed and coverage of the defect and restoration of the oral cavity lining is achieved through the use of mucosal or myomucosal flaps harvested from the alveolar and/or the labiobuccal mucosa or through the use of palatal mucoperiosteal flaps. These local flaps are supplied by branches of the facial artery (angularis oris and superior labial arteries) in the caudal (cheek) area, by the infraorbital artery and its ramus (lateral nasal) for the superior lip, by the inferior labial artery and mental artery for the inferior lip or by the major palatine for hard palate mucoperiosteal flaps. The local flaps may be random or axial pattern flaps depending whether direct artery and vein have been included in the base of the flap.

Large defects of the oral cavity may be mainly closed through local advancement, transposition flap or combination of, and sutured in a single or double-layer technique. Double-layer closure is considered to have a positive influence on the outcome of palatal defect repair and is advocated whenever possible (82). Palatal defects create a communication between the oral and nasal cavities. Therefore, single layer flap reconstruction of palatal defects is characterized by most of the inner surface of the flap facing the nasal passages without any underlying connective tissue support. Moreover, wound edges and suture lines may end up being located over the defect. To avoid this situation, reconstruction with two flaps overlying on each other, their connective tissue in contact, creates a water-tight seal. Creating a double layer of viable connective tissue offers better resistance to adverse oral conditions and provides better healing conditions by increasing support to suture lines, favouring revascularisation when the connective tissue of both flaps is in contact compared to when a single-layer flap is closed over the defect (82). In oral oncologic surgery, a two-flap double-layered technique may not be practical because of insufficient remaining soft tissue to close the defect. However, when using a single oral flap including submucosa or a myomucosal flap, a double-layered closure can be achieved by suturing the flap in two layers, a deep submucosal layer, and a superficial mucosal layer. Large flaps must be harvested while preserving the local (palatine, infraorbital, facial) vascularisation as much as possible. Blood vessels at the rostral end of the flap, which need to be transected for tension-free closure, are ligated with 3-0 or 4-0 polyglactin 910. Flaps are closed in a simple-interrupted pattern using a 5-0, 4-0, or 3-0 absorbable monofilament suture materials such as poliglecaprone 25.

Advancement flaps can be used to repair a palatal defect by advancing the oral mucosa medially. The distance from the edge of the defect to the mucocutaneous junction is measured to evaluate whether sufficient amount of mucosa is available to cover the defect. Uni- or bilateral flaps may be used depending on the size and location of the defect. These full thickness mucosal advancement flaps are usually considered random flaps. Nevertheless, by preserving the main arterial supply (angularis oris, superior labial, lateral nasal, and their anastomoses) during careful deep submucosal dissection they may function similar to an axial pattern flap. Slightly divergent mucosal releasing incisions are made rostrally and caudally with a number 15 blade to design a slightly trapezoidal flap. In the caudal area, care is taken to preserve the angularis oris and superior labial vascularisation. In the rostral part, the lateral and/or dorsal nasal artery may be ligated and cut to enable medial displacement of the flap and tension-free closure. Flap elevation is performed with a periosteal elevator followed by blunt deep submucosal dissection with small curved scissors to the level of the mucocutaneous junction (83). The flap is manipulated with stay sutures and positioned in its final location to assess tension (**Figure 2**). When mucoperiosteum of the hard palate is still available after the resective surgery, a bi-pedicle mucoperiosteal flap may be advanced medially to decrease tension on the opposite oral mucosa advancement flap. Oral advancement flaps may be harvested from the entire lip and cheek and are, subsequently, adequate for closure of large defects (**Figure 3**). Their limitation is the width of oral mucosal tissue available, which may not be sufficient to fully cover a large central oral defect. Dogs with wide and loose lips harbour a greater width of oral mucosa, thus facilitating the surgical closure compared to dogs with a long nose and tight lips.

Several axial pattern transposition flaps have been described in the literature. In 1989, Bozola et al. first described an axial pattern buccinator myomucosal flap based on the buccal and facial arteries to treat oral defects in humans (84). In 1992, Pribaz et al. proposed a modification of Bozola's flap with the ***Facial Artery MyoMucosal (FAMM) Flap*** (85). The FAMM flap is considered a robust and versatile reconstructive option for small to medium size defects of the oral cavity or oropharynx in humans (53, 75, 86). Similarly, axial pattern myomucosal flaps harvested from the oral mucosa and based on collaterals of the facial artery, the angularis oris and/or superior labial arteries, respectively, have been described in dogs (72, 87). In their original description of the angularis oris axial pattern buccal flap in two dogs, Bryant et al. used a skin incision through the cheek to identify the vascular structure and design the buccal mucosal flap (87). Three other published cases reports, describing a total of five cases, used same surgical technique (88–90). A possible rostral advancement of the flap to the level of the canine tooth has been described when the flap is islandized (87). Nevertheless, islandization takes the risk of damage to the vascular supply of the flap at its base and cannot be systematically recommended (72). A wide based flap preserving the mucosal layer over the pedicle and avoiding the island design increases the chance of inclusion of venous drainage, which is fundamental for flap survival in the first weeks (76, 91–93). A longer myomucosal flap may be obtained by not only incorporating the angularis oris but also the superior labial artery. This superior labial myomucosal flap is designed as a peninsular flap with a caudal base in the retromolar area and can extend rostrally to the level of the canine tooth. It is harvested intraorally, after identification of the vascular supply through transillumination or the use of a handheld doppler, with no skin incision. The plane of dissection of the flap lies between the skin and the buccinator/orbicularis oris muscles. The flap also incorporates the superior labial vein. Based on a cadaveric vascular anatomic study, a good clinical outcome of this flap has been recently reported in one dog (72).

These two types of myomucosal flaps differ very little. They are peninsular flaps with a base located caudally, at the level of the mandibular ramus and the masseter muscle (retromolar area). Because they are both based on the facial artery and its collaterals, the board term of FAMM flap, similar to human terminology, could rather be used. The angularis oris artery is directed toward the commissure of the lip whether the superior labial artery continues beyond the commissure in a more dorsal direction along the superior lip and can subsequently be extended further rostrally (**Figure 4**). From the clinical point of view, incorporation of one or both vessels is based on the location and size of the defect to be covered. The commissure and the superior lip are retracted with stay sutures and the first step is to mark the flap on the oral mucosa with a surgical marking pen according to the predefined limits. Harvesting of the flap is made through incision with a n°15 scalpel blade of the mucosa, submucosa and buccinator/orbicularis oris muscles. The flap is elevated with dissection scissors in a layer underneath the superior labial and/or angularis oris artery, which is followed in a retrograde fashion toward the retromolar area. The artery is left attached to the overlying tissue over its entire length. To facilitate gentle dissection, stay sutures are placed on the distal edge of the flap. The flap can be transposed over the defect by rotating it up to a 90° angulation. Rotation up to 180° is theoretically possible,

