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CASE STUDY Agricultural Science Digest

Effect of Atropine Sulphate on Laryngeal Accessibility and Visibility for Swine Intubation

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ABSTRACT

Background: Animal intubation is necessary in painful surgery procedures in order to maintain general anaesthesia and analgesia. In swine, orotracheal intubation is difficult or impossible; all possible measures to improve it need to be explored.

Methods: Two groups of a total of 20 clinically healthy local mixed breed (Sus scrofa domesticus) premedicated swine underwent intubation. The animals were randomly divided into two groups, one using atropine sulphate (A) and one using saline solution (N), prior to endotracheal intubation. Premedication started with intramuscular injection of xylazine, ketamine and fentanyl. Intubation was performed after induction with thiopental sodium administered intravenously with atropine sulphate or saline solution. Larynxes were photographed and filmed and coded images and videos were sent to investigators for evaluation.

Result: In this study, a negative effect of atropine sulphate on the laryngeal visibility, opening and accessibility in local mixed breed anesthetized pigs was identified.

Key words: Anaesthesia, Intubation, Sus scrofa domesticus.

INTRODUCTION

Swine are often used as research models for cardiovascular surgery; these surgical models require balanced anaesthesia in accordance with animal welfare and good laboratory practice. For long, painful procedures, orotracheal intubation is necessary in order to protect the airway and prevent complications (Chum and Pacharinsak, 2012). Successful intubation is vital to the stable maintenance of swine under general anaesthesia. However, achieving endotracheal intubation in pigs is still regarded as being technically difficult for scientists performing the procedure; therefore, the procedure is avoided, or tracheostomies are chosen in order to prevent laryngospasm and further complications (Theisen *et al*., 2009). There is a need to improve the intubation of pigs for inexperienced scientists and veterinarians in all possible ways; protocols for anaesthesia being developed to help prevent certain complications from intubation (Ettrup *et al*., 2011) (Linkenhoker *et al*., 2010). Easier methods of pig intubation using a plastic guide for safe and rapid pig intubation are also described (Janiszewski *et al*., 2014).

Pigs have a small larynx diameter compared to other animals of similar weight. Endotracheal intubation is hampered by poor visualization of the larynx due to the narrow mouth opening, long distance from the snout to the larynx, its anatomical features and protective reflexes (Hartmann *et al.,* 1971, Murison, 2011). For safe anaesthesia, it is important that the intubation of a pig be successful at the first attempt, as subsequent attempts can cause very serious respiratory damage and may be lethal (Oshodi *et al*., 2011, Steinbacher *et al*., 2012). Oropharyngeal manipulation induces nerve blockade-laryngospasm; these reflexes may be inhibited by using anticholinergic agents. It is believed that reduced airway secretion improves larynx visualization and can prevent laryngospasm (Hartmann *et al*., 1971, Steinbacher *et al*, 2012, Gavel and Walker, 2014).

Many authors and guidelines recommend the use of atropine sulphate for its antisialogogous effect, intramuscularly or intravenously, at the beginning of premedication or induction (Calzetta *et al*., 2014); however, its effect has not been fully defined or proven (Steinbacher *et al*., 2012). The aim of this study was to evaluate the effect of atropine sulphate on laryngeal visibility and accessibility, thus facilitating the complex and life-threatening intubation of pigs. Our hypothesis was that atropine affects the accessibility and visibility of a pig larynx improving pig intubation.

MATERIALS AND METHODS

The study was approved with permission (G2-171) from the State Food and Veterinary Service of the Lithuania and was performed according to the European Community guiding

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principles as outlined in the Guide for the Care and Use of Laboratory Animals (NRC, 2011) and associated guidelines of the EU Directive 2010/63/EU for animal experiments from 2020 till 2022. All animal were kept in Lithuanian University of Health Sciences Biological Research Centre. The study included only clinically healthy pig sus scrofa local breed females (nonpregnant, nulliparous), same age 57±1.45 d., same breeding facility, weighing 35±0.89 kg. The animals were randomly divided into two groups (10 pigs/group): the atropine (A) group and the negative control (N) group. Random numbers were generated using the standard= RAND () function in MS Excel 2010 (Microsoft, Brussels, Belgium).

