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# Effects of chemical and mechanical stimulation on laryngeal motion during anaesthetic induction with alfaxalone, thiopentone or propofol in healthy dogs

By

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Submitted in fulfilment of the requirements for the degree
Masters of Science (Veterinary Science (MSc) in the Department of Companion Animal Clinical Studies in the Faculty of Veterinary Science, University of Pretoria

Supervisor: Prof TB Dzikiti
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## Declaration

I, Sandra Labuscagne, hereby declare that the research presented in this dissertation, was conceived and executed by myself, under guidance from my supervisors.

Neither the substance, nor any part of the dissertation has been submitted in the past, or is to be submitted for a degree at the University of Pretoria or any other University.

This dissertation is presented for partial fulfilment of the requirements for degree Master of Veterinary Medicine.

Signature:

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Sandra Labuscagne

Date:

## Contents

## List of Tables

## List of Figures

## List of Abbreviations

| | |
|---|---|
| mg | Milligram |
| mL | Millilitre |
| L | Litre |
| L $L^{-1}$ | Litre per litre |
| L $min^{-1}$ | Litres per minute |
| kg | Kilogram |
| g $L^{-1}$ | Gram per litre |
| µmol $L^{-1}$ | Micromoles per litre |
| mg $kg^{-1}$ | Milligram per kilogram |
| mg $kg^{-1}$ $minute^{-1}$ | Dose rate in milligram per kilogram per minute |
| % | Percentage |
| < | Smaller than |
| > | Greater than |
| = | Equal to |
| °C | Temperature in degrees Celsius |
| CNS | Central nervous system |
| GABA | Gamma aminobutyric acid |
| HCl | Hydrochloride |
| IM | Intramuscular |
| IV | Intravenous |
| LP | Laryngeal paralysis |
| NGGA | Normalised glottal gap area |
| PCV | Packed cell volume reported as volume L per L |
| RG | *Rima Glottidis* |
| RSA | Republic of South Africa |
| TSP | Total serum protein |
| UPBRC | University of Pretoria Biomedical Research Centre |
| ANOVA | Analysis of variance |

## Summary

# Effects of chemical and mechanical stimulation on laryngeal motion during anaesthetic induction with alfaxalone, thiopentone or propofol in healthy dogs

By

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Promoter: Prof TB Dzikiti

Co-promoter: Prof GE Zeiler

Department: Department of Companion Animal Clinical Studies

Degree: Masters of Science (Veterinary Science (MSc)

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

**Study design:** Blinded, randomised, crossover study

**Animals:** Eight adult beagle dogs with median (range) weight and age 15.2 (12.2-19.8) kg and 14 (13-16) months, respectively.

**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 hydrochloride 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 one-week wash-out period between treatments. If deemed inadequately anaesthetised, supplemental boli (25% of induction bolus) of thiopentone (1.8 mg $kg^{-1}$), propofol (0.75 mg $kg^{-1}$) or alfaxalone (0.4 mg $kg^{-1}$) were administered. The calculated induction bolus for each dog was administered over a 60 second period intravenously via a syringe driver and allowed to take effect for 10 seconds. The anaesthetic depth was determined by evaluating jaw tone and palpebral reflexes. Laryngeal examination was performed by the primary investigator (SL) who was blinded to the treatments. Assessment of number of arytenoid motions and vital breaths, among others, began immediately after induction. Chemical and mechanical stimulation were begun 2 minutes after anaesthetic induction (time period 1). Data were collected at 2, 3 and 5 minutes (time period 2) after anaesthetic induction and the Friedman rank sum or repeated measures ANOVA (Analysis of variance) tests were used, when applicable, for statistical analysis.

**Results:** Duration of examination times were significantly different among treatments ($p$ = 0.01). Significant differences were observed regarding the number of arytenoid motions during thiopentone induction combined with chemical stimulation (doxapram hydrochloride) in comparison to alfaxalone ($p$ = 0.0086), thiopentone ($p$ = 0.0108) and propofol ($p$ = 0.0086), when combined with mechanical stimulation at 3 minutes after induction. The laryngeal function score was significantly higher during time period 1 compared to time period 2 for induction with alfaxalone ($p$ = 0.0007), thiopentone ($p$ < 0.0001), and propofol ($p$ = 0.0013) combined with chemical stimulation. No significant differences were observed among treatments or time periods for number of vital breaths recorded during the 3 different time periods, jaw tone, laryngospasm, breath scores, swallowing score or paradoxical motion score.

**Conclusion and clinical relevance:** Doxapram hydrochloride, combined with thiopentone, is the most effective means of stimulating arytenoid motion among the regimens for assessing laryngeal motion in the present study and could improve accuracy of diagnosis of laryngeal

paralysis in dogs. Time period 2 (2-5 minute after conclusion of induction) is the optimal time period for laryngeal evaluation. Misdiagnosis of laryngeal paralysis can be avoided by identifying the ideal time period for evaluation. Induction with thiopentone combined with doxapram hydrochloride facilitated increased respiratory efforts, ample arytenoid motions, and adequate arytenoid exposure conducive to laryngeal function evaluation in healthy non-premedicated beagle dogs. The dosages and administration rates used in the present study were effective in achieving adequate depth of anaesthesia for laryngeal function evaluation, without inducing respiratory depression, confirming viable application for diagnosis of laryngeal paralysis.