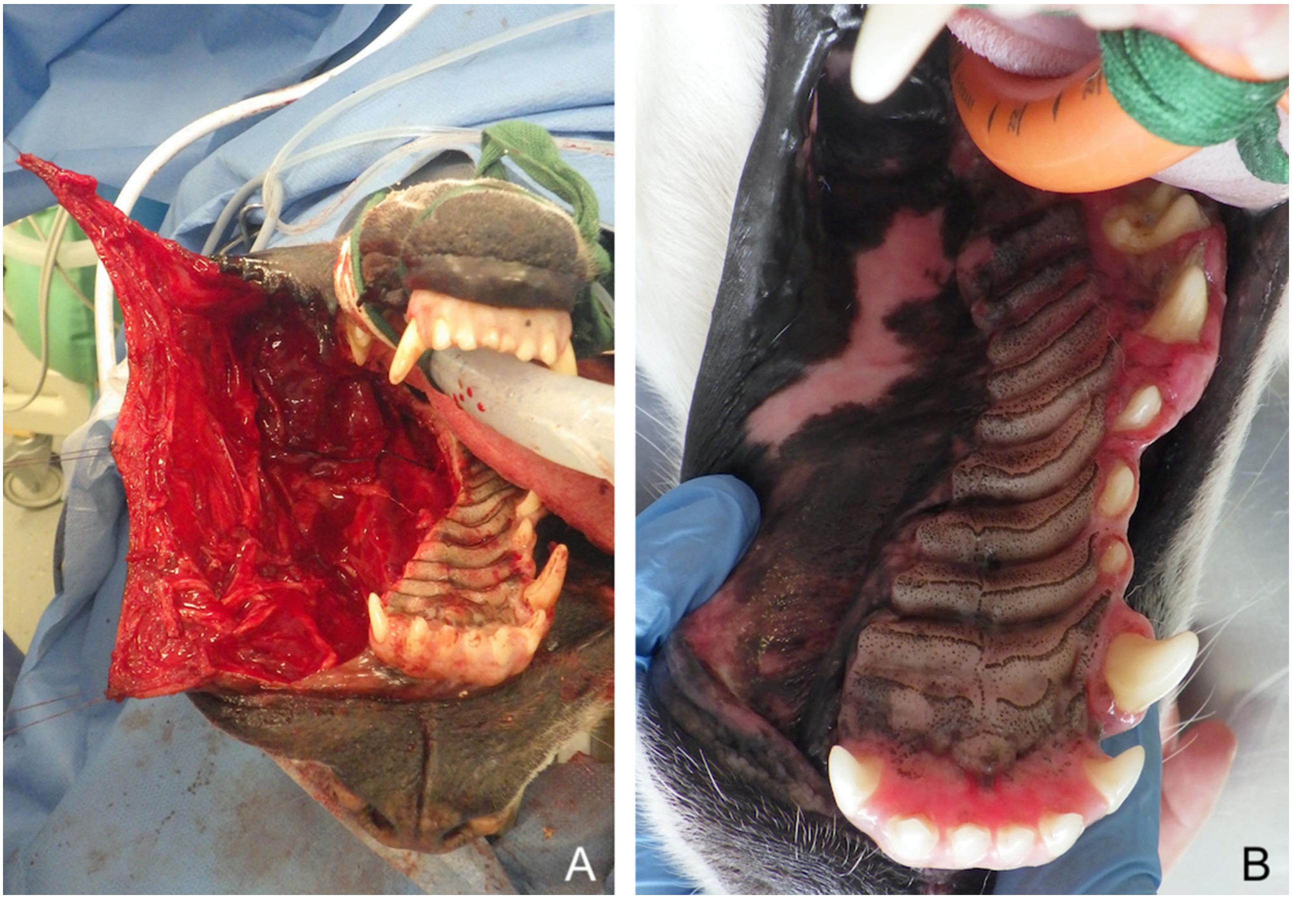

FIGURE 2 | Large myomucosal advancement flap for closure of a unilateral maxillectomy defect (fibrosarcoma) in a dog. **(A)** Harvesting of the flap and manipulation with stay sutures. **(B)** 12-months postoperative aspect.

in case of oropharyngeal defects, but the arc of rotation should be minimal as during transposition over the palate its base needs to be twisted along its long axis (72). The main limitation of these myomucosal flaps is the width of cheek/lip mucosa available. Dogs with a long nose and narrow cheek and lips are less likely to provide a fair amount of mucosa compared to dogs with hanging lips. The width of mucosa located between the attached gingiva and the muco-cutaneous junction is the maximal width of the flap that can be used. The flap is either transposed in a parasagittal or transverse direction (90° rotation). When transposed along the long axis of the defect, the width of the flap should be larger than the width of the defect and the length of the flap larger than the length of the defect. When transposed with a 90° rotation, the width of the flap should be larger than the length of the defect and the length of the flap larger than the width of the defect. The donor site is either left exposed and undergoes secondary healing or is closed for primary healing. Closing of the donor site may result in a tight lip if most of the lip mucosa has been harvested. Whenever possible, at least 1 cm of oral mucosa is left at the mucocutaneous junction to facilitate closure of the donor site (**Figure 5**).

A modification of the ***superficial cervical myo-cutaneous flap*** (SCMC) has been studied by Dundas et al. for the closure of caudal partial-thickness defects located in the palate in an experimental setting (94). This flap is based on the prescapular perforating branch of the superficial cervical artery (formerly omocervical trunk), arising from the subclavian artery between the omotransversarius and trapezius muscles (66, 94). A 6–8-cm-wide islandized flap centered on this arterial branch and its corresponding vein and incorporating the contralateral angiosome was outlined. The vascular supply of the flap was studied on cadavers and a two-stage procedure was performed on 3 experimental dogs. In stage 1, the flap was elevated and its survival was observed after ligation of the opposite perforating blood vessel and de-epithelialization of its distal end. In stage 2, 7 days later, the necrotic tissue at the distal end of the flap was trimmed and the flap was implanted in the oral cavity through parapharyngeal tunnelization. The three flaps showed good survival post implantation with a small area of partial dehiscence at the rostral corner of the flap in one dog. Nevertheless, it should be noticed that in this experimental setting only partial defects of the caudal oral cavity were studied and that a loss of 20–25% of the originally anticipated flap length was observed presumably secondary to the two-staged protocol (94). As to our knowledge the outcome of this procedure in a clinical setting has not been further documented, caution is advised prior

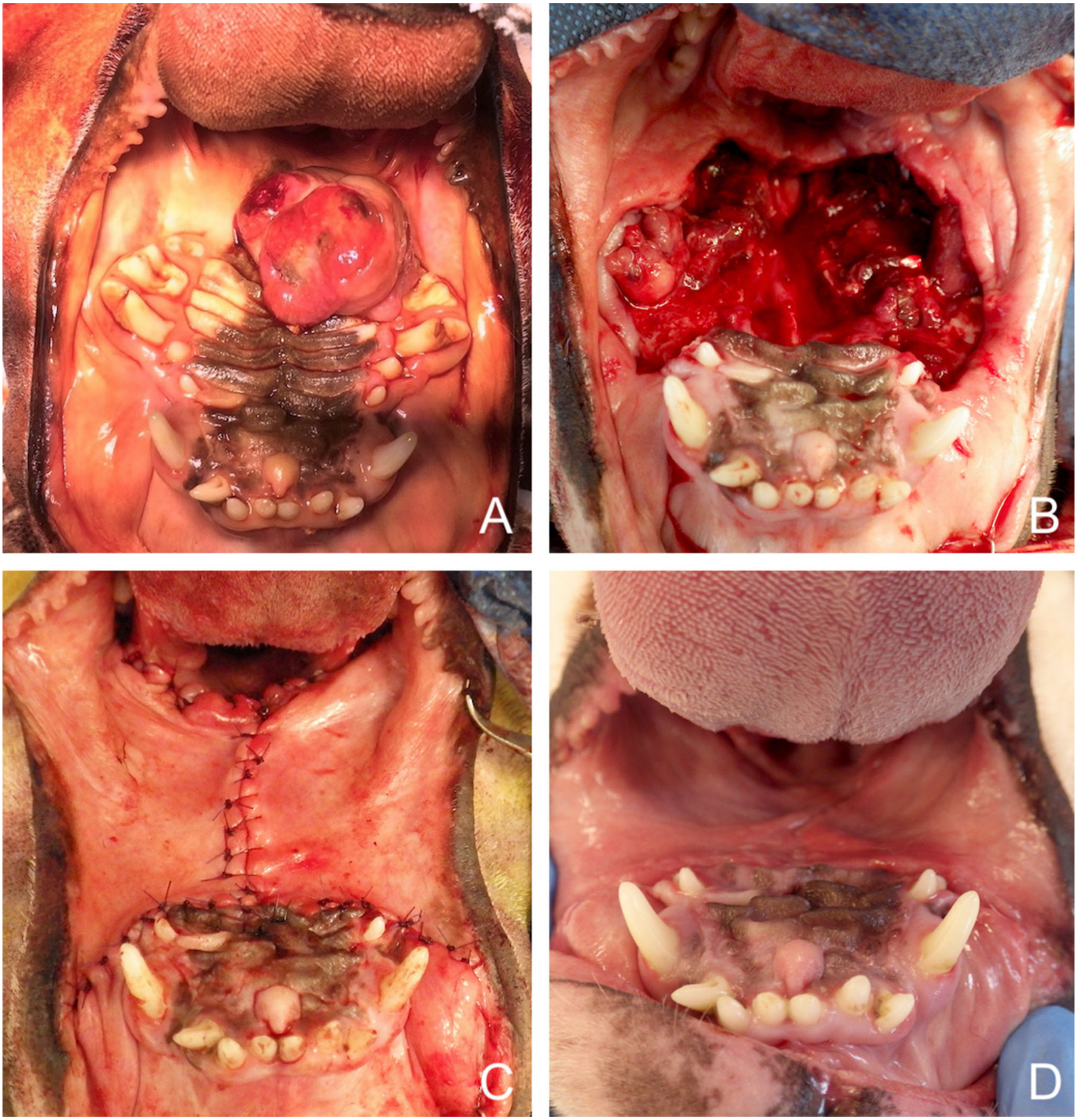

FIGURE 3 | Bilateral myomucosal advancement flaps for closure of a large central maxillary defect after removal of a multilobular tumour in a dog. **(A)** Preoperative aspect of the tumour. **(B)** Radical maxillectomy. **(C)** Immediate postoperative aspect. **(D)** 5-months postoperative aspect.

to extrapolating this protocol to clinical situations with large oronasal defects.

