Effects of chemical and mechanical stimulation on laryngeal motion during alfaxalone, thiopentone or propofol anaesthesia in healthy dogs

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Abstract

Objective: To compare the effect of chemical and mechanical stimulation on arytenoid cartilage motion during anaesthetic induction with alfaxalone, thiopentone or propofol.

Study design: Masked, randomized, crossover study.

Animals: A group of eight adult Beagle dogs.

Methods: Anaesthesia was induced with thiopentone (7.5 mg kg^{-1}), propofol (3 mg kg^{-1}) or alfaxalone (1.5 mg kg^{-1}) intravenously (IV), which were concurrently paired with either chemical (doxapram at 2.5 mg kg^{-1} IV) or mechanical (gentle pressure to the corniculate process of the right arytenoid cartilage using a cotton bud) stimulation for enhanced assessment of laryngeal motion, in random order, with a 1 week wash-out period between treatments. If deemed inadequately anaesthetized, supplemental boli of thiopentone (1.8 mg kg^{-1}), propofol (0.75 mg kg^{-1}) or alfaxalone (0.4 mg kg^{-1}) were administered. Assessment of number of arytenoid motions and vital breaths, among others, was initiated immediately after induction. Chemical (doxapram) and mechanical stimulation were begun 2 minutes after anaesthetic induction. Data were collected at 2, 3 and 5 minutes after anaesthetic induction and the Friedman rank-sum or repeated-measures analysis of variance tests were used when applicable for statistical analysis.

Results: The duration of examination time was shorter among treatments combined with chemical stimulation (p=0.001). Examination time during induction was longer for alfaxalone-chemical (8.9 minutes) and -mechanical (10.9 minutes) compared to both induction with thiopentone-chemical (3.8 minutes) and propofol-chemical (4.0 minutes). The median number of arytenoid motions for both thiopentone (67) and propofol (59) induction combined with chemical stimulation was significantly higher in comparison to that of alfaxalone (1), thiopentone (2) and propofol (2), when combined with mechanical stimulation at 3 minutes after induction.

Conclusion and clinical relevance: Among the regimens for assessing laryngeal motion assessed in the present study, combinations of thiopentone or propofol with doxapram are the most effective means of stimulating arytenoid motion and could improve the accuracy of diagnosis of laryngeal paralysis in dogs.

Keywords: alfaxalone; chemical stimulation; laryngeal paralysis; mechanical stimulation; propofol; thiopentone;

Introduction

Clinical diagnosis of laryngeal paralysis (LP) in dogs is usually performed under a light plane of anaesthesia (Gross et al., 2002, Jackson et al., 2004) by evaluating arytenoid cartilage abduction during the inspiratory phase of breathing. Historical and physical examination findings in conjunction with orolaryngoscopy improve the sensitivity for LP diagnosis (Kitshoff et al. 2013). Chemical and mechanical stimulation can be used to enhance the accuracy of diagnosis of LP in anaesthetized dogs.

Drugs used to induce anaesthesia may influence laryngeal function by diminishing the laryngeal reflex (MacPhail 2014). Hence, the characteristics of an ideal anaesthetic drug for diagnosis of LP include: 1) minimal effect on laryngeal function, 2) adequate jaw muscle relaxation and 3) circumvention of induction apnoea while providing ample regular inspiratory efforts. The desired light plane of anaesthesia for orolaryngoscopy can be achieved using anaesthetic induction drugs such as thiopentone, propofol or alfaxalone (Smalle et al. 2017). The effects of these drugs are well documented, but there is a lack of consistency in descriptions of ideal dosages for clinical application. There is also a paucity of information on complementary diagnostic aids that can be used to improve accuracy in LP diagnosis. The use of either chemical or mechanical stimulation to enhance laryngeal reflex responses has been proposed (Miller et al., 2002, Tobias et al., 2004). Doxapram hydrochloride is a known stimulant of the medullary respiratory centre and has been proposed to improve abduction ability of the arytenoid cartilages (Tobias et al. 2004). Although an optimal dose of doxapram hydrochloride for enhancement of LP diagnosis has not been established, previous studies cited a dose range of 1.1–2.2 mg kg^{-1} intravenously (IV) (Miller et al., 2002, Tobias et al., 2004). Tobias et al. (2004) investigated the effect of doxapram as a chemical stimulant on laryngeal function of normal and LP-affected dogs. The study used neuroleptanalgesia premedication drugs (acepromazine and butorphanol), which could have influenced the function of the crico-arytenoideus dorsalis muscle. Miller et al. (2002) also examined doxapram's effect on LP diagnosis in healthy dogs, but used premedication (glycopyrrolate and acepromazine maleate), thus complicating the comparison of findings between the studies. There is a scarcity of research relating to mechanical stimulation as an additional diagnostic aid for LP in dogs. Stimulation of the larynx and the tracheal mucosa has been proposed to increase motion of the laryngeal abductor (Poliacek et al. 2008). This scarcity warrants further investigation of different types of mechanical stimulation, including pressure, water, air and temperature.

