RESEARCH PAPER

Blind *versus* ultrasound-guided maxillary nerve block in donkeys

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Abstract

Objectives To describe the 'blind' and ultrasound-guided approaches to block the maxillary nerve in donkeys. To compare the success and complication rates between the 'blind' and ultrasound-guided techniques based on staining of nerves and other structures in cadavers and assessing level of analgesia in live animals.

Study design Prospective anatomical and experimental study.

Animals Eighteen cadaver heads and nine adult live donkeys.

Methods Phase 1: the anatomical characteristics of the maxillary nerve and its related structures were investigated within the pterygopalatine fossa in five cadavers. Phase 2: 0.1 mL of methylene blue dye was injected blindly and via ultrasound guidance in 13 cadavers to stain the left and right maxillary nerves, respectively. Nerve staining and dye spreading were evaluated through cadaver dissection. Phase 3: the former procedures were applied in nine live donkeys using lidocaine hydrochloride 2% and the onset of analgesia was verified through needle pricking at the naris.

Results Ultrasound-guided deposition of methylene blue dye in cadavers and lidocaine injection in live animals were successful in all instances (accuracy = 100%) without inadvertent vascular penetration. Using the 'blind' technique, misdirection and intravascular deposition of dye were reported in four cadavers (accuracy = 69.2%) and neurovascular trauma was observed in live donkeys (five cases). Loss of cutaneous sensation in the ipsilateral naris was earlier in the ultrasound-guided approach (10.9 ± 1.8 minutes) than in the 'blind' technique (27.8 ± 3.2 minutes; $p < 0.001$).

Conclusions and clinical relevance An ultrasound-guided maxillary nerve blockade proved very practical and can be used to block the maxillary nerve with a high degree of accuracy while avoiding vascular penetration. Further studies are mandatory to validate its analgesic effectiveness in clinical situations.

Keywords donkey, maxillary, nerve block, ultrasound guided.

Introduction

Donkeys play a crucial role in agriculture and farm work, particularly in developing countries where they exist in large populations (Lizarraga et al. 2004). More than 40 million donkeys are distributed all over the world (Joubert et al. 1999); however, scant information specific to this species exists in the literature (Lizarraga & Beths 2012). Until recently, donkeys were considered as small horses, although anatomical and physiological differences have been reported for each (Taylor et al. 2001).

In horses, the maxillary nerve (a major branch of the trigeminal nerve) provides sensory innervation to the ipsilateral dental structures of the maxilla and premaxilla, the paranasal sinuses and naris. Several vital structures are intimately related to the maxillary nerve where it crosses the pterygopalatine fossa including the maxillary artery and vein, the

infraorbital artery, the buccal artery, the descending palatine artery, the deep facial vein and the ventral border of the orbit (König & Liebich 2014). 'Blind' blockade of the maxillary nerve can be achieved in the pterygopalatine fossa before it enters the maxillary foramen (Bardell et al. 2010); however, this might be a challenge because of the poorly defined landmarks and complex anatomy of the area (Staszyk et al. 2008). The technique was associated with repeated needle placement and excessive volumes of local anesthetics, and resulted in undesired side effects, with neurovascular trauma frequently encountered (Staszyk et al. 2008; Bardell et al. 2010).

Ultrasound-guided regional anesthesia has gained popularity in veterinary practice (O'Neill et al. 2014). The increased adoption of ultrasound-guided regional anesthesia techniques in veterinary medicine may be observed in numerous articles retrieved by a quick literature search using PubMed as a data source. Ultrasonographic-guided nerve blockade allows for a real-time imaging of the target nerves eliminating the need for repeated needle insertion, minimizing tissue damage (Sites & Brull 2006), reducing the risk of inadvertent vascular injury (Gray 2006) and shortening the block performance time (Williams et al. 2003). Furthermore, a real-time visualization of the spread of local anesthetic solution over the target nerve is quite possible, thus allowing a closer needle insertion to the target nerve and subsequently reducing the required amount of local anesthetic compared with the conventional blind techniques (Casati et al. 2007; Oberndorfer et al. 2007).