Facial Defects

Oral tumours with aggressive behaviour may require lip/cheek resection to achieve tumour-free margins. Various flap techniques may be used to close the full-thickness defect and restore function. Ideally, both the mucosal and the skin layer need to be reconstructed. This is mostly possible with the use of local flaps providing skin and oral mucosa from adjacent lip and cheek. When using loco-regional flaps to close large lip/cheek defects, oral mucosa may not be available in sufficient quantity. Options for reconstruction of the oral mucosal lining include using the remaining nearby oral mucosa resulting in a tighter

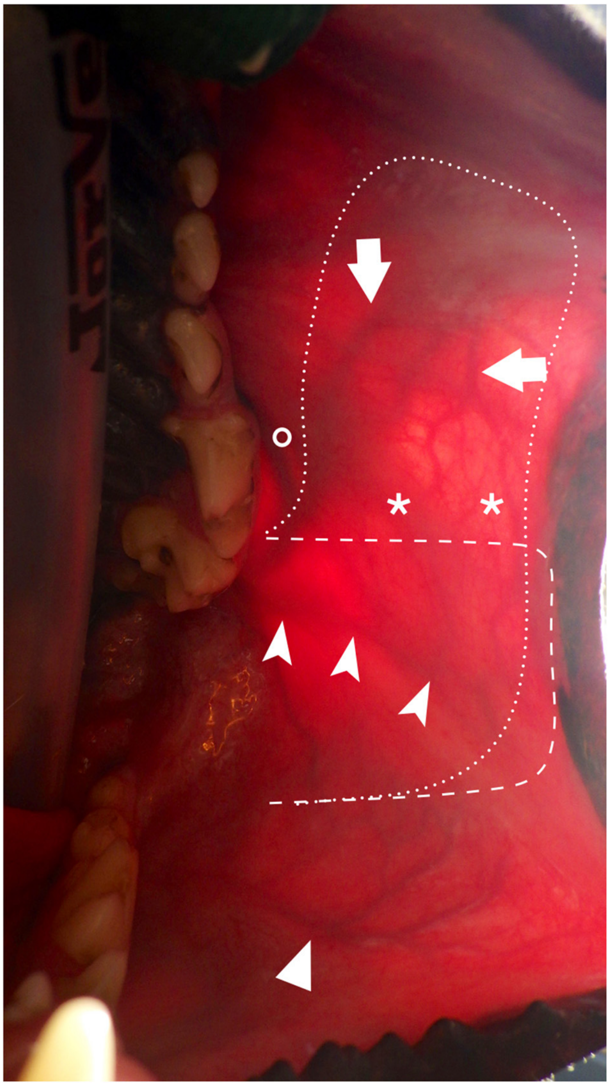

FIGURE 4 | Transillumination intraoral view of the main arteries of the cheek and lips and surgical outline. (arrow: SLA; chevron arrowhead: AOA; simple arrowhead: ILA; asterisks: anatomoses between SLA and AOA; interrupted dash line: limits depicting the facial artery myomucosal (FAMM) flap centered on the AOA; interrupted dot line: limits depicting the facial artery myomucosal (FAMM) flap incorporating the AOA and SLA; circle: parotid duct papilla. AOA, angularis oris artery; SLA, superior labial artery; ILA: inferior labial artery.

lip with a shorter vestibule, closing the defect with skin only without a mucosal layer, which may result in wound contraction and poorer cosmetic results, using a mucosal graft or using an inversed skin flap (41, 95).

Delicate technique and proper flap design are essentials to avoid tissue damage and ischemia. The flap must be handled with care favouring the use of stay sutures rather than toothed forceps or skin hooks to hold the margins (96, 97). A scalpel blade is preferred to monopolar electrosurgery for skin incisions as the latter has been shown in dogs to be associated with delayed healing and more complications within the first 7 days (98). Adequate undermining and sutures are other essential components of a successful tension-free closure. Sutures should not be too tight as with the development of post-operative edema they may strangulate tissue margin and induce ischemia (96).

Most *local flaps* are based over the subdermal plexus circulation unless a direct artery and vein (perforating vessels) are fortuitously included in the base of the flap (becoming an axial pattern flap) (95). Closing of the defect implies first realigning the mucocutaneous junction by placing a single interrupted absorbable suture on the mucosal side at the distal tip of the flap, closing the oral mucosa as a first layer with interrupted absorbable sutures and the skin as a second layer with interrupted non-absorbable sutures. In large dogs, a third intermediate suture layer can be placed on the superficial muscular layer. Removal of the skin sutures around the mouth, especially in cats or dogs with bad behaviour, may require sedation/anaesthesia. To avoid this trouble and for cost-effectiveness, the poliglecaprone-25 suture material remaining after closure of the mucosa can be used to close the skin layer using either an intradermal or a cutaneous suture pattern. No statistically significant cosmetic difference and no statistically significant difference in wound complications (infection, hematoma or dehiscence) have been shown in facial surgery in humans between skin closure with poliglecaprone-25 or polypropylene (99, 100).

Full-thickness labial/buccal advancement flaps can be used to close a defect of the superior or the inferior lip. A skin incision is made parallel to the long axis of the defect either dorsally (maxillary defect) or ventrally (mandibular defect) and extended caudally as far as necessary to make use of tissue elasticity to stretch the flap over the defect. By doing so, the commissure of the lips is moved rostrally (**Figure 6**). For defects located on the rostral part of the lip, the base of the flap may not extend beyond the commissure provided enough tissue is available for tension-free closure. Only one longitudinal full-thickness incision of the lip is required, either dorsally for the superior lip or ventrally for the inferior lip. Opposite to the skin incision, the oral mucosa is incised away from the mucogingival junction to facilitate mucosal closure. For defects involving the middle or caudal part of the lip, the base of the flap needs to extend to the cheek or further caudally in the masseter region. Two slightly divergent full-thickness incisions are performed. One is ventrally or dorsally located and the second is at the level of the commissure. These full-thickness flaps are based either on the superior or inferior labial vascularization. The infraorbital/lateral nasal vessels (superior lip) or the caudal mental branch of the inferior alveolar artery (inferior lip) may need to be ligated and cut for flap mobilisation. As previously described, numerous anastomoses occur between these branches and the superior or inferior labial arteries forming a dense arterial network (70). When making the full-thickness incision at the commissure of the lip toward the cheek, care is taken to preserve the angularis oris blood vessels. Further backwards, the parotid (Stensen) duct and the buccal ventral and dorsal branches of the facial nerve, running on the surface of the masseter, must be identified and preserved (**Figure 7**).

A ***full-thickness buccal rotation flap*** is a semi-circular flap that rotates into the adjacent recipient bed. Although the flap is

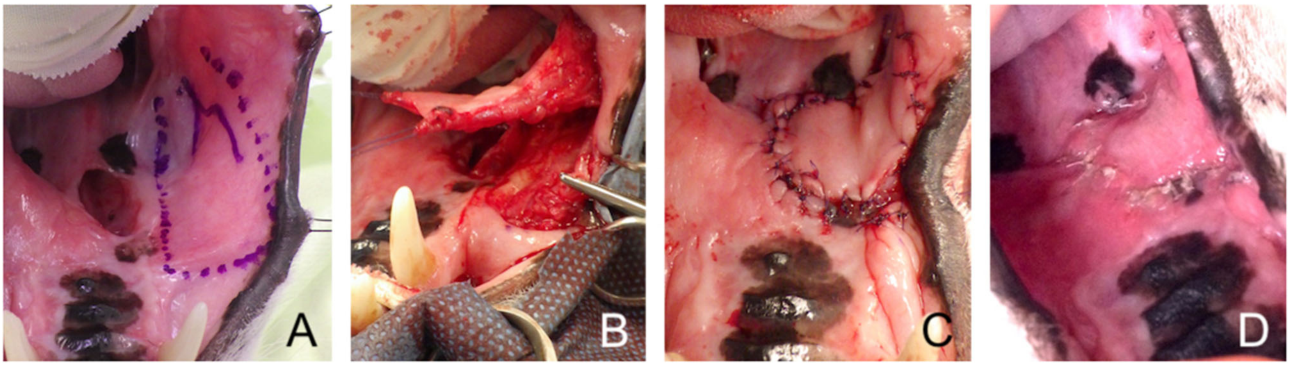

FIGURE 5 | Facial artery myomucosal (FAMM) flap. **(A)** Revision surgery of a large palatal defect; surgical limits of the FAMM flap depicted with a surgical marker. **(B)** Harvesting of the myomucosal flap. **(C)** Immediate postoperative appearance. **(D)** 3-weeks postoperative aspect.