The purpose of the present study was to compare the effects of chemical and mechanical stimulation on arytenoid cartilage motion during anaesthetic induction with three drugs (alfaxalone, thiopentone or propofol) at currently recommended doses in healthy dogs.

Materials and methods

The study was approved by the Animal Ethics Committee of the University of Pretoria, Pretoria, South Africa (Project number: V106-16). A group of eight healthy castrated male Beagle dogs housed and cared for at the University of Pretoria Biomedical Research Centre (UPBRC) were enrolled into the study. The Beagle dogs were purposefully bred for research studies, and the number of dogs selected in the present study would allow comparison to

other studies (Gross et al., 2002, Smalle et al., 2017). The dogs had never been diagnosed with any upper or lower airway disease and were considered to have normal laryngeal function and anatomy. The dogs underwent a preanaesthetic physical examination and routine haematology and biochemistry were analysed 24 hours prior to induction and subsequent data collection week, to confirm their health status. Randomization of the order of treatments (1–6) per dog in this prospective crossover trial was performed with Winpepi Version 11.20 (Brixton Health, Israel). A wash-out period of 1 week was enforced between treatments, which were as follows: 1) alfaxalone–chemical stimulation (Alf-chem); 2) thiopentone–chemical stimulation (Thio-chem); 3) propofol–chemical stimulation (Prop-chem); 4) alfaxalone–mechanical stimulation (Alf-mech); 5) thiopentone–mechanical stimulation (Thio-mech); and 6) propofol–mechanical stimulation (Prop-mech).

Food, but not water, was withheld overnight (8–12 hours) prior to data collection. On the day of the anaesthetic procedure, the dogs were housed in dedicated holding pens within the UPBRC facilities and allowed to rest for 1 hour to minimize excitement or stress. Each dog was assessed as being 'excited' (active on the table and resisting physical restraint) or 'not excited' (compliant with minimal resistance to physical restraint) before the experimental procedure commenced. Each dog was weighed using an electronic scale (JS series; Jadever Weightec Inc., ON, Canada) and its identity verified by a microchip (Pocket Reader EX; Identipet, South Africa). The anaesthetic induction drugs were drawn up at the pre-determined dose into 20 mL syringes [B. Braun; B. Braun Medical (PTY) Ltd, South Africa] and a low-volume extension set was attached (Extension set REF 011-C150; Poly Medicure Ltd/ICU Medical SA, South Africa). Each extension set was covered with black general-purpose electrical trunking material to obscure visibility and mask the primary investigator. Each anaesthetic induction drug was administered from a separate electronic syringe driver [B. Braun Perfusor Space; B. Braun Medical (PTY) Ltd]. The predetermined volume was set for each dog individually according to body weight and administered over the pre-defined induction period. To further mask the primary investigator's view, the syringe drivers were positioned outside the investigators field of view and obscured by a blanket screen. The dogs were positioned on the procedure table 10 minutes prior to induction and a 21 gauge IV cannula (Jelco; Smiths Medical, UK) was placed and secured into the right lateral saphenous vein. An injection-port stopper was secured to the IV cannula and provided access for the IV injection of the anaesthetic induction and chemical stimulation drugs. Lactated Ringer's solution (Ringer's Lactate; Fresenius Kabi, South Africa) (2 mL) was used to flush the injection-port after administration of a drug bolus. The anaesthetic induction drugs were administered at these dosages: 1) alfaxalone [Alfaxan-CD RTU 1%; Jurox (Pty) Ltd/Kyron laboratories (Pty) Ltd, South Africa]; induction bolus 1.5 mg kg^{-1}; supplemental bolus 0.4 mg kg^{-1}; 2) thiopentone (Thiopentone Sodium Fresenius 0.5 g 20 mL^{-1}; Fresenius Kabi South Africa); induction bolus 7 mg kg^{-1}; supplemental bolus 1.8 mg kg^{-1}; and 3) propofol [Fresenius Propoven 1% (50 mL); Fresenius Kabi South Africa]; induction bolus 3 mg kg^{-1}; supplemental bolus 0.75 mg kg^{-1}.