Both the 'blind' and ultrasonographic-guided maxillary nerve block techniques have been reported in horses (Staszyk et al. 2008; Bardell et al. 2010; O'Neill et al. 2014); however, no reports have been found in donkeys. Therefore, the objectives of this study were: to describe a 'blind' technique to approach the maxillary nerve; to describe an ultrasonographic approach to localize and block the maxillary nerve; to describe the ultrasonographic appearance of this nerve; and finally, to assess the reliability of the ultrasound-guided and 'blind' techniques for blocking of the maxillary nerve in donkeys. It was hypothesized that ultrasound guidance would be useful for optimizing needle position, and the real-time visualization of the maxillary nerve and its related structures would improve the success rates and safety of this technique in comparison with the 'blind' technique.

Materials and methods

This study was approved by the Institutional Animal Care and Use Committee of Beni-Suef University, Egypt.

Animals

The study was carried out in three phases. In the first and second phases, 18 fresh cadaver heads were used for the anatomical and cadaver trial studies. Heads were obtained from donkey cadavers euthanized for reasons unrelated to head pathologies and unconnected to this study. Heads were decapitated at the atlanto-occipital joint and used immediately for various procedures. Based on the cadaveric trial study, nine live healthy adult donkeys were used in the third phase of the study to compare the accuracy and onset of analgesia for the 'blind' and ultrasound-guided approaches of the maxillary nerve. Animals were apparently healthy with no signs of head or neurological disorders.

Phase 1: anatomical study (n = 5)

A longitudinal skin incision was created parallel to the zygomatic arch that extended from the temporomandibular joint to the facial crest. The skin and subcutaneous fascia were separated from the underlying tissues and reflected dorsally and ventrally to expose the cutaneous faciei and zygomaticus muscles. Both muscles were incised, dissected carefully and reflected apart to depict the masseter muscle. The latter was transected just ventral to its origin from the zygomatic arch and the facial crest to disclose the extraperiorbital fat body that was bluntly dissected and removed to investigate the vascular and neural structures in the triangle formed by the rostral edge of the mandible, the maxillary tuber and the facial crest (pterygopalatine fossa). Photographs were taken to facilitate interpretation and correlate the anatomical structures with the corresponding ultrasonographic findings.

Phase 2: cadaver trial study (n = 13)

In this phase, the examination area (ventral to the lateral canthus of the eye and caudal to the facial crest) on both sides of the head was shaved, and cleaned with warm water and soap. The right side was saturated with alcohol and covered with coupling gel. Specimens were positioned on a table simulating a standing sedated donkey. The right maxillary nerve was injected via ultrasound

guidance, while the left one was blindly injected based on anatomical landmarks and experience gained from the first phase. Ultrasonographic examination was conducted using an Eickemeyer MAGIC 5000 Digital ultrasound machine (Eickemeyer Veterinary Equipment, Germany) with a 5−10 MHz T-shaped linear transducer. All examinations and injections were performed by the same individual (UH).

On the right side, ultrasonographic examination and localization of the maxillary nerve were performed using the angled method as described in horses (O'Neill et al. 2014). The transducer was positioned caudal to the facial crest just below the level of the lateral canthus with the ultrasound beam directed rostroventrally toward the last maxillary cheek tooth of the contralateral head side. The hyperechoic area between the maxillary tuber and the junction of the frontal and palatine bones was scanned from dorsal to ventral to localize the maxillary and sphenopalatine foramina until a hypoechoic break (foramina) was identified. An 18 gauge, 90 mm spinal needle (Becton Dickinson, Germany) with a stylet was introduced about 1 cm ventral to the ultrasound transducer and in the long axis of the ultrasound beam (in-plane approach) aiming for the junction of the dorsal rim of the maxillary and sphenopalatine foramina with the perpendicular plate of the palatine bone (Fig. 1). The needle was advanced deeply and observed ultrasonographically while piercing the skin, subcutaneous tissue, deep fascia, masseter muscle and the extraperiorbital fat body until the maxillary nerve and its associated vascular structures were recognized. Aspiration was then attempted and approximately 0.1 mL of methylene blue dye (Methylenblau; Burkhard Pielarski, Germany) was injected. Once the dye was injected, the stylet was replaced to ensure complete injection of the dye and eliminate further discoloration of the adjacent or superficial structures during needle withdrawal.

On the left side, the technique for 'blind' blockade of the maxillary nerve was carried out using the perpendicular method as described in horses (Bardell et al. 2010) with reference to information gained from the previous anatomical dissection phase. The spinal needle was inserted perpendicular to the skin surface 2 cm ventral to the zygomatic arch on a line running perpendicular to the dorsal head contour through the lateral canthus of the eye (Fig. 2). The needle was advanced through the masseter muscle and subsequently through the extraperiorbital fat body until the tip of the needle struck the perpendicular plate of the palatine bone. Aspiration was attempted prior to injection of the dye and the stylet was replaced prior to needle withdrawal to prevent dispersion of dye to the adjacent or superficial structures.