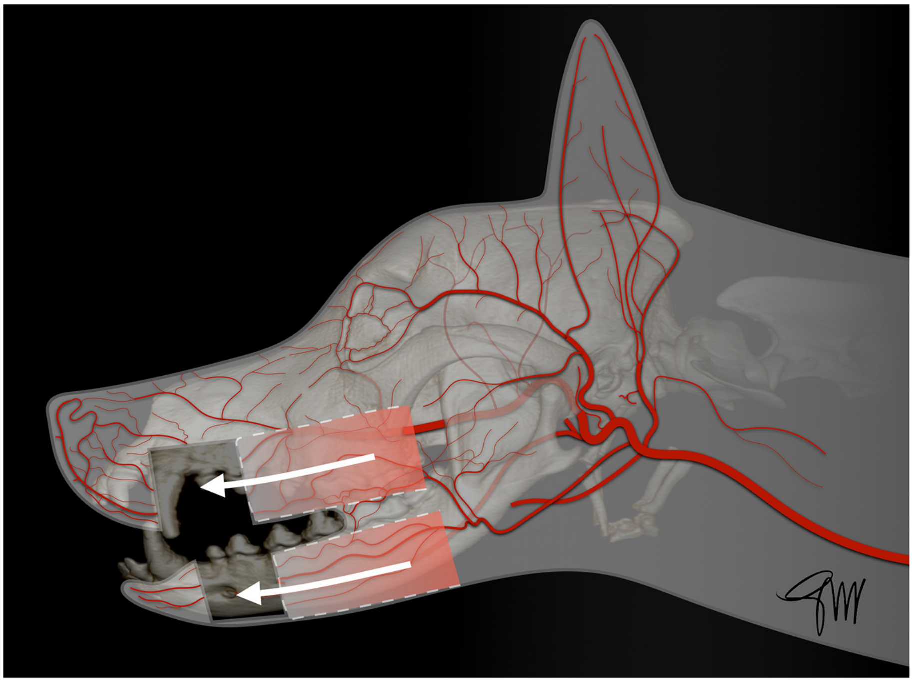

FIGURE 6 | Drawing depicting full-thickness labial/buccal advancement flaps for upper or lower lip defects.

rotational in its direction, it also spans the defect by stretching the elastic tissues (76). The ideal defect for a rotation flap is triangular in shape. The flap is rotated at a maximum of 90° from the axis of the defect. The height-width ratio of the triangle ideally should be 2:1. The length of the flap should be 4 times the width of the base of the triangular defect (76). Large lip defects with insufficient remaining tissue on the same jaw can be reconstructed with a rotation flap using the lip of the opposite jaw. This technique has been described both for the superior and inferior lips as well as for nasal reconstruction (41, 42, 95, 101). Through the rotation of tissue, the commissure is moved forward dorsally or ventrally resulting in a slight facial asymmetry with a unilateral shortening of the opening between the lips (*rima oris*). This is considered a minor cosmetic change (**Figure 8**).

The ***caudal auricular myocutaneous axial-pattern flap*** can be used to close defects in the caudal part of the face, namely the cheek, the orbital area, and the parietotemporal area (upper face) (102). The base (cranial border) of the flap is centered between the lateral aspect of the wing of the atlas and the vertical ear canal. It is based on the sternocleidomastoideus branches of the caudal auricular artery and vein, providing blood supply to the platysma muscle, with perforating branches into the subcutaneous tissue, and the overlying skin. The plane of dissection lies just over the cervical neck fascia (**Figure 9**). Anastomoses with the superficial cervical artery may extend the flap design up to the spine of the

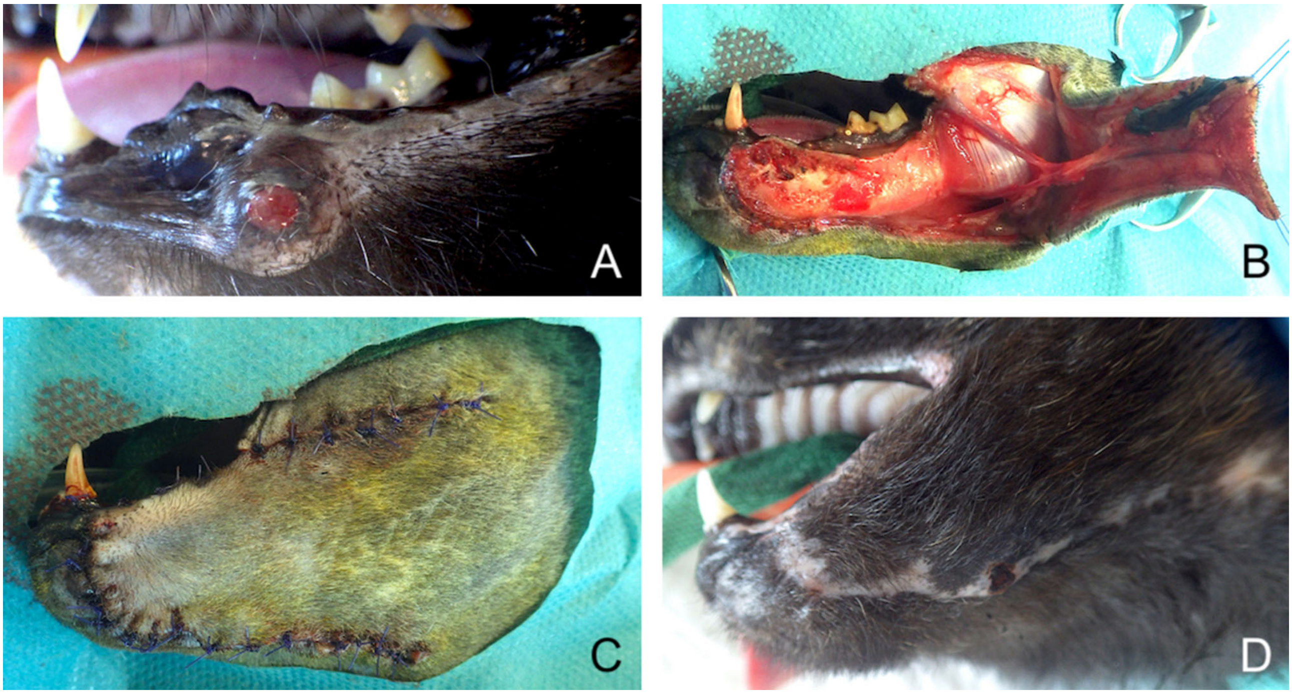

FIGURE 7 | Full-thickness local advancement flaps for closure of a defect secondary to surgical removal of a recurring lower lip trabecular adenocarcinoma. **(A)** Preoperative aspect of the tumour. **(B)** Harvesting of the flap based on facial vascularisation. **(C)** Immediate postoperative aspect. **(D)** 1-month postoperative aspect.

scapula (67, 103). Similar anatomy has been described in dogs and cats (68). In the original experimental study and description of the technique in dogs a mean flap length survival of 85.2% was reported (103). In a similar study in cats using 3 cm-wide flaps and various lengths of 6, 9, and 12 cm, distal flap necrosis was reported in 75% of the cases, with a mean survival length of 93.8, 81.9, and 84.4%, respectively. Following this experimental study, two client-owned cats experienced a good outcome after transposition on aural and periorbital defects, but using wider flap dimensions of 4 × 10, and 5 × 12 cm, respectively (67). Subsequently, the caudal auricular myocutaneous axial-pattern flap has also been successfully used to close defects following exenteration, or upper eyelid reconstruction in dogs, and cats (104–107). Recently, a multicentric retrospective study in 16 dogs and 12 cats has shown a relatively high complication rate with necrosis of the distal aspect of the flap in 62.5% of the dogs and 41.7% of the cats, requiring revision surgery in 72.7% of the dogs and 50% of the cats with complications. Flap necrosis involved 10–25% of the flap in dogs and 25–50% of the flap in cats. It should be noticed that flap dimensions in this retrospective study was superior to that of the experimental study, with a mean width of 6.5 cm in dogs and 5 cm in cats and a mean length of 19 cm in dogs and 16 cm in cats. Decreasing the length of the outlined flap, as well as avoiding as much as possible any factors that could impair blood supply (e.g., excessive tension, or torsion of the pedicle, thrombosis, infection) is recommended in order to enhance the flap reliability (102).

The ***superficial temporal myocutaneous axial-pattern flap***, first described by Fahie et al. (68) in dogs and cats, is based on the cutaneous branch of the superficial temporal artery, originating at the base of the zygomatic arch (68). The anatomical landmarks and viability of the flap have been experimentally studied in dogs (69). The base of the flap corresponded approximately to the length of the zygomatic arch, as the width of the flap is limited rostrally by the eye and caudally by the ear (**Figure 10**). At 7 days post-operatively, flaps extending to the midline of the head showed significantly better length survival (91.8%) than flaps extending to the contralateral zygomatic arch (69.5%) or flaps in the control group with vessel ligation. No palpebral deficit was reported though a cranial branch arising from the auricular plexus was transected to facilitate flap rotation. The donor site was closed by a simple cranial advancement (myo)cutaneous flap. Due to the risk of extensive necrosis of the distal tip, it was concluded that extending the flap beyond the contralateral mid-dorsal orbital rim, and rotation >90° was not recommended (69). Subsequently, the superficial temporal myocutaneous flap was successfully used in client-owned dogs and cats to close defects affecting the maxillary, orbital or nasal area (69). The rostral extent of the flap in the maxillary area is dependant of the specific head shape. Dogs and cats with a short nose and a large rounded head may benefit the most from this technique (**Figure 11**).

