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A Feasibility Study for a Novel Trans-infraorbital Canal Approach to the Maxillary Nerve in Pigs (*Sus domesticus*)

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Experimental maxillofacial surgery is commonly performed in pigs; however, locoregional anesthesia of this area has not been described. This study evaluated the feasibility of a novel maxillary nerve block approach. In part I, cadavers were used to determine anatomic landmarks and assess maxillary nerve dye staining by using 0.03 mL kg^{-1} of a 1:10 mixture of commercial food dye and 0.5% bupivacaine. In part II, 10 additional pig cadavers underwent bilateral ultrasound-guided maxillary nerve blocks by using trans-infraorbital canal needle placement. The maxillary nerve was harvested and scored based on degree of staining (0 and 1, absent or incomplete staining; 2, staining; >1 cm circumferentially). Intracranial and intraconal spread of dye was evaluated. A Kruskal–Wallis test was used to compare infraorbital canal length estimated either externally via landmarks, internally via ultrasound, or actually measured after dissection. In 18 of 20 (90%) injections, successful staining (score = 2) of maxillary nerves was obtained for a nerve length of 2.4 ± 0.3 cm. Two of 20 cases (10%) had inadequate staining (score <2). At dissection of these 2 cases, the needle tip was observed to have collided with an unerupted tooth (third molar). No intracranial or intraconal spread of dye was observed. We detected no statistical differences between the estimated external, estimated internal, or actual dissection methods for measurement of infraorbital canal length (P = 0.3). Ultrasound-guided trans-infraorbital maxillary nerve block in pigs is a feasible technique, warranting further work to evaluate its in vivo efficacy and safety.

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Introduction

Pigs have distinct advantages that support their wide use for translational research—similar dento-alveolar structure to humans, spontaneous disease processes resembling those in humans, similar osseous regeneration properties, and more.[11] Despite their frequent use, providing adequate perioperative analgesia in pigs is limited by the practical challenges of long-term intravenous access and maintenance, unvalidated pain scoring techniques and the paucity of pharmacokinetic, and pharmacodynamic studies for analgesic drugs and dosages, which are often extrapolated from human or companion veterinary experience and may not have been validated or yielded clinically desirable results.

Locoregional techniques in which local anesthetics are used for local or regional infiltration to block nerve transmission could mitigate these problems by reducing the need for repeated administration of analgesics and would thereby also reduce the need to handle. Because pigs are being used with increasing frequency in dental and reconstructive surgery research,[2] a reliable technique for locoregional anesthesia of the maxillofacial region would be a valuable addition to the analgesic armamentarium for this species.

Innervation of the maxillofacial area appears to be similar in humans, dogs, and pigs.[1,4] In dogs, several methods have been described for anesthetizing the maxillary nerve, including lateral percutaneous, intraoral, posterior extraconal, and modified infraorbital approaches[7,8,16]; however, the current literature does not describe an approach to the maxillary nerve in pigs. The 2 aims of this study were to confirm the regional anatomy, including the course of the maxillary nerve and its surrounding landmarks as depicted in porcine anatomic references and to potentially identify an approach to the maxillary nerve using a combination of anatomic landmarks and ultrasound guidance by injection of a marker dye. The hypothesis of this study is that the maxillary nerve can be successfully approached and consistently stained under ultrasound guidance with the aid of anatomic landmarks.

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Materials and Methods

Animals. The pig cadavers used in this study were obtained from an unrelated terminal study and therefore IACUC approval was not required. All work was conducted in an AAALAC-accredited facility in compliance with the Animal Welfare Act and *Guide for the Care and Use of Laboratory Animals* and the unrelated terminal study was approved by IACUC of Cornell University (Ithaca, NY). Before euthanasia, the pigs had not undergone any procedures involving the head. The study was conducted in 2 parts.