An analytical, prospective and blinded study design was chosen for this study. All animals underwent anaesthesia and analgesia using the same protocol depending on animal weight. Animals were premedicated intramuscularly with xylazine hydrochloride 3 mg kg^{-1} (Sedaxylan, Eurovet Animal Health, The Netherlands), ketamine hydrochloride 20 mg kg^{-1} (Ketamidor, Ricchter Pharma, Austria) and fentanyl citrate 3 µg kg^{-1} (Fentanyl, Kalceks, Latvia) mixed in one syringe and were always assessed after injection. Immediately after the animal reached the surgery anaesthesia stage, it was transferred to a preparation table and a 16-gauge intravenous catheter (BD Venflon, Sweden) was inserted into the auricular vein. Induction was obtained with thiopental sodium 8 mg kg^{-1} (Thiopental, VUAB, Czech Republic). Subsequently, atropine sulphate 0.04 mg kg^{-1} (Atropine sulphate, Sanitas, Lithuania) was administered intravenously for each animal in group A, or the same amount of saline solution (NaCl 0.9%, B Braun, Germany) was administered for each animal in group N. After induction, a laryngoscope was inserted into the mouth cavity and used to perform laryngoscopy using an endoscope (Olympus, Germany); a 5- to 10-second-long procedure was performed in order to create images and a short video. Animals were excluded from the study if intubation failed, tracheostomy was required, or other complications occurred.

For each animal, four different investigators were involved as follows: A first investigator (VZ) administered the treatment based on the randomization table. This investigator was the only person aware of the treatment group allocation, responsible for anaesthetic protocol during the study. Two experienced investigators, veterinary medicine doctors used to casual animal anaesthesia and intubation (horses, sheep, goats, rabbits and guinea pigs) (RG; AK) and one beginning investigator, a veterinary student experienced as a veterinary technician in a small animal clinic (EJ), all unaware of the treatment, were responsible for larynx evaluation after the procedure. The investigators did not know the animal groups; after the study they received 20 coded images and videos of swine larynges. During evaluation, the following parameters for easy intubation were assessed: diameter, accessibility, visibility and amount of saliva and mucus. Laryngeal scores were graded as follows: score 1: possibly difficult complicated intubation due to a narrow laryngeal opening, restricted visibility and accessibility and a large amount of saliva; score 2: broader laryngeal opening, limited accessibility and poor visibility and a small amount of saliva; score 3: extensive laryngeal opening, clear accessibility, visible posterior origin of the vocal cords and a moist mucosa; score 4: comprehensive laryngeal opening, complete accessibility, a full view of the glottis and moist mucosa; score 5: best and easiest intubation with widespread laryngeal opening, complete accessibility and visibility and wet mucosa. Data were decoded after analysis; images that were most often graded with a certain score are shown in Fig 1.

Sample size calculations were made using G*Power 3.1.9.7 software, choosing power level 0.9, alpha measures at 0.05 and beta levels at 80; a total sample size of n= 20 or 10 animals per group was required. Measures of the effects of the treatment groups were compared between groups with score levels using the t-test difference between two independent means (two groups) (StatSoft 7.0, USA). Data in the text are presented as the mean ± standard deviation; the statistical level of significance was set at $p<0.05$.

RESULTS AND DISCUSSION

All local breed pigs were used in this study; after the laryngoscopies were intubated successfully at first attempt, no complications occurred during the procedures. Pigs underwent general anaesthesia for experimental purposes not related to this study. There were no differences in the age, sex and body weight among groups in the study.