The dosages of the induction drugs were as per the study of Smalle et al. (2017). The calculated induction bolus was administered over a 1 minute period IV using a syringe driver and allowed to take effect for 10 seconds before assessment of anaesthetic depth. While the syringe driver was set on standby mode, jaw tone and palpebral reflexes were evaluated. Adequate depth of anaesthesia was defined as the ability to open the jaws without any accompanying resistance in the form of chewing, conscious swallowing or head avoidance movement away from laryngeal examiner; absence of the lateral palpebral reflex and a regular breathing pattern. If a dog was deemed inadequately anaesthetized, a supplemental

bolus was administered over a 10-second period followed by a further 10-second waiting period before assessing depth of anaesthesia again. This sequence was repeated until the depth of anaesthesia was deemed adequate and the induction end point reached. Soon after, the maxilla was hooked over a padded anaesthetic frame and the mandible lowered to introduce an illuminated laryngoscope blade (Macintosh size 3, Satin™ Fiber Optic Macintosh Laryngoscope Blades, American Diagnostic Corporation, NY, USA) ventral to the epiglottis into the vallecula at the base of the tongue. The laryngoscope blade tip was directed ventrally, to expose the rima glottides (RG) and then the laryngeal evaluation, by the primary investigator, commenced. The onset of inspiration was verbally communicated so as to assist the primary investigator in ascertaining whether arytenoid abduction and inspiration were coordinated. Chemical stimulation treatment was performed at a fixed time period: 2 minutes after the induction end point. The chemical stimulant (doxapram hydrochloride) was administered IV at a dose of 2.5 mg kg^{-1}, over a period of 30 seconds, after which the IV cannula was flushed with lactated Ringer's solution. Mechanical stimulation was performed at 2, 3 and 5 minutes after the induction end point by applying pressure, for a period of 5 seconds, to the right corniculate process of the arytenoid cartilage, using a cotton bud. Examination of the larynx continued until the examination end point was reached. The examination end point was considered reached once chewing or conscious swallowing attempts were observed. Then the dogs were moved to a warm, dry, padded area and recovered under observation.

Data collection

During the induction period, the number of supplemental boli required was recorded. Examination time was defined as the period from the start of administration of the induction bolus until the examination end point had been reached. Laryngeal examination was divided into three recorded time periods: 1) Time period 1: defined as the period from start of laryngeal examination until 2 minutes (induction); 2) Time period 2: commenced at the end of Time period 1 (or end of induction period) and spanned 3 minutes or until the examination end point (early recovery) was reached. During this time period, mechanical or chemical stimulation was applied; and 3) Time period 3: commenced at the end of Time period 2 and continued until the recovery end point had been reached.

The total number of arytenoid abductions and vital breaths (deep inspiratory efforts) were recorded per time period. The ability to evaluate the RG was scored using a subjective laryngeal exposure score (Appendix A). Also, the characteristics and quality of arytenoid motion were subjectively scored (Appendix B). The scoring systems were adapted from Smalle et al. (2017).

Statistical analysis

Data were analysed using the R Statistical Software (Version 3.2.3; The R Foundation for Statistical Computing, Austria). Data were assumed to be non-parametric because of the small sample size and are expressed as median (range). The Friedman rank sum test was used to test for statistically significant differences amongst treatments for data on preanaesthetic clinical evaluation observations, duration of laryngeal function evaluation time, duration of total time and the number of supplemental boli required for induction of anaesthesia. If significant differences were observed, a pairwise Wilcoxon rank sum test with a Bonferroni adjustment for multiple testing was conducted. Repeatedly measured variables (number of vital breaths, number of arytenoid motions, laryngeal evaluation breath score, jaw tone score,

swallowing score, laryngeal spasm score, laryngeal function score and paradoxical motion score) were tested for statistically significant differences among groups using the repeated-measures analysis of variance (anova) by ranks followed by *post-hoc* analysis (Tukey test with a Bonferroni adjustment for multiple testing). Statistical significance was set at $p < 0.05$

Results

Observations regarding examination time, number of supplemental boli, vital breaths and arytenoid motions for the respective time periods are detailed in Table 1. Examination time was longer for Alf-chem and -mech compared to both Thio- and Prop-chem. The examination time was also longer for mechanically stimulated treatments compared to chemically stimulated treatments. No differences were observed among treatments regarding number of vital breaths.