Part 1: Confirming the regional anatomy and identifying a potential approach to the maxillary nerve. For the first part of the study, 10- to 12-wk-old female Yorkshire pig cadavers weighing approximately 50 kg were frozen immediately after euthanasia and thawed before dissection. Two of these 10 cadavers were positioned in lateral recumbency and dissected bilaterally to

identify the pathway of the maxillary and other nerves that provide sensory input to the maxilla. Dissections were compared with porcine-specific anatomic atlases.[1,4] A skin incision was made at the level of the buccal commissure and was extended caudally around the zygomatic arch and orbit. The skin flap was reflected caudally to expose the underlying tissues. The levator labii maxillaris and the caninus muscles were cut and reflected to expose the infraorbital foramen and infraorbital nerve. The malaris and masseter muscles were dissected from the zygomatic arch and reflected ventrally. The zygomatic arch and mandibular coronoid process were excised. The orbital fascia separating the extraconal from the intraconal compartment was identified and preserved, whereas the extraocular muscles were released and removed along with the periocular adipose tissue; the optic nerve was transected allowing the globe to be enucleated. Finally, the orbital fascia was removed along with the lateral pterygoid muscles to expose the maxillary and other nerves.

The maxillary nerve is the largest division of the trigeminal nerve and was identified in a location similar to that of humans and dogs (Figure 1). A common nerve trunk exited the cranial cavity through the foramen orbitorotundum where it divided into the ophthalmic and the maxillary nerves. The maxillary nerve coursed along the pterygopalatine fossa to enter the infraorbital canal. At the level of the pterygopalatine fossa, the maxillary nerve divided further into the zygomaticofacial and the accessory zygomaticofacial branches, innervating the lower eyelid and the medial canthus of the eye; furthermore, it contributed branches to the zygomaticotemporal nerve, a branch of the ophthalmic nerve that provides sensory innervation to the skin of the temporal region. Parallel to the medial angle of the orbit, and before entering the posterior opening of the infraorbital canal, the maxillary nerve gave off the caudal maxillary alveolar and pterygopalatine nerves that provide sensory innervation to the teeth, palate, and nasal cavity.[1,4] The anterior opening of the infraorbital canal (i.e., the infraorbital foramen) was located just dorsal to maxillary premolar 4/molar 1 in the transverse plane of the buccal commissure, whereas the posterior opening of the infraorbital canal was located just dorsal to maxillary molar 3 in the transverse plane of the medial canthus of the eye.[3]

The remaining 8 of the 10 cadavers were used to identify a potential method for approaching the maxillary nerve for perineural injection. Based on the dissections above, cadavers were placed in lateral recumbency with the side to be injected uppermost; a random number generator was used to decide whether the left or right maxillary nerve would be injected first. Identification of the infraorbital foramen was attempted intraorally by using digital palpation of the foramen over the vestibular mucosa and extraorally by using both digital palpation and ultrasound imaging techniques. An intraoral (caudal to the last molar tooth) and a lateral percutaneous approach to the maxillary nerve, similar to that described in dogs, were also attempted.[8]

The trans-infraorbital, ultrasound-guided approach to locate the infraorbital foramen and subsequent ultrasound needle identification within the pterygopalatine fossa was the technique chosen for the present study. Furthermore, a comparison of external anatomic landmarks with ultrasound-guided measurements and actual dissection-based measurements of