The ***facial/angularis oris myocutaneous transposition flap*** is a very versatile flap enabling the closure of facial soft tissue defects in dogs and cats (108–112). It can be used to close both maxillary and mandibular defects. The anatomical boundaries of this U-shaped skin flap are the lip commissure at the level of the medial canthus of the eye (base), the ventral aspect of the zygomatic arch (dorsally), the ventral margin of the caudal mandible (ventrally)

FIGURE 8 | Full-thickness local rotation flap for closure of a lower lip defect in a dog. **(A)** Oral malignant melanoma of the lower rostral lip. **(B)** Harvesting of the flap. **(C)** Immediate postoperative appearance. **(D)** 6-months postoperative appearance.

and the wing of the atlas (caudally) (111). The base of the flap is vascularized by direct cutaneous arteries arising from the superior, inferior labial arteries, and the angularis oris artery, which are branches of the facial artery; these branches penetrate into and through the platysma muscle (111). The distal end of the flap is also vascularized by two direct arteries entering in a rostral direction: the transverse facial artery and a cutaneous branch of the masseteric artery; they anastomose with a separate cutaneous branch of the angularis oris artery running caudally toward the ear canal area (111, 112) (**Figure 12**). Flap design must be carefully planned prior to the resective surgery by measuring the resulting defect size and its distance from the base of the flap to ensure full coverage of the defect. The boundaries of the resection and of the flap are delineated with a sterile surgical marker. A template created by cutting a piece of surgical drape may be used to help design the flap. Skin and superficial muscle are incised with a scalpel blade and dissection is performed deep under the platysma using blunt dissection scissors in caudal to rostral direction. Care is advised when approaching the angle of the mouth to preserve angularis oris vessels. A dorsoventral line drawn from the medial canthus and perpendicular to the axis of the mandibular body can be used as a safe rostral limit (111). Two small series of angularis oris myocutaneous transposition flap have been published in dogs (108, 112). Most of the cases were used to treat middle-face defects with a flap rotated dorsally. Losinsky et al. (112) reported the outcome of 9 flaps in 8 dogs. Results were good with no major complications observed. Partial incisional dehiscence of the distal end of the flap was noticed in 3 dogs with 2 of the dogs showing small area of necrosis (<1 cm); the defect healed by second intention in one of the dogs or after

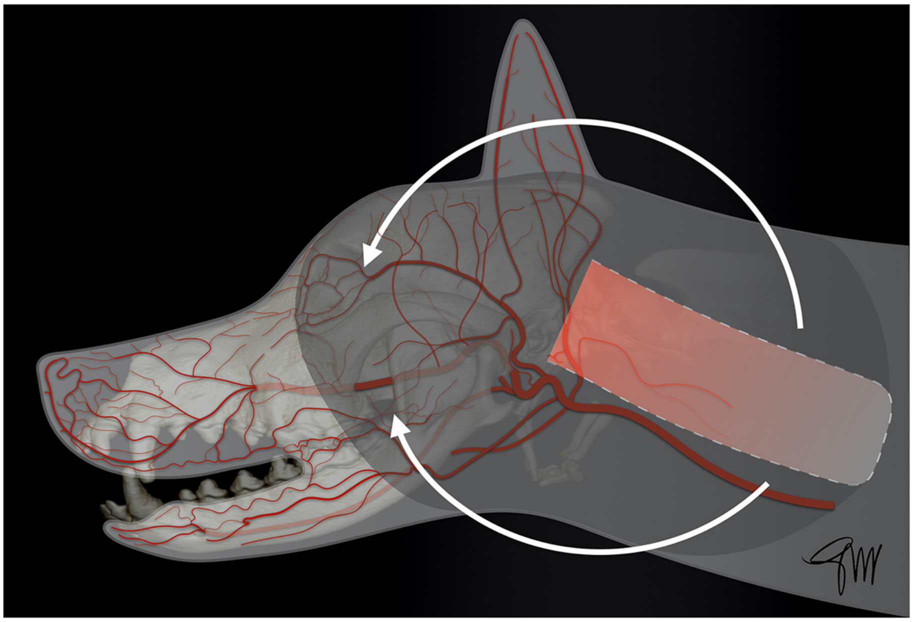

FIGURE 9 | Drawing depicting the surgical limits and extent of rotation of a caudal auricular myocutaneous axial-pattern flap.

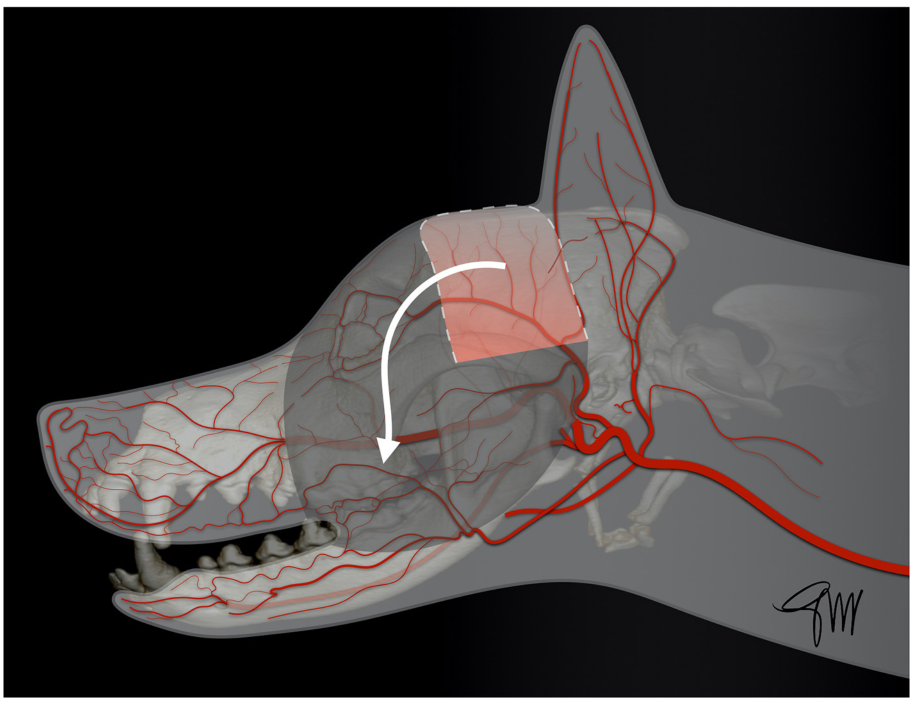

FIGURE 10 | Drawing depicting the surgical limits and extent of rotation of a superficial temporal myocutaneous axial-pattern flap.

minor revision surgery in two of them (112). Frasson et al. (108) reported the outcome of the flap in 6 dogs. Three of the 6 flaps showed minimal suture dehiscence but all flaps healed without necrosis of any portion of the flaps and did not require revision surgery (108). In both series, the caudal limit of the flap was either the vertical external ear canal or the wing of the atlas depending of the defect to be covered (**Figures 13**, **14**). Distal flap dehiscence and/or necrosis was not associated with a more caudal extent of the flap. Mild to moderate flap oedema was the most common minor complication (108, 112). A modification of