Table 1. The median (range) examination time, number of supplemental boli, vital breath score and arytenoid motions observed for time periods 1 (T1), 2 (T2) and 3 (T3) during oral laryngoscopy in dogs anaesthetized with thiopentone, propofol or alfaxalone. T1, period from start of laryngeal examination until 2 minutes; T2, commenced at the end of T1 (or end of induction period) and spanned 3 minutes or until the examination end point (early recovery) was reached; T3, commenced at the end of T2 and continued until the recovery end point had been reached

Treatment		Alf-chem	Thio-chem	Prop-chem	Alf-mech	Thio-mech	Prop-mech
Examination time (minutes)		8.9 (7.7–10.0)*,†	3.8 (3.2–4.2)‡,§	4.0 (3.6–4.8)‡,§	10.9 (9.8–11.6)*,†,¶	12.2 (6.0–18.5)	5.0 (4.1–6.8)§
Supplemental boli (*n*)		3 (3–3)**	4 (3–5)	4 (3–5)	3 (3–4)	4 (4–4)‡	4 (3–4)
Vital breaths (*n*)	T1	0 (0–1)	1 (0–2)	1 (0–2)	0 (0–0)	1 (0–2)	1 (0–1)
	T2	16 (1–39)	3 (0–17)	8 (2–39)	0 (0–1)	3 (2–3)	2 (1–8)
	T3	2 (1–6)	0 (0–0)	2 (2–2)	2 (1–5)	7 (6–8)	1 (1–6)
Arytenoid motions (*n*)	T1	0 (0–0)	1 (0–2)†	2 (1–5)††	0 (0–0)	1 (0–2)	1 (0–2)
	T2	36 (26–53)	67 (35–168)§,¶,**,‡‡	59 (50–148)§,¶,**,‡‡	1 (0–2)*,†	2 (1–3)*,†	2 (1–8)*,†
	T3	46 (6–114)	170 (170–170)	3 (3–3)	2 (1–25)	11 (7–59)	1 (1–1)

*Significant statistical difference ($p<0.05$) compared to Thio-chem. †Significant statistical difference ($p<0.05$) compared to Prop-chem. ‡Significant statistical difference ($p<0.05$) compared to Alf-chem. §Significant statistical difference ($p<0.05$) compared to Alf-mech. ¶Significant statistical difference ($p<0.05$) compared to Prop-mech. **Significant statistical difference ($p<0.05$) compared to Thio-mech. ††Statistically significant difference ($p<0.05$) compared to time period 2. ‡‡Statistically significant difference ($p<0.05$) compared to time period 1.

Thio- and Prop-chem treatments demonstrated higher numbers of arytenoid motions compared to mechanical stimulation for all induction agents. Arytenoid motions were higher for time period 2 compared to time period 1 for Thio- ($p = 0.006$) and Prop-chem ($p = 0.003$). No differences in arytenoid motions were observed for Alf-chem compared to other treatments between time periods.

Observations regarding laryngeal exposure and function scores for the respective time periods are detailed in Table 2. Fig. 1 illustrates the distribution of the laryngeal exposure scores for the respective time periods. The laryngeal evaluation swallowing score was higher during time period 2 compared to time period 1 for Thio-chem ($p = 0.019$). No other

differences regarding laryngeal evaluation scores (jaw tone, swallowing, laryngeal spasm and breath scores) were observed among treatments (1–6) or time periods within a treatment.