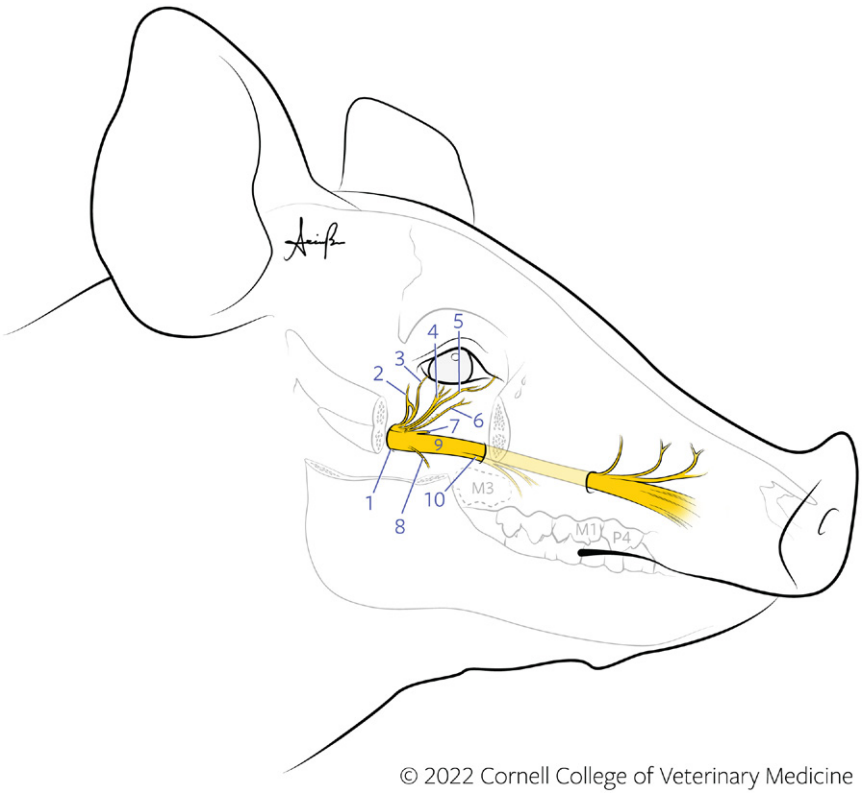

Figure 1. Original schematic of the pathway of the porcine maxillary nerve. 1. Foramen orbitorotundum (common point of exit of the maxillary and ophthalmic nerves from the cranial cavity). 2. Lacrimal nerve. 3. Zygomaticotemporal branch of the ophthalmic nerve. 4. Zygomaticofacial branch of the maxillary nerve. 5. Accessory zygomaticofacial branch of the maxillary nerve. 6. Ventral branch of oculomotor nerve. 7. Caudal maxillary alveolar nerve (cut). 8. Minor palatine nerve (cut). 9. Maxillary nerve. 10. Pterygopalatine nerve. The unerupted third molar is depicted as a possible impediment to needle advancement within the infraorbital canal. Medical illustration(s) created by Allie Buck, of the Educational Support Services unit of the Cornell University College of Veterinary Medicine; used with permission of Cornell University College of Veterinary Medicine.