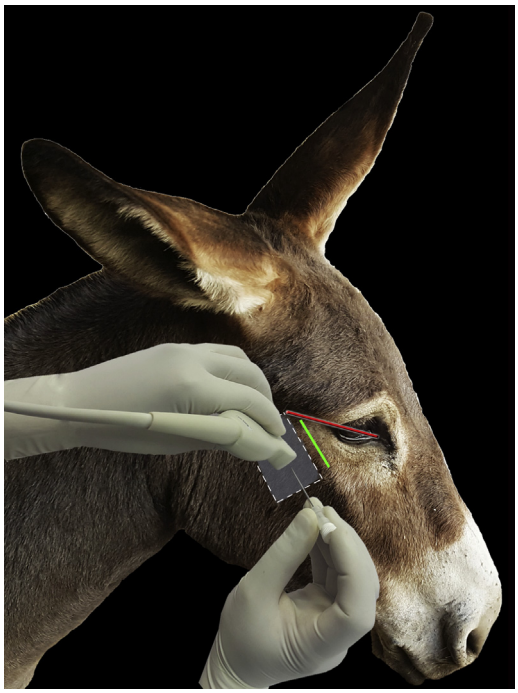

Figure 1 Acoustic window for ultrasound-guided maxillary nerve blockade (dotted white lines). The transducer was positioned caudal to the facial crest (green line), ventral to an imaginary line connecting the medial and lateral canthi and extending beyond the facial crest (red line). The needle is inserted about 1 cm ventral to the probe. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The insertion site of the needle into the skin in each puncture on each side of the head was marked and photographed to describe the portal of entry, anatomical landmarks and acoustic window used during injection. Dissection of cadavers was performed within 1 hour after injection by an independent observer (MGT) who was unaware of the approach employed in each instance and recorded the accuracy of dye deposition in relation to the target (maxillary nerve) and the possibility of malposition or injection into or injury of the adjacent structures.

Phase 3: experimental nerve blockade in vivo (n = 9)

To assess the reliability and accuracy of the 'blind' and ultrasound-guided nerve blockade, nine adult

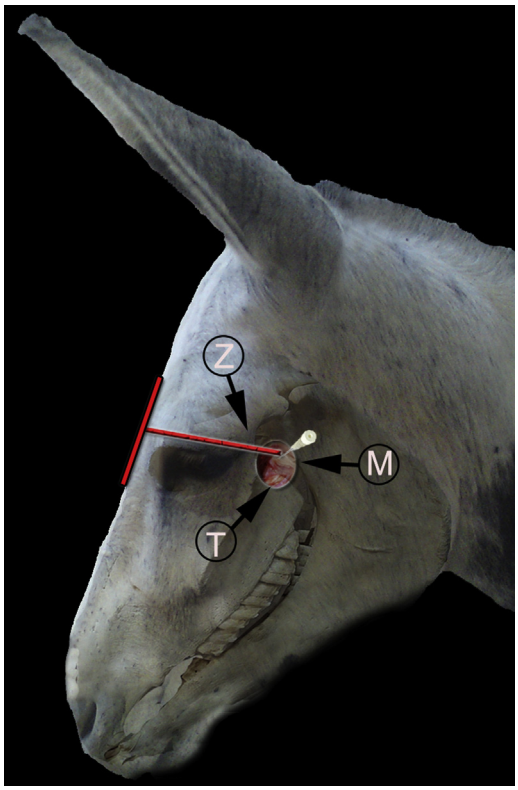

Figure 2 Schematic representation of a donkey head and skull demonstrating the position of a spinal needle for 'blind' blockade of the maxillary nerve. 'Blind' approach to the maxillary nerve is achieved by inserting the needle in the triangle formed by the zygomatic arch (Z), rostral rim of the mandible (M) and the maxillary tuber (T) on a line running perpendicular to the dorsal head contour through the lateral canthus of the eye (red lines). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

healthy donkeys were used. Animals were sedated using 20 μg kg^{-1} detomidine hydrochloride (Detogesic; Zoetis GmbH, Germany) intravenously. The examination area was shaved and aseptically prepared; 1 mL of lidocaine hydrochloride 2% (Lidocain Hcl 2%; B. Braun Melsungen AG, Germany) was injected subcutaneously to provide analgesia of the injection site. The maxillary nerve was approached ultrasonographically on the right side using the acoustic window established earlier or blindly on the left side as described previously. After negative aspiration, 20 mL of lidocaine hydrochloride 2% was injected slowly in each side.