Table 2. Laryngeal examination variables and laryngeal function score [median (range)] observed for time periods 1 (T1), 2 (T2) and 3 (T3) during oral laryngoscopy in dogs anaesthetized with thiopentone, propofol and alfaxalone. T1, period from start of laryngeal examination until 2 minutes; T2, commenced at the end of T1 (or end of induction period) and spanned 3 minutes or until the examination end point (early recovery) was reached; T3, commenced at the end of T2 and continued until the recovery-end point had been reached

Treatment		Alf-chem	Thio-chem	Prop-chem	Alf-mech	Thio-mech	Prop-mech
Laryngeal evaluation jaw tone score*	T1	0 (0–1)	1 (1–1)	1 (0–1)	1 (0–1)	0 (0–1)	1 (0–1)
	T2	1 (1–1)	2 (2–2)	2 (1–2)	1 (0–2)	1 (1–1)	2 (1 –2)
	T3	1 (1–2)	2 (2–2)	1 (1–2)	1 (1–2)	1 (1–1)	1 (1–1)
Laryngeal evaluation swallowing score*	T1	0 (0–0)	0 (0–0)†	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–1)
	T2	0 (0–0)	1 (1–1)‡	0 (0–1)	0 (0–0)	0 (0–0)	1 (1–1)
	T3	0 (0–1)	1 (1–1)	1 (1–1)	0 (0–1)	0 (0–1)	1 (1–1)
Laryngeal function score*	T1	7 (7–7)†,§	7 (7–7)†	7 (7–7)†	7 (7–7)§	7 (7–7)§	7 (7–7)
	T2	3 (2–5)‡	2 (2–3)‡	3 (2–3)‡	6 (2–7)	5 (2–6)	4 (3–5)
	T3	4 (3–5)‡	3 (3–3)	3 (3–3)	5 (3–5)‡	3 (2–3)‡	5 (4–6)

*The laryngeal evaluation jaw tone (range 0–3), swallowing (range 0–1) and laryngeal function scores (range 0–7) are defined in Appendices A and B respectively. †Statistically significant difference (p<0.05) compared to time period 2. ‡Statistically significant difference (p<0.05) compared to time period 1. §Statistically significant difference (p<0.05) compared to time period 3.

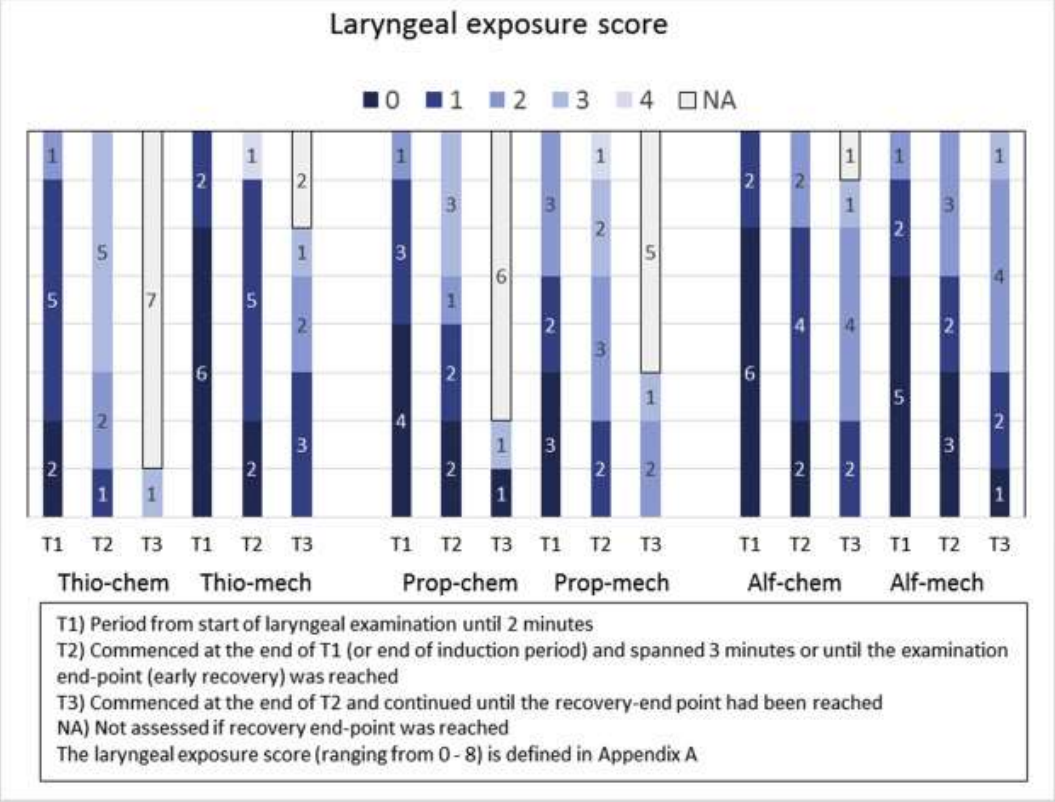

Figure 1. Frequency table summarizing the laryngeal exposure scores for chemical and mechanical stimulation during time periods 1 (T1), 2 (T2) and 3 (T3). The figure indicates the number of dogs assigned a specific exposure score for the six treatments administered.