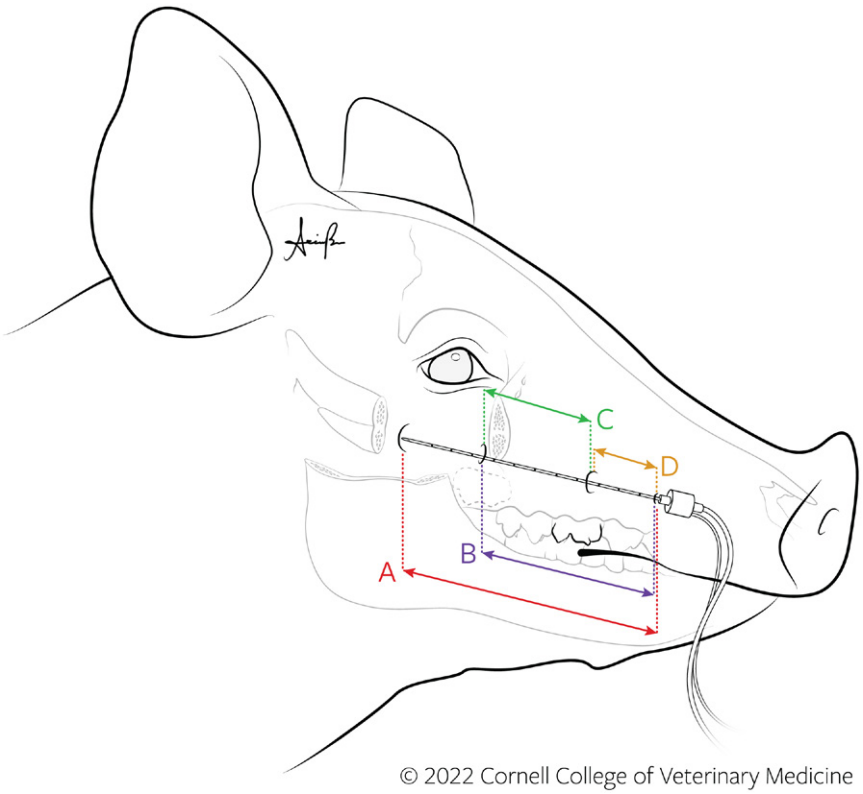

Figure 2. Original schematic depicting the measurements taken during experimental data collection. (A) Skin incision to foramen orbitorotundum. (B) Skin incision to posterior opening of the infraorbital canal. (C) Length of infraorbital canal. (D) Skin incision to anterior opening of the infraorbital canal (infraorbital foramen). B, C, and D can be used to determine the length of the infraorbital canal in 3 ways: landmark-based estimated length is taken as a measure of the distance between the commissure of the mouth and the medial canthus of the eye ($C_{External}$), ultrasound-based estimated length is equal to B minus D ($C_{Internal}$), and dissection-based measurement is directly measured as C_{Actual}. Medical illustration(s) created by Allie Buck, of the Educational Support Services unit of the Cornell University College of Veterinary Medicine; used with permission of Cornell University College of Veterinary Medicine.

the length of the infraorbital canal was performed. Based on current literature, the length of the infraorbital canal was estimated by measuring the length of an imaginary rostrocaudal line just dorsal to the maxillary arcade, beginning at the level of the buccal commissure rostrally and ending at the level of the medial canthus of the eye caudally[3,4] (Figure 2, distance C). Because length was measured using these external anatomic landmarks, it was designated distance $C_{External}$. All ultrasonography and injections were performed by a single anesthesiologist experienced in ultrasound-guided regional anesthesia. The area over the lateral aspect of the maxilla was imaged with a 13- to 6-MHz linear array probe (HSL25×; Fujifilm Sonosite, Bothell, WA) positioned in a rostrocaudal direction at the level of the buccal commissure dorsal to premolar 4/molar 1 to identify the infraorbital foramen (Figure 3A). A 21-gauge, 10-cm insulated echogenic needle with markings at 1 cm (NB2110; MILA International, Florence, KY) and integrated tubing prefilled with 1% methylene blue dye (Methylene Blue, 1%; Consolidated Chemical & Solvents, Quakertown, PA) was inserted through the skin just rostral to the infraorbital foramen and advanced from rostral to caudal (in-plane technique) into the infraorbital foramen (Figure 3A). The distance between the needle insertion point in the skin and the infraorbital foramen was also measured (Figure 2, distance D). The needle was then advanced dorsomedially into the infraorbital canal. The markings on the needle shaft allowed measurement of depth of insertion. Once the needle had traversed approximately 1/3 of the calculated length of the infraorbital canal, the ultrasound probe was changed to a 8- to 5-MHz microconvex array probe (C11×; Fujifilm Sonosite, Bothell, WA) that was positioned ventral to the medial canthus of the eye at the posterior opening of the infraorbital canal (Figure 3B). The needle insertion continued until the tip was visible via ultrasound at the posterior opening of the canal, just entering the pterygopalatine fossa (Figure 3B). The distance in centimeter between the skin and needle tip was measured (Figure 2, distance B). Distance B minus distance D was equal to the length of the infraorbital canal (distance C); because this distance was measured using ultrasonographic internal landmarks, it was designated distance $C_{Internal}$. After negative aspiration, 0.03 mL kg^{-1} (approximately 1.8 mL) of dye was injected. This volume was based on previous reports of volumes used for maxillary nerve blocks in people.[10,13]

The needle was left in place with the tip at the caudal opening of the infraorbital canal, and dissections were performed as described above for the first 2 pigs. The length of the infraorbital canal measured after dissection was designated distance C_{Actual}. This was considered to be our primary standard technique for measuring the length of the infraorbital canal. The maxillary nerve was identified and removed en bloc. The needle was then advanced further caudally until either resistance was met, indicating the tip had reached the foramen orbitorotundum, or until the needle could not be inserted further. The distance from skin to foramen orbitorotundum was measured in centimeter (Figure 2, distance A).