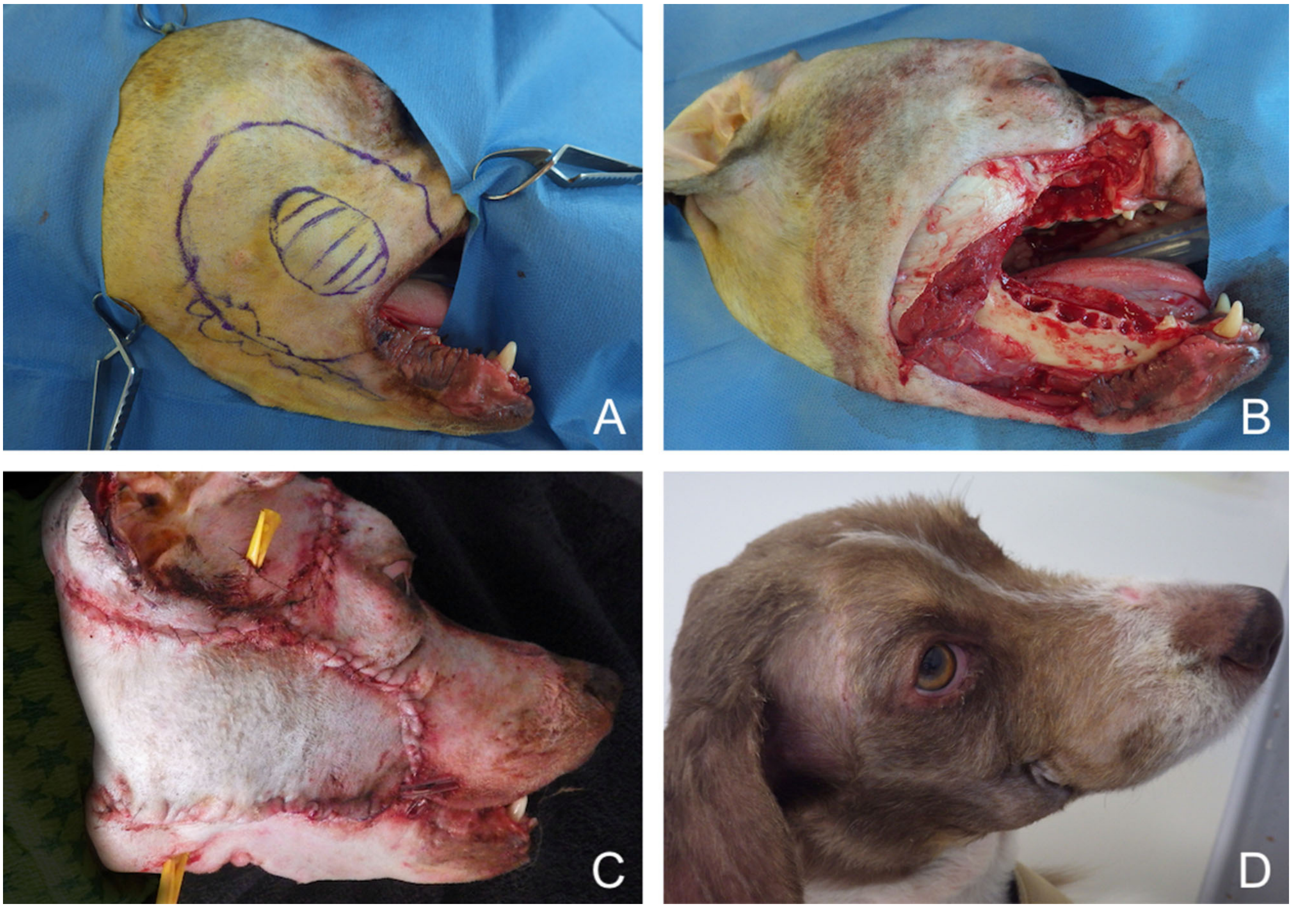

FIGURE 11 | Closure of a large facial composite defect with a superficial temporal myocutaneous axial-pattern flap in a dog with an invasive oral malignant melanoma. **(A)** Surgical planning. **(B)** Peri-operative aspect after removal of the cheek, caudal maxilla. **(C)** Immediate postoperative aspect. **(D)** 1.5-months postoperative aspect.

this flap has recently been documented in a small series of three dogs to reconstruct large palatal defect. A full-thickness bridging incision is made ventrally at the level of the last mandibular molar tooth to allow flap transposition into the oral cavity. Though hair regrew on the surface of the flap within the oral cavity, no major complication was reported in this short series (113).

The ***temporal myofascial flap*** was described in the dog by Tomlinson and Presnell (114) for closure of a 3-cm-diameter orbitonasal defect secondary to exenteration of the orbit and radiation therapy (114). Another paper has reported the use of this flap for closure of a 15 mm-diameter caudal oronasal fistula in a canine patient after caudal maxillectomy with exenteration and radiation therapy (115). Additionally, an experimental study published in 1996 assessed the feasibility and the outcome of this technique following total unilateral maxillectomy in thirty cats (116). The myofascial flap is based on the rostral and caudal deep temporal arteries. A curvilinear skin incision is made between the orbit and the ear. The temporal fascia covering the temporal muscle is exposed. The temporalis is harvested circumferentially from the temporal fossa by incising its attachment to the temporal line up to the sagittal crest dorsally, to the nuchal crest caudally, to the zygomatic arch laterally and to the caudal orbital margins rostrally (114–116). During elevation of the flap, the deep temporal artery and vein located ventro-medially, as well as the attachments of the muscle over the coronoid process of the mandible are carefully preserved. Transposition of the flap in the oral cavity is performed after tunnelization under the orbit. The flap is sutured over the defect with the fascia facing the oral cavity (116). Ostectomy of a portion of the zygomatic arch, or osteotomy of the coronoid process of the mandible have been suggested to facilitate mobilization of the flap (114, 116). Good clinical outcome has been reported in cats despite a longer healing time compared to humans. Coverage by a smooth oral mucosa was achieved in experimental cats 18–24 weeks after surgery (116). Indications of temporalis muscle flap in humans comprise the repair of defects involving the ipsilateral maxilla and palate, the lateral oropharyngeal wall and the retromolar area (117, 118). So far, there is limited documentation of the clinical use of this flap to repair large oronasal fistula in dogs and cats.

More distant axial-pattern flaps such as the ***superficial cervical myo-cutaneous flap*** have been suggested, though not yet fully investigated for facial reconstructions to the authors' knowledge (79). As transposition to the caudal oral

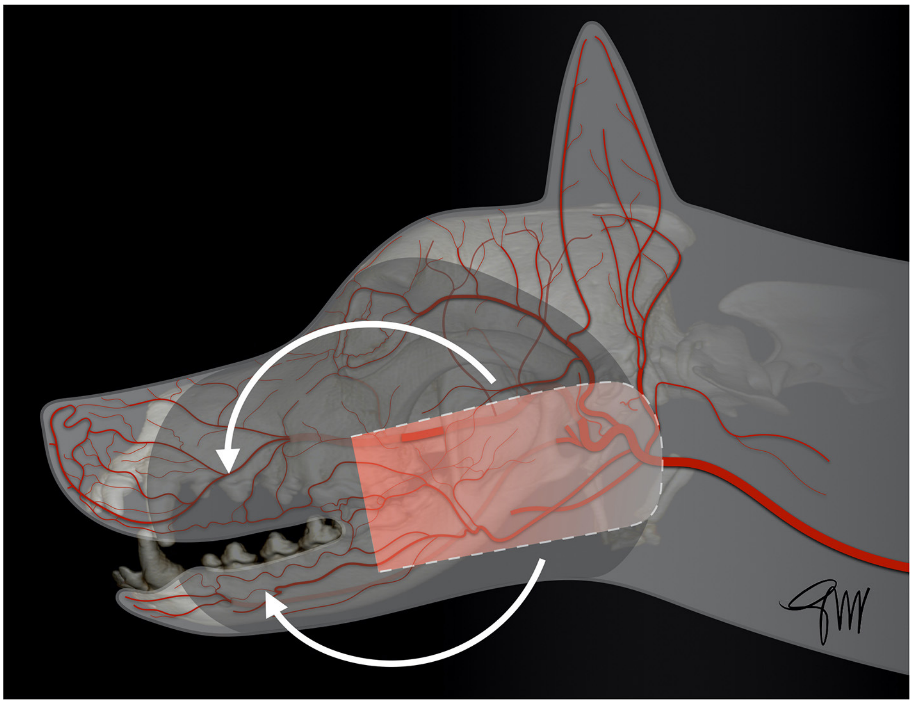

FIGURE 12 | Drawing depicting the surgical limits and extent of rotation of a facial (angularis oris) transposition flap.

cavity has already been described (see previous section), the reconstruction of defects involving the upper face and caudal aspect of the lower and midface (cheek) might be possible (94).

DECISIONAL ALGORITHM

To help the clinician planning the reconstructive phase following resective oromaxillofacial surgery, we proposed a decisional algorithm based on the current literature (**Figure 15**). When considering the reconstruction of oromaxillofacial defects, the easiest and most suitable technique by increasing order of difficulty should be considered first (53, 60). As the complexity of procedure may further impact the duration of the anaesthesia and the outcome, the clinician should also focus on its reliability as well as its time- and cost-effectiveness. The decisional process starts with a good appraisal of the resective procedure in order to plan the reconstructive modality accordingly (13–29). Small defects involving superficial soft tissue layers can sometimes be managed by second intention healing, providing inspection and special care of the wound are satisfactory, with no high risk of fibrosis or site infection. Direct closure, local random flaps or skin/mucosal grafts are generally preferred. Some deeper, moderately extensive defects may also be advantageously treated by direct closure or local random flaps whenever possible. For more severe (medium to large) defects, locoregional axial pattern flaps constitute the technique of choice. They may be combined with other techniques. They enable the restoration of an anatomic barrier between the oral cavity, the nasal cavity and/or the cutaneous layer in order to preserve function. The choice of the proper flap is dependent on the location of the defect. As a general rule, the closest available flap is preferred as a more distant harvesting site may require a longer flap to reach the defect to be covered and may be associated with a higher risk of distal flap necrosis. When a specific procedure may lead to an excessive risk of dehiscence (e.g. undersized, compromised vascularization, excessive tension), or when functional impairment may be associated, the next flap in the decisional algorithm should be considered. Flaps with low scientific support, and those necessitating highly trained teams and fully-equipped facilities (e.g., free vascularized flaps) are considered at last resort. Flaps based on the facial artery seem the more versatile and reliable and, due to their anatomical proximity, are currently used in most of the situations in first-intent closure of oromaxillofacial defects.