Laryngeal function score was higher during time period 1 compared to time period 2 for all chemical stimulated treatments. Paradoxical motions, which could be attributed to the high dose of doxapram, were deemed absent (score 0) for all treatments, with no differences observed among treatments or time periods. All dogs recovered well, without any side effects such as muscle rigidity, seizures, excitation or mortalities observed.

The median weight and age were 15.2 (12.2–19.8) kg and 14 (13–16) months, respectively, at commencement of the study.

Discussion

The chemical stimulant, doxapram hydrochloride, was beneficial in stimulating intrinsic laryngeal motion compared to the form of mechanical stimulation used in the present study. The difference in the impact of mechanical or chemical stimulation as adjuncts to anaesthetic induction drugs during laryngeal function evaluation is highlighted by the higher number of arytenoid motions observed during Thio- and Prop-chem compared to Alf-, Thio- and Prop-mech during time period 2 of the present study. The study by Smalle et al (2017), which did not include chemical or mechanical stimulation as adjunct to anaesthetic induction drugs, did not report any difference in arytenoid motion between alfaxalone, thiopentone or propofol.

The median examination times for all three induction drugs were shorter when combined with chemical stimulation compared to mechanical stimulation. Doxapram hydrochloride is a central nervous system stimulant, which transiently increases respiratory rate and tidal volume, by increasing electrical activity in the inspiratory and expiratory centres of the medulla (Arrioja 2001). The central nervous system stimulation caused by doxapram hastens recovery from general anaesthesia (Evers et al. 1965). Subjectively, the depth of the inspiratory phase appeared greater after doxapram hydrochloride administration compared to observations during mechanical stimulation. This could imply that the number of vital breaths is not influenced by either chemical or mechanical stimulation. However, in light of the shortened median examination time for chemical stimulation compared to mechanical stimulation, shorter durations between individual vital breaths were observed. Therefore, a greater inspiratory effort would be expected for chemical stimulation. This is in agreement with the study by Miller et al. (2002), which concluded that doxapram not only increased the size of the RG but may also aid in uncovering more subtle changes in laryngeal function because of increased inspiratory effort. This renders chemical stimulation with doxapram more beneficial over mechanical stimulation as an aid to arytenoid motion evaluation during the 2–5 minute period (time period 2 of the present study) after induction for both thiopentone and propofol induction agents. Doxapram was administered at a dosage of 2.5 mg kg^{-1} IV, which is higher than those reported in previous studies that used 1.1 and 2.2 mg kg^{-1} IV (Miller et al., 2002, Tobias et al., 2004).

We recommend laryngeal function evaluation during time period 2 (2–5 minute period after induction) over time period 1 (period from start of laryngeal examination until 2 minutes) when using any of the anaesthetic induction agents investigated herein. The time period of 2–5 minutes after anaesthetic induction was identified as the optimal time period for laryngeal evaluation because of the higher number of arytenoid motions, vital breaths and desirable arytenoid function scores observed within some treatments. The number of arytenoid motions was statistically higher during this time compared to the earlier time period soon after induction for Thio- and Prop-chem. The laryngeal function score was significantly higher during early end-induction time, and was characterized by complete immobility of arytenoid

cartilage and vocal folds for all induction agents combined with chemical stimulation compared to the time period of 2–5 minutes after anaesthetic induction. Laryngeal function scores after chemical stimulation in time period of 2–5 minutes after anaesthetic induction were synchronous with symmetrical movement of the arytenoid cartilages and full abduction not being achieved with every inspiratory effort.

The median number of vital breaths was higher during time period of 2–5 minutes after anaesthetic induction compared to early end-induction time period and the recovery period (time period 3 of the present study) under chemical stimulation for all induction agents. We therefore recommend that laryngeal function evaluation be performed during time period of 2–5 minutes after anaesthetic induction when using any of the anaesthetic induction agents investigated herein. The aforementioned is highly recommended for propofol compared to alfaxalone to mitigate the increased potential for induction apnoea, as reported by Keates & Whittem (2012).