Part II: Evaluating the trans-infraorbital ultrasound-guided approach and identifying potential complications. For this part of the study, ten 8-wk-old female Yorkshire cross pig cadavers weighing approximately 30 kg were obtained immediately after euthanasia from an unrelated terminal study. Within 4 h after

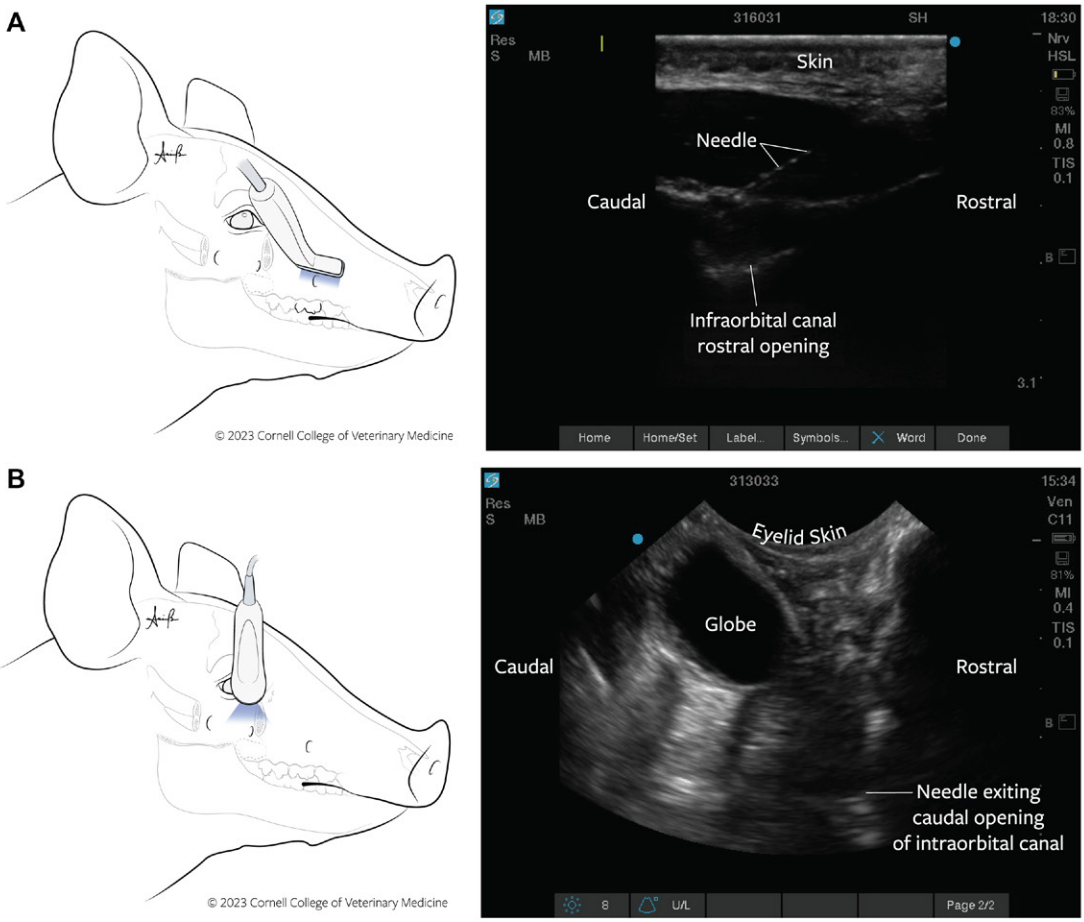

Figure 3. (A) Side-by-side image of a 6- to 13-MHz linear array ultrasound transducer probe positioned in a rostrolateral-caudomedial direction over the anterior opening of the infraorbital canal (infraorbital foramen, which lies dorsal to the buccal commissure at the level of the fourth premolar/first maxillary molar) and the ultrasound image obtained with this probe after insertion of a 21-gauge, 10-cm insulated echogenic needle. (B) Side-by-side image of a 5- to 8-MHz microconvex array ultrasound transducer positioned in a rostral to caudal direction over the medial aspect of the orbital rim with the beam directed toward the posterior opening of the infraorbital canal and the ultrasound image obtained with this probe after a 21-gauge, 10-cm insulated echogenic needle was advanced through the infraorbital canal and into the pterygopalatine fossa. Medical illustration(s) created by Allie Buck, of the Educational Support Services unit of the Cornell University College of Veterinary Medicine; used with permission of Cornell University College of Veterinary Medicine.