The blind or ultrasound-guided technique was judged as successful when there was complete loss of cutaneous sensation of the ipsilateral naris. Sensation was verified at the naris of each side via a pin prick test using a 22 gauge (2.5 cm long) needle before lidocaine injection (baseline) and at intervals of 2 minutes after injection until loss of sensation was recorded for each side. The needle was inserted through the skin and the response of the animal to such stimulus was recorded. Analgesia was considered successful when the animal tolerated the skin puncture and showed no response to pricking. When the animal showed movement of the head, neck and/or trunk in an attempt to avoid the noxious stimulus of the needle, analgesia was considered unsuccessful. The time elapsed from injection of the local anesthetic to recording a positive response (loss of sensation) was considered as the time of onset of local anesthetic. Donkeys were evaluated for three successive days following injection to assess for potential complications such as infection, hematoma or neurological disorders.

Statistical analysis

Based on data derived on the accuracy of maxillary nerve staining from the trial study with 13 cadavers, a sample size calculation indicated that at least nine donkeys would be required to detect a significant difference ($p < 0.05$) with a power of 80%. Accuracy of maxillary nerve staining in cadavers was calculated as the number of cadavers in which the maxillary nerve was successfully stained divided by the total number of cadavers in which this access was attempted. Data analysis was performed using a statistical software program (SPSS for Windows Version 16; SPSS Inc., IL, USA). The age and weight of animals and onset of analgesia values were tested for normal distribution using the Kolmogorov–Smirnov test. An independent sample *t*-test was used to determine the main effect between the onset of action in the blind and ultrasound-guided techniques. A $p < 0.05$ was considered significant. Data are reported here as mean ± standard deviation or as proportions.

Results

The age of the donkey cadavers was 10 ± 4 years and weight was 202 ± 34 kg. The age of the live donkeys was 9 ± 2 years and weight was 200 ± 19 kg.

Phase 1: anatomical study

In this part of the study, gross dissection was performed following the expected pathway of the needle toward the target nerve. The masseter muscle was transected

at its origin and the extraperiorbital fat body was carefully removed exposing the pterygopalatine fossa. The portal of needle insertion into the pterygopalatine fossa was bounded by a bony triangle formed by the zygomatic arch dorsally, the mandible caudally and the maxillary tuber rostrally. An area of about 8 cm × 8 cm caudal to the facial crest and ventral to the lateral canthus of the eye was found to be an appropriate acoustic window for ultrasound-guided block of the maxillary nerve. Blunt dissection of this area showed numerous structures vulnerable for injury during needle insertion. Those structures included the maxillary nerve, the maxillary artery, the infraorbital artery, the descending palatine artery, the buccal artery, the deep facial vein and the periorbita (Fig. 3). The mean distance from skin surface to the target nerve was about 5.5 ± 0.5 cm.

Phase 2: cadaver study

Ultrasound-guided injection of the maxillary nerve using cadaver heads was practical, reliable and accurate. The maxillary nerve was successfully identified in all cadavers (13/13) and properly stained in all instances (accuracy = 100%), which was confirmed through cadaver dissection (Fig. 4). Accidental penetration of vascular components was not detected in any of the dissected cadavers, where none of the blood vessels were mottled with the blue dye in any of the inspected cadavers. In all specimens, it was possible to identify the bony boundaries of the pterygopalatine fossa as well as observe the needle where it penetrated through tissues until it became close to the target nerve. On ultrasonograms, the deep facial vein, the infraorbital artery and the maxillary nerve were readily distinguished. The two vascular components appeared as well-differentiated oval to round hypoechogenic structures surrounded by a hyperechogenic thin rim, while the maxillary nerve was identified as a bright hyperechogenic structure located at a depth of approximately 5 cm (Fig. 5).