Investigators awarded higher scores to the larynx images and videos of animals treated with saline solution

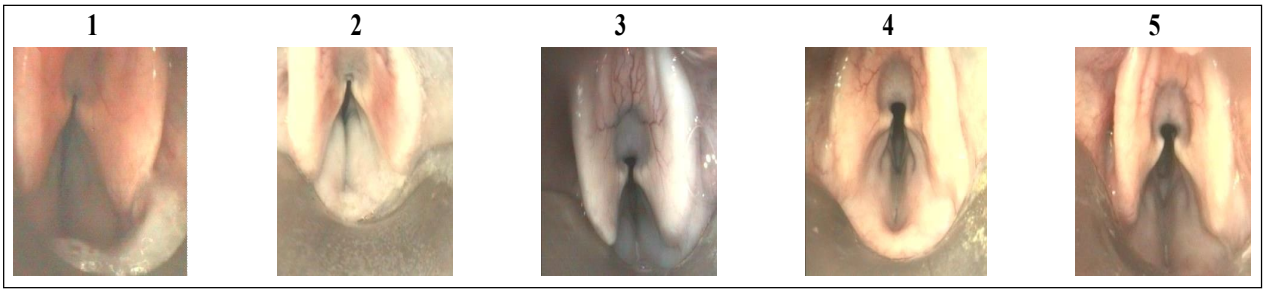

Fig 1: Laryngeal scoring after blind larynx grading in our study.

and lower scores were awarded to the larynx images of animals treated with atropine sulphate. Some of the images taken in this study are shown in Fig 1; these images were nominated most often with a certain score.

After evaluation and analysis, data were decoded and evaluation scores were assessed. Overall evaluation scores differed significantly $p<0.05$ between the A and B groups. Images and videos of larynges from group A-atropine treated were graded with lower scores 2.79±1.42, in comparison with the N group-saline treated pig's larynges were graded to 4.14±1.16; these results are shown in Fig 2.

Pigs are increasingly used for research purposes for various surgical procedures; to ensure the animal's welfare, good balanced anaesthesia is important to reduce the animal's pain and suffering during and after procedures (Chum and Pacharinsak, 2012). The pigs used in our study and their weight correspond to the weight of pigs of this age in normal farms (Naha *et al*., 2017). Due to anatomical and technical features, pig intubation is still complicated even for experienced professionals, requiring an average of 1.2 attempts for successful intubation (Janiszewski *et al*., 2014). The best intubation recumbency (Theisen *et al*., 2009), the use of a plastic guide (Hartmann *et al*., 1971) and the best anaesthesia protocols are still being developed and complications described in order to improve the intubation of pigs (Zigmantaite *et al*., 2018) (Kurita *et al*., 2022). Ketamine is the primary anaesthetic drug, mainly used for pig anaesthesia; a well-known ketamine potential side effect is hypersalivation, which can lead to laryngospasm (Heinz *et al*., 2006); therefore, the use of atropine in combination with ketamine is often recommended in anaesthesia protocols (Calzetta *et al*., 2014). Atropine sulphate is known to reduce the risk of laryngospasm by its antisialogogous action decreasing secretions (Gavel and Walker, 2014). It is now known that muscle relaxants have the greatest effect in preventing laryngospasm (Theisen *et al*., 2009) (Hernández-Cortez, 2018). The most recent study in paediatric anaesthesia suggested that atropine was only indicated in special situations such as for newborns or premature patients, with greater activity upon parasympathetic tone and it also can be used for its anticholinergic effects to reduce ACH-mediated bradycardia that can accompany endotracheal intubation (Hernández-Cortez, 2018). In clinical practice, atropine sulfate is also used to anesthetize dogs, but its exact action is not clear (Saikia *et al*., 2022) (Kumar *et al*., 2020). Some new studies describing anesthesia in dogs use another drug from the same group, glycopyrrolate (Bayan and Sarma, 2022).

Scores from 1 to 5 were used to grade different larynges, using the Cormack-Lehane human larynx evaluation (Krage, *et al*., 2010; Cormack and Lehane, 1984) system, which is used to evaluate larynx scores and possibly intubation complications in humans. An unexpected finding in this study was that atropine had no effect on laryngeal opening, accessibility and visibility in premedicated pigs. As was demonstrated in this study, atropine sulphate affected the saliva amount and mucosae moisture; the highest value for saliva was without atropine injection. However, saliva levels in pig anaesthesia are not a major problem compared to other salivating animals (Meyer *et al*., 1964). The results of our study indicate that saliva did not affect the laryngeal visibility and opening. This may be due to the atropine mechanism of blocking the myoneural junctions of the smooth muscle connections (Harrison and Vanik, 1963), possibly causing closeness of the larynges. Others considered the effect of atropine on laryngospasm as a