euthanasia, 0.03 mL kg^{-1} (approximately 1 mL) of food dye (Blue Food Color; McCormick Culinary, Hunt Valley, MD) was injected as determined in part I. Within 15 min of dye injection, dissections were carried out as described in part I. A single investigator performed all injections and dissections. A second investigator graded nerve staining and stain distribution using a 0- to 2-point scale, where 0 indicated no visible stain uptake, 1 indicated partial, noncircumferential staining or staining <1 cm in length, and 2 indicated complete circumferential staining of the nerve for ≥1 cm length. Injections were considered successful if a score of 2 was obtained. The observer also recorded any staining of adjacent structures. The apex of the orbital cavity was removed at the level of the foramen orbitorotundum to determine intracranial spread of dye.

Statistical analysis. ***Part I.*** Data analysis was performed using GraphPad Prism Version 8.0a (GraphPad Software, La Jolla, CA). Data were assessed for normality using the Shapiro–Wilk test. To determine whether the length of the infraorbital canal estimated using external landmarks ($C_{External}$) compared with ultrasonography ($C_{Internal}$) was different from the actual length measured after dissection (C_{Actual}), scores were compared using the Kruskal–Wallis rank-sum test.

Part II. Staining scores (0, 1, or 2) were expressed as a percentage, and the average lengths of nerve staining as mean ± SD were reported. Differences between left- and right-sided measurements were analyzed by using the Mann–Whitney *U* test. Significance for both parts of the study was set at $P < 0.05$.

Results

Part I. The infraorbital foramen of the pigs was not digitally palpable externally because of thick, robust skin and subcutaneous tissues. Moreover, pigs have no significant buccal vestibule, which makes intraoral palpation of the infraorbital canal opening impossible. Because of the narrow and deep oral cavity that restricted access and visibility, an intraoral (caudal to last molar) approach to the maxillary nerve was not attempted. Similarly, the lateral percutaneous approach was not feasible due to the zygomatic arch and the mandibular ramus completely overlying the pterygopalatine fossa.

In part I, staining of the maxillary nerves with 1% methylene blue was inconsistent in the first 3 pig cadavers despite saturation of other surrounding structures with dye. Therefore, we changed from 1% methylene blue to a commercial blue food dye for the remaining 5 cadavers. After this change, the same technique yielded adequate staining of both nerve tissue and adjacent structures. Given this finding, commercial food dye was used for part II.

Part II. The maxillary nerve was successfully stained (score = 2) in 18 of 20 (90%) injections with a mean length of staining of 2.4 ± 0.3 cm. In 2 of 20 (10%) injections, the staining of the maxillary nerve was considered inadequate: one right-sided maxillary nerve had a score of 1, with a noncircumferential staining of 2.5 cm in length, and another right-sided maxillary nerve had a score of 0. In these 2 occasions, resistance to needle advancement was encountered at the level of the pterygopalatine fossa, and ultrasound visualization of the needle tip was subjectively poor. After dissection, needle tips were found to have collided against unerupted third molar teeth, and a large amount of dye was seen in the anterior opening of the infraorbital foramen.

The dye reached the opening of the orbitorotundum foramen in 16 of 20 (80%) injections. Three of the other injections at this level (3 of 20; 15%) had a staining score of 1, and one (5%) had a score of 0. Two of these corresponded to unsuccessfully stained maxillary nerves, whereas the other 2 corresponded to successfully stained maxillary nerves. Intraconal, intracranial, or optic nerve staining was not seen in any subject.

When external anatomic landmarks were used to guide needle placement, the median $C_{External}$ (distance between the commissure of the mouth and the medial canthus of the eye) was 3.5 (2.5–4) cm. When ultrasound imaging was used to guide needle placement, the median $C_{Internal}$ (distance between the anterior opening of the infraorbital canal and ultrasound needle visualization within the orbit) was 3.5 (1.5–4) cm. When confirming needle placement with actual internal anatomic measurements after dissection, the median C_{Actual} (distance between the anterior opening of the infraorbital canal and direct needle tip visualization in the posterior opening of the infraorbital canal after dissections) was 3.5 (2.5–4) cm. The values obtained for these 3 placement methods were not statistically different ($P = 0.3$). No differences were detected between measurements or success rate when comparing left and right sides ($P = 0.3$ and $P = 0.5$, respectively).