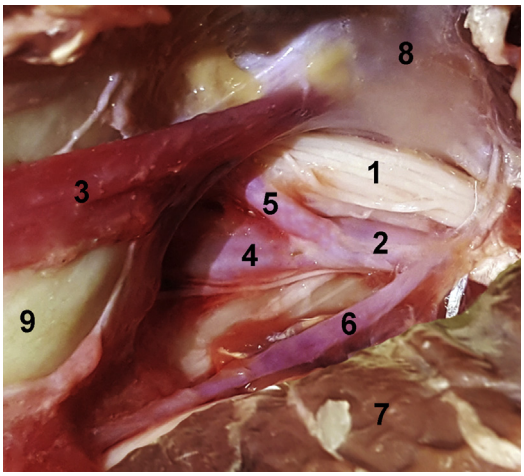

Figure 3 Gross dissection of the pterygopalatine fossa in a donkey after reflection of masseter muscle and removal of the extraperiorbital fat body. 1, maxillary nerve; 2, maxillary artery; 3, deep facial vein; 4, descending palatine artery; 5, infraorbital artery; 6, buccal artery; 7, masseter muscle; 8, periorbita; 9, maxillary tuber.

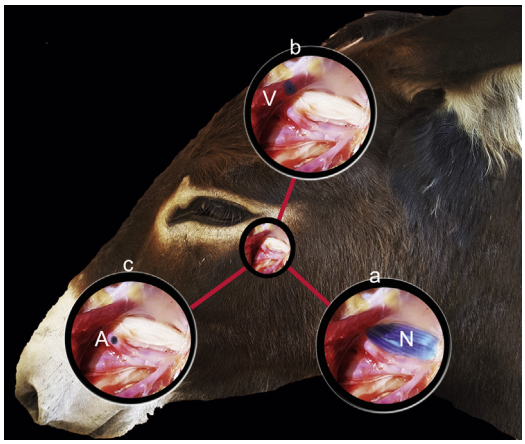

Figure 4 Gross dissection of the pterygopalatine fossa following injection of the methylene blue dye showing (a) successful staining of the maxillary nerve (N); (b) misdirection and staining of the deep facial vein (V); and (c) faulty deposition of the dye in the infraorbital artery (A). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

In the 'blind' injection trials of the maxillary nerve, localization of the anatomical landmarks as well as determining the point of needle insertion was not easy and was time-consuming. However, it was possible to insert the needle in the pterygopalatine fossa in all instances. The blindly injected dye was found surrounding the maxillary nerve in nine of 13 cases (accuracy = 69.2%) at dissection. Although aspiration prior to injection was always unproductive, accidental penetration and staining of the deep facial vein were detected in one specimen, and the infraorbital artery was inadvertently punctured in three specimens (Fig. 4).

Phase 3: experimental nerve blockade *in vivo*

Both ultrasound-guided and 'blind' techniques for blocking of the maxillary nerve were well tolerated in live donkeys.

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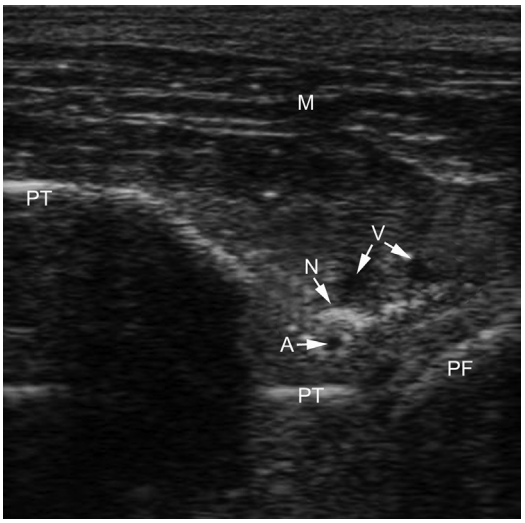

Figure 5 Ultrasonogram of the pterygopalatine fossa in a donkey beneath the masseter muscle (M) demonstrating the maxillary nerve (N) related to branches of the deep facial vein (V), the infraorbital artery (A) and bounded by the perpendicular plate of the palatine bone (PT) and fascia of the periorbital cone (PF).