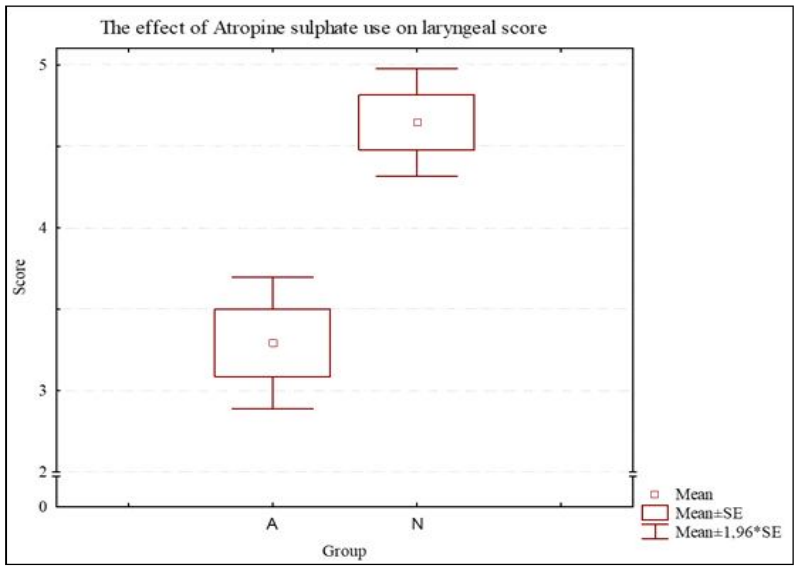

Fig 2: Overall evaluation scores in group A and N. Data shown in smallest square as mean values, the standard deviation is reflected in the error bars for two different treatment groups. Groups A and N represent data from the atropine sulphate treated and the saline treated groups, respectively.

prevention to be a reduction in the superior laryngeal nerve (SNL) stimulation (Rosen, 1960), causing the distortion of the laryngeal anatomy (Wiles, *et al*., 1989). There are rather few publications regarding atropine's effect on larynges; one reported that atropine had a negligible effect and blocked laryngeal reflexes under controlled conditions (Harrison and Vanik, 1963).

The first published data of the effects of atropine on laryngospasm prevention were between 1960 and 1980; it was already stressed that one should not expect atropine to prevent laryngospasms (Rosen, 1960). Other authors also stated that at a dose rate of 1.2 mg per anesthetized cat, atropine failed to alter the coughing response (Harrison and Vanik, 1963). Another study used atropine sulphate for cat premedication and stated that atropine did not affect the stimulated formation of laryngospasm using ether (Rex, 1971).

For coughing or laryngospasm prevention, some authors suggested using local anaesthetics intravenously in early 1951 (Gilbert *et al*., 1951) (Steinhaus and Gaskin, 1963); in 1970, a topical effect was investigated (McCulloch *et al*., 1992). Proper laryngospasm prevention requires balanced anaesthesia management (Ionescu and Ved, 2019); the use of local anaesthetics (Qi *et al*., 2016, Talbi, 2015) and muscle relaxants is required (Collins *et al*., 2019).

In terms of the limitations of the study, there is very little literature on the subject and little research has evaluated the effect of atropine on laryngeal visibility and accessibility. The methodology of this study was not perfect, but the study was conducted diligently and sincerely. From the current perspective, it would be very good to measure the time taken to intubate differently treated pigs and thus evaluate the effect of atropine; we hope to do so in the future experiments. We add to the recommendations of other authors to treat atropine sulphate in special conditions where the sensitivity of the parasympathetic nervous system and the possible risk of bradycardia are suspected.

CONCLUSION

In conclusion, atropine sulphate used for induction of local breed pigs had significant effect on laryngeal visibility, accessibility and opening. The negative effect of atropine sulphate did not interfere with the successful intubation of pigs.

Conflict of interest: None.

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