From the skin, and through the infraorbital canal, the needle could be advanced into the orbital cavity for 8.5 (6.5–9) cm in 17 of 20 (85%) injections until resistance to further needle advancement was met; however, in 3 cadavers (15%) the needle never met resistance and was found intracranially after dissections intracranially.

Discussion

In pigs, the maxillary nerve can be successfully targeted through the infraorbital foramen using ultrasound guidance, despite its size and in contrast to other species. In dogs, a percutaneous approach to the infraorbital canal can be performed without imaging,[8] but this was not possible in pigs. With ultrasound guidance, identification of the infraorbital foramen and insertion of a blunt, graduated needle into the canal were easily performed. The centimeter graduations aided in measuring depth of needle insertion into the canal. In addition, the blunt tip needle provided greater safety (as it is less likely to tear or penetrate the neurovascular bundle) and avoided entrapment in the periosteum of the canal and subsequent resistance to advancement.

Ultrasound guidance with a microconvex probe technique was used to identify the needle placement in the pterygopalatine fossa; injectate deposition in this location can be more precisely confirmed in this manner. However, reliance on ultrasound guidance presents a 2-fold challenge. First, some programs may have only a linear probe that is used to perform a variety of locoregional techniques and may lack the equipment needed to directly visualize needle placement within the pterygopalatine fossa. Second, even with ultrasound guidance, anatomic landmarks should be identified for verification of an adequate length of needle advancement to avoid operator error, as the consequence of such an error could result in intracranial placement of the needle with aggressive advancement. The distance from commissure of the mouth to the perpendicular from the medial canthus was consistently correlated to the distance from needle insertion into the infraorbital canal until emergence into the pterygopalatine fossa. Using both modalities of verification is the best way to avoid adverse events.

Depending on the age of the pig, the unerupted third molar tooth was also a potential impediment to full needle advancement through the infraorbital canal; this accounted for the 10% failure rate. The incidence of this complication might vary based on age, breed, and sex of the pigs. The problem can be avoided in general by readjusting the angle of insertion of the needle. If both visual confirmation of the needle on ultrasound imaging and anatomic measurement references were used as guidelines, the injection attempts consistently dyed the maxillary nerve. Despite the dye systematically reaching the orbitorotundum foramen, none of the procedures resulted in intracranial spread of the dye. Because both maxillary and ophthalmic nerves exit the orbitorotundum foramen as a common trunk, the presence of dye in this location could also indicate that the ophthalmic branch of the trigeminal nerve would be stained, although this was not objectively evaluated in our study.

Methylene blue 1% has been frequently used in cadaveric veterinary studies of regional anesthesia to determine the volume of dye that will adequately stain the target nerve(s).[5,6] We had initially used this dye in the present study, but because of inconsistent nerve and tissue staining during the pilot study, food dye was substituted. Food dyes have been used successfully in human and veterinary literature for nervous tissue and bacterial staining.[14,15,17]

Some limitations to this study include the specifics of the cadaver population chosen. Cadaveric tissue quality does influence the diffusion of injectate, and this could be integral to the future work for dye characterization and an objective scoring method development.[12] The pigs in part II of the study were juveniles of similar breed, age, and weight. However, pigs of different breeds may have significant conformational differences, and the anatomic references from this study may not be appropriate. This approach may also be difficult to use in boars because of the presence of tusks. The age group and breed of pig that we used here provides a baseline for future research on locoregional analgesia in settings that commonly perform maxillofacial surgeries performed.[9,11] The use of cadavers rather than live animals is another limitation to this study; the spread of dye and extent of nerve staining in cadavers may not mimic the dissemination of local anesthetics in the live animal.[12]

This study investigated the regional anatomy of the maxillary nerve in pigs and used injection of dye and subsequent dissection to validate a successful way to approach to the nerve. The maxillary nerve was successfully stained in 90% of injections. Further research is necessary to evaluate the clinical efficacy of this block in vivo and the potential side effects in pigs undergoing maxillofacial surgery.

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Conflict of Interest

The authors have no conflicts of interest to declare.

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