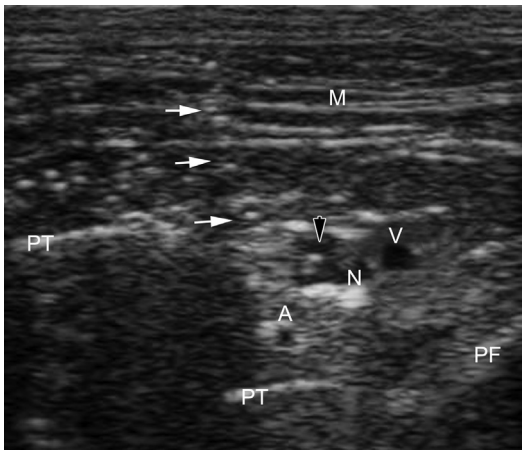

Figure 6 Ultrasonogram of the pterygopalatine fossa in a donkey demonstrating the pathway of the spinal needle (white arrows) through the masseter muscle (M) and underlying tissues as well as the spread of the local anesthetic (black arrow) around the maxillary nerve (N). A, infraorbital artery; PF, fascia of the periorbital cone; PT, perpendicular plate of the palatine bone; V, branches of the deep facial vein.

In the ultrasound-guided method, it was possible to visualize the same structures as observed in the cadavers. There were no differences between the ultrasonographic appearances of the maxillary nerve and the surrounding structures in live or dead animals; however, pulsation of the infraorbital artery in live animals was a useful landmark. In all cases, it was possible to follow the movements as well as the deep advance of the needle toward the target nerve in real time via ultrasonography. Negative aspiration confirmed accuracy and skipping of vascular structures. Once the tip of the needle was observed close to the maxillary nerve, the local anesthetic solution was slowly injected and could be observed in real time in all cases (Fig. 6). This blockade produced desensitization of the right naris in all animals within about 10.9 ± 1.8 (range, 8−14) minutes. No gross or ultrasonographic abnormalities were noted either during or following this procedure.

In the 'blind' block method, identification of the bony landmarks was possible in all cases. The needle was advanced about 5 cm deep and head jerking with increased resistance to injection was observed in two animals. Aspiration was attempted before each injection and blood was aspirated in three donkeys. In those animals, the needle was withdrawn and reinserted until aspiration was negative. Desensitization of the left nostril was achieved in all cases and onset of action was reported in about 27.8 ± 3.2 (range, 22−32) minutes. All the donkeys recovered uneventfully from the procedures, and did not show signs of neurological disorders as a result of a nerve injury.

The onset of analgesia was shorter in the ultrasound-guided approach compared with the blind technique ($p < 0.001$).

Discussion

To our knowledge this is one of the first studies describing and comparing the 'blind' and ultrasound-guided approaches for maxillary nerve blockade in donkeys. The results of this study showed that it is possible to use either ultrasound or anatomical landmarks to approach the maxillary nerve in the pterygopalatine fossa. This study was carried out in three phases: an anatomical study; a cadaver pilot study; and an experimental trial study. A similar study design has been reported for the ultrasound-guided blockade of the sciatic and femoral nerves in dogs (Echeverry et al. 2010) and calves (Michela et al. 2014).

In this study, gross dissection of cadavers was valuable for determining the anatomical landmarks, appropriate acoustic window and depth of needle required to approach and block the maxillary nerve in donkeys. The location of the maxillary nerve and its associated structures was similar to that reported

in horses (Staszyk et al. 2008); therefore, the maxillary nerve was approached using similar anatomical landmarks (Bardell et al. 2010) and acoustic window (O'Neill et al. 2014) as described in horses. The depth of the maxillary nerve in relation to the skin surface in donkeys was about 5.5 ± 0.5 cm; therefore, the needle was inserted to a depth of almost 5 cm in both the blind and ultrasound-guided techniques to avoid injury of both the nerve and adjacent structures.

In this study, randomization of sides was not considered to avoid the limitations described in horses (Bardell et al. 2010) concerning the difference between the left and right sides when the operator is right handed. The ultrasound-guided approach was performed on the right side because it was easier for the right-handed operator to hold the transducer in the left hand and the injection needle in the right hand. The angled method (Bardell et al. 2010) was favored for the ultrasound-guided technique because it was possible to keep the needle and ultrasound probe in the same plane (in-plane approach) so the needle length and needle tip could be visualized while advancing the needle toward the nerve. Furthermore, an angled approach resulted in frequent deposition of the injectate rostrally and dorsally (Bardell et al. 2010), which was visible ultrasonographically. The perpendicular method was favored for the 'blind' approach on the left side because the anatomical landmarks for this technique were palpable and easily identified in contrast to the angled technique. In addition, the success rates (number of full hits) in the perpendicular technique in horses on the left side were double compared with the right side (Bardell et al. 2010).