PERIOPERATIVE CARE

Perioperative care is considered of fundamental importance in head and neck surgery and has been shown to influence the outcome in humans (119, 120). As little is documented in veterinary medicine, the veterinary patient may benefit from human recommendations.

Antibiotics

Antibiotic prophylaxis is recommended for orofacial reconstructive surgery with exposure to the oral or nasal

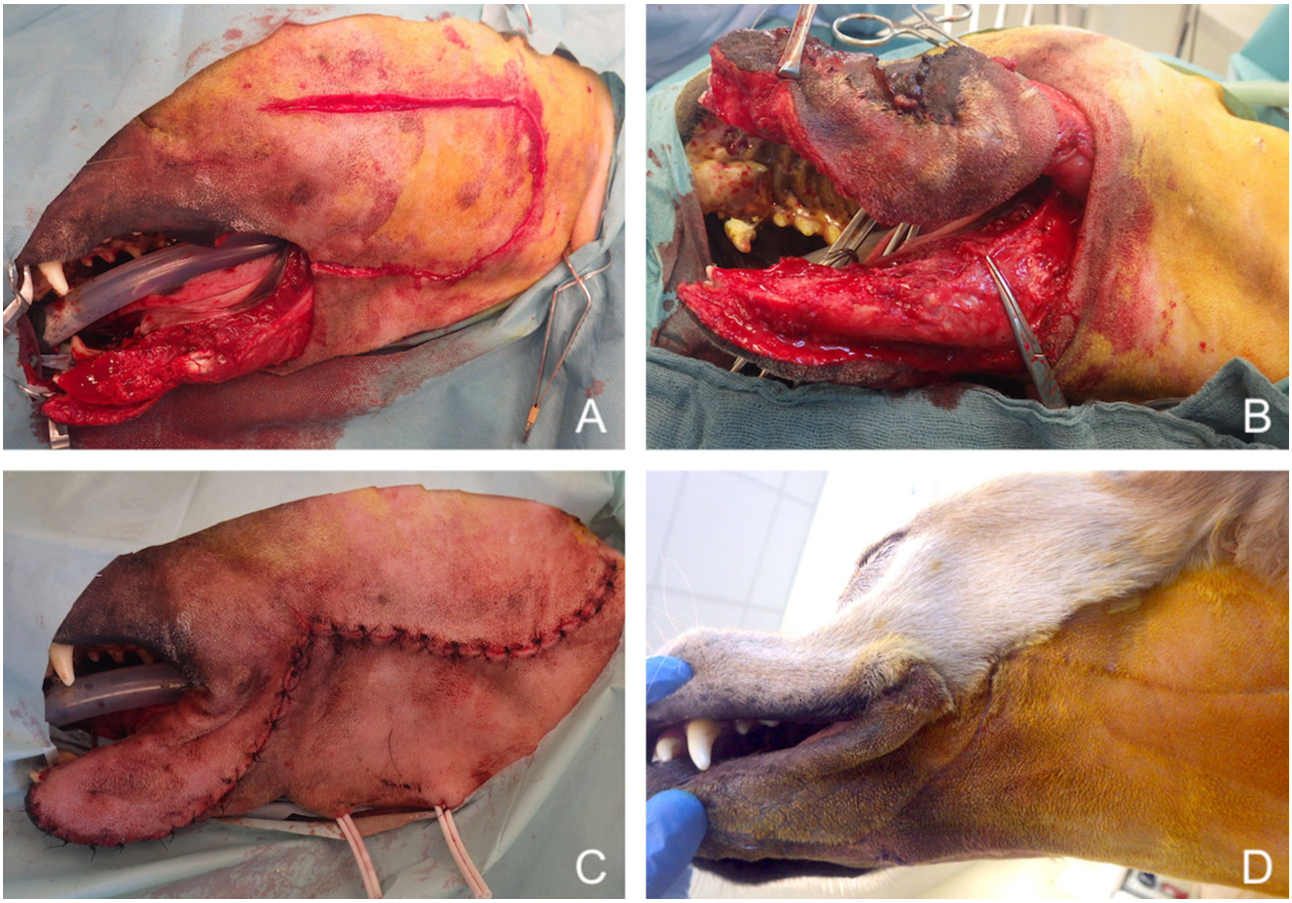

FIGURE 13 | Reconstruction of a composite defect of the lower face with a facial (angularis oris) myocutaneous flap. **(A)** Composite resection of the body of the mandible and lower lip in a dog presenting with a neurofibrosarcoma. **(B)** Surgical preparation of the flap. **(C)** Immediate postoperative appearance. **(D)** 9-months postoperative appearance.

environment (clean-contaminated surgery) because of the high risk of surgical site infection (119, 121, 122). Type and duration of antibiotic treatment has been debated in humans. Currently, ampicillin-sulbactam, amoxicillin-clavulanate and cefazolin are considered antibiotics of choice and are used postoperatively up to 24–48 h (75, 119–123). Some studies have shown that clindamycin should be avoided postoperatively in free flap surgery in humans as it was found associated with a poorer outcome due to its ineffectiveness against gram negative bacteria (121, 122). Reports on the use of antibiotics in head and neck surgery in dogs and cats are scarce. Human recommendations on the use of perioperative antibiotics are followed in some reports (6, 88, 112, 113). In a large retrospective study concerning axial pattern flaps in dogs and cats antibiotics were used postoperatively in 88% of the animals and surgical site infections was reported in 28% of the patients (81). Preoperative and postoperative antibiotic treatments were not found to have a significant impact on the outcome nor on the complication rate in this retrospective study (81). As no specific study has been conducted in veterinary medicine, our recommendation is to follow human protocols on antibiotic use when performing head and neck reconstructive surgery.

Drain Placement

Head and neck operations are associated with clinically significant postoperative morbidities such as haematoma, seroma, wound infection, and flap complications. Surgeons may choose to use *drainage systems* to reduce these complications (124, 125). However, the decision to place a drain is mostly based on individual cases and surgeons' experience and preference (126). A recent national survey in Ireland has shown that there were poorly defined guidelines and considerable heterogeneity amongst surgeons in term of indication for insertion (127). No significant difference in complication rate was found between active compared to passive drainage after free flap reconstruction in head and neck surgery in humans (124). Great heterogeneity amongst studies is also found in the veterinary literature. In a recent retrospective study on subdermal skin flaps in dogs and cats, an active drainage was placed in 11% of the cases and a passive one in 26% of the cases according to the surgeon preference (78). In a retrospective study on axial pattern flap