Previous studies by Jackson et al. (2004) indicated that thiopentone displayed more predictable arytenoid motion during late recovery compared to propofol. Recovery from thiopentone primarily occurs because of redistribution of the drug into well-perfused tissue and fat (Waelbers et al. 2009). The present study suggests that the addition of chemical stimulation allowed more predictable arytenoid motions for thiopentone earlier during the evaluation. The ideal examination time should last sufficiently long enough for laryngeal evaluation to allow the clinician an accurate LP diagnosis, but not too long to avoid keeping a dog anaesthetized unnecessarily.

A significant difference was observed in the examination time among treatments. The median examination time for all induction agents combined with chemical stimulation displayed a decrease compared to mechanical stimulation, with a more marked decrease observed for thiopentone. This suggests that the median examination time of thiopentone was shortened by chemical stimulation compared. Previous studies by Smalle et al. (2017) noted that the long examination time for thiopentone was a disadvantage, but this can be mitigated by the addition of chemical stimulation.

The interaction between doxapram hydrochloride and alfaxalone appeared to be less marked, which could be attributed to the fact that alfaxalone has the least depressive effect on the respiratory system compared to the other induction agents.

Examination times were longer under alfaxalone anaesthesia compared to propofol and are in agreement with the study of Maney et al. (2013), where a single bolus of alfaxalone resulted in a longer recovery time compared to a single bolus of propofol. Previous studies by Ferre et al. (2006) have found that the total body clearance of alfaxalone is high in the dog (55 mL $minute^{-1}$ kg^{-1}). Alfaxalone does not appear to accumulate and has high clearance leading to rapid recovery with most studies equating its recovery times to those of propofol. The higher induction dose used for alfaxalone and the lower dose used for propofol compared to previous studies could contribute to the examination time differences. However, the same clinical end point was achieved rendering the dosages of drugs equipotent. The use of alfaxalone at a lower dosage could probably provide improved clinical outcome. Using a higher dosage of propofol during chemical stimulation may result in improved length of examination time preventing the rapid awakening seen in the study, but could result in suppressed arytenoid motion. Intrinsic laryngeal function can be altered by anaesthetic drugs, masking subtle changes in laryngeal function, which can convolute the accurate diagnosis of

LP. Therefore, it is paramount to evaluate laryngeal motion under only a light plane of anaesthesia, which should be interpreted in combination with both historical and physical examination observations for an accurate diagnosis.

Orolaryngoscopy in anaesthetized dogs remains the gold standard (Broom et al., 2000, Radlinsky et al., 2004) in LP diagnosis; however, the high likelihood of reduced respiratory efforts associated with anaesthetic drugs may mask the true picture regarding laryngeal motion potentially leading to misdiagnosis of LP. The drug dosages administered in this study were based on the reports of Smalle et al. (2017) and compared favourably to previous studies (Gross et al., 2002, Jackson et al., 2004) that proposed lower dosages than historically recommended. The rate of administration is a key variable as rapid administration rate may increase the occurrence of apnoea, especially with the use of propofol as induction agent. In the present study, the total drug dosages and administration rates were observed to be adequate for accurate evaluation of laryngeal motion and diagnosis of LP and could be used in clinical practice to achieve repeatable outcomes.

The small sample size ($n = 8$) could be a potential limitation of the present study; however, the limitation was remedied by implementation of a randomized crossover trial. The number of dogs used in the present study compares favourably to numbers ranging between 6 and 8 used in similar previous studies (Gross et al., 2002, Smalle et al., 2017). Only healthy, non-premeditated dogs were used for the study, which may be a shortcoming and warrants a need for further investigation on dogs confirmed to have LP.

Doxapram hydrochloride has been reported to cause various side effects including central nervous system excitement and paddling, and is contra-indicated in dogs with hypertension or increased intracranial pressure. None of these effects (excitement or paddling) were observed during the present study. Hastened awakening was observed in some dogs, notably during use of propofol. Another limitation of the study was that the mechanical stimulation applied was limited to the application of pressure to the right corniculate process of the arytenoid cartilage, whereas many other forms of mechanical stimulation (by air or water compression of the caudal laryngeal nerves motor innovation) may be considered for future research. However, we did not observe an increase in arytenoid motions with repeat mechanical stimulation. A recent study by Radkey (2017) compared the effect of acepromazine and butorphanol on the quality of laryngeal examination. Radkey (2017) concluded that doxapram hydrochloride would overcome any negative impact from this neuroleptanalgesic combination, further supporting the importance of chemical stimulation during laryngeal evaluation. Further studies on arytenoid function assessment using similar experimental design and induction dosages to the present study, but in premedicated dogs are warranted to investigate whether that would improve the diagnosis of LP over the existing protocols.