In this study, a cadaveric pilot study was employed as a model to assess and compare the accuracy of the ultrasound-guided and 'blind' injection techniques to block the maxillary nerve in donkeys. The accuracy rates for injections using the 'blind' technique were not as high as desired (69.2%) and the ultrasound-guided technique provided a higher degree of accuracy (100%). Similar findings were reported in horses (Bardell et al. 2010; O'Neill et al. 2014).

To the author's knowledge, this is one of the first studies investigating the use of ultrasound guidance for nerve blockade in donkeys. A T-shaped linear transducer (5−10 MHz) and an in-plane approach were used (Campoy et al. 2010; Echeverry et al. 2010; Michela et al. 2014). This approach allowed visualization of the length and needle tip while progressing to the nerve. There was no difference between the ultrasonographic appearance of the maxillary nerve in fresh cadavers and live donkeys. Similar findings were described in dogs (Echeverry et al. 2010). In horses (O'Neill et al. 2014), the infraorbital artery was not visualized as cadavers were frozen and later thawed; however, use of fresh cadavers in this study enhanced visualization of the vascular structures in the pterygopalatine fossa.

The success of neural blockade relies mainly on placing the needle as close as possible to the nerve without causing damage to the target nerve (Campoy et al. 2010). In all donkeys, both ultrasound-guided and 'blind' techniques produced analgesia in the ipsilateral naris; however, the onset of analgesia was earlier in the ultrasound-guided technique (10.9 ± 1.8 minutes). Similar findings were reported for ultrasound-guided nerve blockade of the maxillary nerve in horses (O'Neill et al. 2014) and the sciatic nerve in calves (Michela et al. 2014). This is possibly the result of direct deposition of the local anesthetic solution in contact with the target nerve, enabling rapid infiltration of the local anesthetic solution throughout the maxillary nerve, thus allowing a quicker onset and longer analgesic effect (Echeverry et al. 2012). The higher success rates in live animals (100 %) compared with cadavers (69%) and delayed onset of analgesia in the 'blind' technique (27.8 ± 3.2 minutes) might be a consequence of the deposition of a large volume of local anesthetic solution (20 mL) at a further distance from the nerve that required longer time to diffuse and block the nerve (Tremaine 2007).

In this study, no signs of either vascular or neural injury were observed during the ultrasound-guided maxillary nerve blockade in live donkeys, as the nerve and the adjacent vascular structures were clearly discriminated. Moreover, visualization of the periorbital cone limits the possibility of inadvertent penetration or injection of local anesthetic into the intraorbital structures that might cause globe prolapse, retrobulbar hematoma, neurological signs and/or cardiac arrest if the local anesthetic was injected into the dural cuff of the optic nerve (Staszyk et al. 2008). In the blind study, inadvertent vascular penetration was reported in four cadavers and blood was aspirated in three live animals. Furthermore, nerve trauma was reported in two animals manifested by head jerking and increased resistance during injection of the local anesthetic.

Finally, although a shorter onset time appeared to be achieved with the ultrasound-guided approach compared with the 'blind' technique, potential limitations need to be mentioned. The first is the success

of an ultrasound technique is user dependent. Ultrasound is a unique skill that requires training and experience in order to become proficient (Falyar 2010). The second is performing the nerve blockade on the side most comfortable for the clinician. The third is the low number of animals used in this study. Further investigations are needed in larger populations to evaluate both the dose and duration of analgesia in these techniques, and validate the effectiveness in clinical patients.

In conclusion, this study showed that the ultrasound-guided approach to block the maxillary nerve in donkeys offered many advantages over the 'blind' technique including accurate and easy nerve localization, direct visualization of the needle and local anesthetic spread, avoidance of inadvertent vascular injury and rapid onset of analgesia. The results of this study suggest that maxillary nerve block in donkeys could be easily achieved clinically using an ultrasound-guided technique; therefore, additional studies are necessary to evaluate this technique in clinical patients.

Authors' contribution statement

UH and MGT: conceived the study and prepared the manuscript; MGT: dissected cadavers dissection and assessed sensation in live animals; UH: injected dye in cadavers and anesthetic solution in live animals and performed ultrasound examinations; All authors contributed equally to the planning, execution, data analysis and interpretation and have read and approved the final version submitted.

Conflict of interest statement

This study was not funded by any external body. None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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