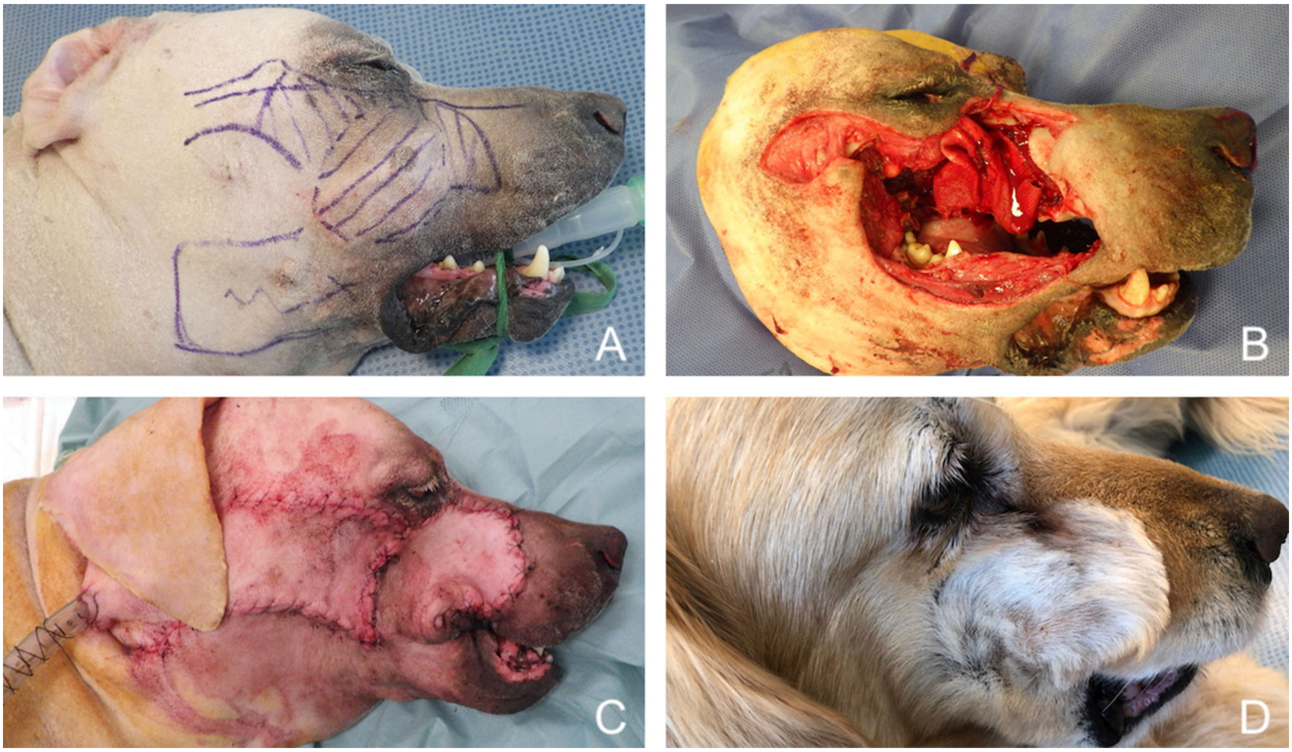

FIGURE 14 | Reconstruction of a composite defect of the mid face with a facial (angularis oris) myocutaneous flap. **(A)** Surgical planning of the surgery delineating the surgical limits (oblique dash line) and the outline of the flap. **(B)** Composite resection of the orbitozygomaticomaxillary area in a dog presenting with an osteosarcoma. **(C)** Immediate postoperative appearance. **(D)** 4-months postoperative appearance.

in dogs and cats, wound drains were used in 66% of the dogs and 38% of the cats and removed after a median of 2 days in dogs and 3 days in cats (81). The placement of a drain and the duration of the drain were not shown to have a significant impact on the outcome nor the complication rate (81). In another study on caudal auricular axial pattern flap in 16 dogs and 12 cats, an active drain was used only in 18% of the dogs and 8% of the cats (102). Therefore, until studies will have been performed to compare the outcome with or without drains as well as the influence of the type of drain, the decision will remain in the surgeon's hands based on the individual case.

Pain Management

Efficient pain management relies on a multimodal approach combining strong opioids, non-opioid analgesics, and peripheral or neuraxial local anaesthetics acting on different sites of the pain pathway (82, 89). In our institution, and in agreement with others, perioperative analgesia is achieved through a combination of locoregional anaesthesia using ropivacaine and constant rate infusion of drugs (opioids, lidocaine, ketamine, or dexmedetomidine) for the first 24–48 h (6, 62, 78). Other drugs that may be used include non-steroidal anti-inflammatory drugs and gabapentinoids. In a recent systematic review in humans, gabapentin, and pregabalin showed significant beneficial effect on perioperative pain relief and analgesic consumption in head and neck surgery procedures within the first 24 h (90, 91). The degree and duration of the postoperative pain management is based on the type of surgery and on the patient's pain score according to current recommendations (92). The less painful the animal, the faster food intake, and recovery are.

Nutrition

Patients suffering from oral tumour may have already lost weight at the time of surgery. Preoperative malnutrition in humans is a well-documented risk factor for perioperative complications and poor outcome (81). Decision to resume oral intake varies between surgeons and institutions but is commonly 5–7 days postoperatively in surgery involving the upper aerodigestive tract in humans (119). As placement of an esophageal feeding tube at time of surgery is a quick and straightforward procedure, we routinely use them in maxillofacial surgery. Large retrospective studies in dogs and cats have shown that most complications are minor (tube displacement, infection of stoma) and can be easily managed (128, 129). A feeding tube allows fast recovery and food and drug administration to the animal while preserving the oral cavity from any mechanical activity. Though there is no study to evaluate postoperative outcome with or without placement of a feeding tube, in agreement with other authors we favour this approach (6, 88, 89).

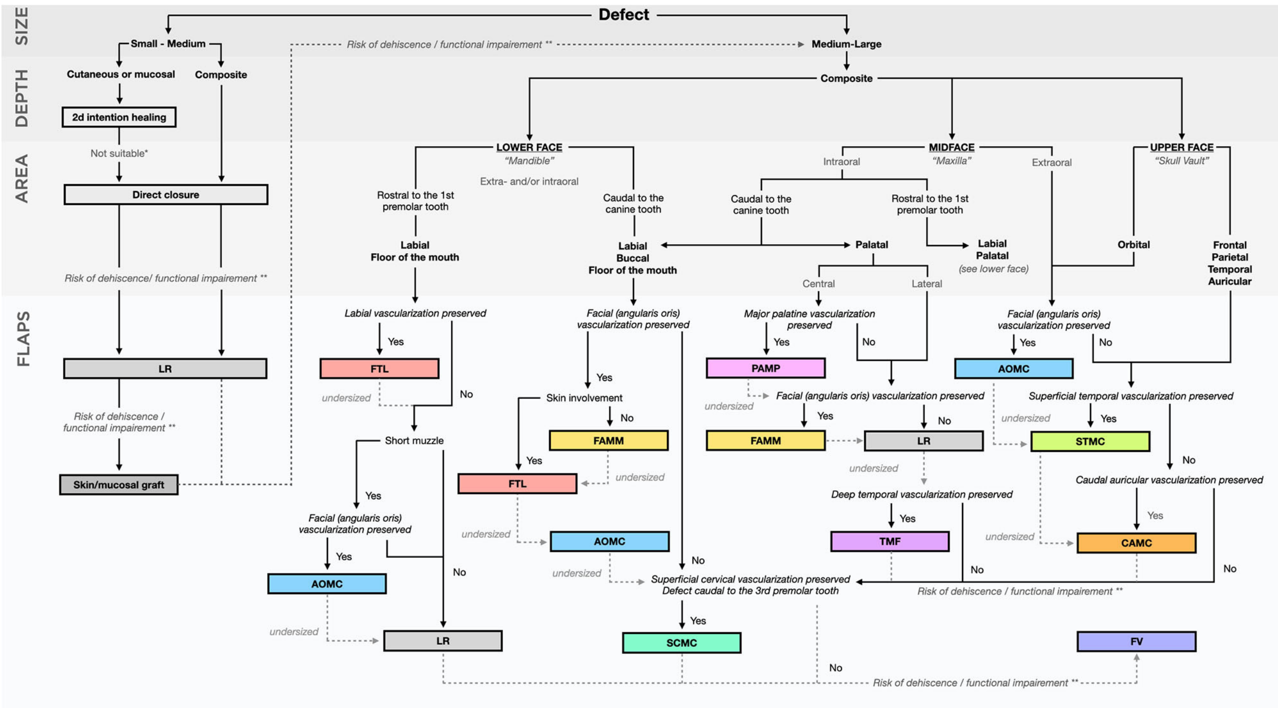

FIGURE 15 | Decisional algorithm. *e.g., risk of periorbicular fibrosis/retraction, infection, bone exposure. **e.g., risk of oronasal communication, occlusal trauma, inability to eat, wound dehiscence, upper airways stenosis/obstruction.

CONCLUSION

Oromaxillofacial surgery has become a specific branch of veterinary surgery, which must take into account specific oral anatomic and functional considerations. Management of large defects resulting from oncologic resective procedures requires excellent knowledge of the head and neck anatomy, and of the different reconstructive options available. Locoregional axial-pattern flaps constitute an important component of the therapeutic armamentarium in oromaxillofacial surgery as they allow for immediate reconstruction of the defects while preserving function and achieving good cosmetic results. These are key elements for the owner's acceptance of the resective surgery. Recently, emphasis has been placed on locoregional mucosal or skin axial pattern flaps based on branches of the facial artery (angularis oris, superior labial artery). These flaps seem versatile and reliable and, due to their anatomical proximity, are best used for reconstruction of orofacial defects. More retrospective studies are needed to fully evaluate the full potential of these techniques. Most of the orofacial reconstructive techniques used are aimed at closing the defect and focus on soft tissue reconstruction. The development of reliable and cost-effective flaps, allowing one-step composite reconstruction, appears to be the next step for the future of maxillofacial reconstructive surgery in small animals.

AUTHOR CONTRIBUTIONS

MG: literature searches, drafting and critical revision for intellectual content of the manuscript, idea and production of the schemes and diagram. DR: review of the article. PH: idea to write a review on locoregional flap reconstruction, proposition to submit to Frontiers in Veterinary Science, literature searches, drafting, critical revision for intellectual content, editing of the manuscript, and selection of the figures.

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