Conclusion

The use of doxapram hydrochloride (2.5 mg kg^{-1} IV) as a chemical stimulant was more effective in stimulating arytenoid motion compared to the tactile mechanical stimulation used in the present study. Furthermore, administration of doxapram shortened the examination time of all induction agents. The ideal time to evaluate laryngeal function is 2–5 minutes after induction of anaesthesia. The use of either thiopentone or propofol in combination with doxapram was associated with increased respiratory efforts, ample arytenoid motions and adequate arytenoid exposure conducive to laryngeal function evaluation in healthy non-premedicated Beagle dogs; and could possibly improve accuracy in diagnosis of LP.

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Authors' contributions

SL: design, data collection, data management, data interpretation, and preparation of manuscript; BD: design, data collection, statistical analysis and preparation of manuscript; GZ: design, data collection and preparation of manuscript.

Conflict of interest statement

The authors declare no conflict of interest.

Appendix A. Composite scoring system used to subjectively evaluate the ability to see the rima glottidis during oral laryngoscopy in dogs anaesthetized with thiopentone, propofol and alfaxalone followed by chemical or mechanical stimulation of the larynx

Laryngeal exposure score (0 best score; 8 worst score)

Breathing score

Score	Definition
0	Deep respirations, normal respiratory rate, strong attempt
1	Moderate respirations, respiratory rate and attempt
2	Shallow respiration, slow respiratory rate, weak attempt
3	No spontaneous respiration

Jaw tone score

Score	Definition
0	No jaw tone, easy to open
1	Slight jaw tone, easy to open
2	Moderate jaw tone, some difficulty opening
3	Excessive jaw tone, difficult to open

Swallowing

Score	Definition
0	Absent
1	Present

Laryngospasm

Score	Definition
0	Absent
1	Present

Note. From "Effects of thiopentone, propofol and alfaxalone on laryngeal motion during oral laryngoscopy in healthy dogs," by Smalle et al., 2017, *Vet Anaesth Analg*, *44*, p. 427–434. Copyright 2017, Elsevier Ltd. Reprinted with permission.

Appendix B. Composite scoring system used to subjectively evaluate the quality of arytenoid abduction activity during oral laryngoscopy in dogs anaesthetized with thiopentone, propofol and alfaxalone followed by chemical or mechanical stimulation of the larynx

Laryngeal function score (0 best score; 7 worst score)		
Category score	**Description**	**Subcategory**
0–1	All arytenoid cartilage movements are synchronous, staccato and symmetrical and full arytenoid cartilage abduction can be achieved and maintained.	0: Arytenoid abduction activity is noted with every inspiratory effort. 1: Arytenoid abduction activity is not noted with every inspiratory effort.
2–3	All arytenoid cartilage movements are synchronous and symmetrical. Full abduction of the arytenoid cartilages is not achieved.	2: Moderate abduction of arytenoid cartilages (15–30° to the midline of the rima glottidis). 3: Sluggish abduction of arytenoid cartilages (<15° to the midline of the rima glottidis).
4–5	Arytenoid cartilage movements are asynchronous and/or larynx is asymmetrical at times but full arytenoid cartilage abduction can be achieved and maintained	4: Transient asynchrony, flutter or delayed movements are observed. 5: Transient asymmetry of the rima glottidis, but there are occasions, typically after swallowing or strong, deep respiratory efforts, when full symmetrical abduction is achieved and maintained.
6–7	Complete immobility of the arytenoid cartilages and vocal fold.	6: Unilateral i) Right ii) Left 7: Bilateral

Note. From "Effects of thiopentone, propofol and alfaxalone on laryngeal motion during oral laryngoscopy in healthy dogs," by Smalle et al., 2017, *Vet Anaesth Analg*, *44*, p. 427–434. Copyright 2017, Elsevier Ltd. Reprinted with permission.

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