***Keywords:*** alfaxalone, chemical stimulation, laryngeal paralysis, laryngoscopy, mechanical stimulation, propofol, thiopentone.

## Chapter 1: Literature review

Diagnosis of laryngeal paralysis (LP) is usually done under anaesthesia and, therefore poses a challenge regarding the choice of anaesthetic drugs that will minimally influence laryngeal function.

LP can be defined as the inability of either one or both arytenoid cartilages to abduct during inspiration due to dysfunction of the recurrent laryngeal nerve or *crico-arytenoid dorsalis* muscle (Tobias et al. 2004; Millard and Tobias 2009; Kitshoff et al. 2013). Clinical signs of LP include coughing, exercise intolerance, weak hoarse bark (Parnell 2010) and respiratory distress, which is exacerbated during excitement or exercise (Monnet and Tobias 2012). A typical inspiratory stridor may result due to failure of the arytenoid cartilages to abduct during inspiration, causing increased resistance to air flow and turbulence through the *rima glottidis* (RG). The severity of the clinical signs can be correlated to the extent of LP. The aetiology of LP can be congenital or acquired. Certain breeds such as the Bouvier des Flandres, Siberian huskies, Bull terriers and white-coated German shepherd dogs are predisposed to the congenital form that is due to an autosomal dominant trait that usually presents clinically before 12 months of age (Monnet and Tobias 2012). Congenital LP polyneuropathy has also been reported in large-breed dogs (Braund et al. 1994; Mahony et al. 1998; Gabriel et al. 2006). Laryngeal paralysis is the most common acquired disease process involving the larynx of middle to old-age large-breed dogs (MacPhail 2014). Aetiologies for acquired LP include trauma (bite wounds, surgical trauma, and mediastinal tumours) to the recurrent laryngeal nerve or vagus nerve (Monnet and Tobias 2012; Kitshoff et al. 2013). Neuropathies, brain stem diseases and hypothyroidism have also been reported as common aetiologies (Monnet and Tobias 2012; Kitshoff et al. 2013). Breeds such as the Labrador retriever, Rottweiler and Irish setter are predisposed to geriatric onset LP polyneuropathy (GOLPP) (Monnet and Tobias 2012). In

contrast to the congenital form, acquired idiopathic LP is more common among middle-aged dogs (Millard and Tobias 2009; Kitshoff et al. 2013; MacPhail 2014;).

Laryngeal paralysis is usually diagnosed under a light plane of general anaesthesia (Gross et al. 2002; Jackson et al. 2004) by direct visualisation of the arytenoid cartilages. Orolaryngoscopy in conjunction with historical and physical examination improves sensitivity of LP diagnosis (Kitshoff et al. 2013). This light plane anaesthesia can be achieved using intravenous anaesthetic induction drugs such as thiopentone, propofol or alfaxalone (Smalle et al. 2017). Laryngeal examination by oral laryngoscopy is the gold standard in definitive diagnosis of LP. Alternative diagnostic techniques include transoral video-endoscopic laryngoscopy, transnasal video-endoscopic laryngoscopy, echolaryngography or computed tomography (CT); but these methods have yet to be proven superior (Radlinsky et al. 2004; Stadler et al. 2011; MacPhail 2014). Laryngoscopy allows visualisation of the *rima glottidis* but can impair laryngeal function due to retraction of the tongue and compression of the epiglottis. Laryngeal function can be influenced by the anaesthetic induction drugs causing a loss of laryngeal reflex (MacPhail 2014). If the plane of anaesthesia is too deep, the arytenoid cartilages may remain in the para-median or adducted position leading to a false positive diagnosis of LP. An ideal anaesthetic drug for aiding diagnosis of LP should cause relaxation of the jaw muscles adequate to maximise visualisation without hindering the normal function of the *crico-arytenoideus dorsalis* muscle. The ideal anaesthetic drug should maintain ample regular inspiratory efforts and circumvent induction apnoea (McKeirnan et al. 2014). Depression of intrinsic laryngeal motion due to anaesthetic drugs can lead to an inaccurate diagnosis of LP. Anaesthetic drugs can alter the size of the RG (Jackson et al. 2004). There is a paucity of information in literature regarding the suitability of most available anaesthetic drugs in aiding the clinical assessment of intrinsic laryngeal function. Drugs that can be

considered for induction of anaesthesia during diagnosis of LP include alfaxalone, propofol and thiopentone.

Thiopentone (5-ethyl-5-pentan-2-yl-2-sulfanylidene-1,3-diazinane-4,6-dione) is an ultra-short-acting intravenous thio-barbiturate that has historically been the drug of choice for aiding diagnosis of LP by laryngoscopy in dogs. The ultra-short action of thiopentone can be attributed to its redistribution into well-perfused tissues initially and into body fat later on (Brodie et al. 1952; Waelbers et al. 2009). Thiopentone binds to ligand-gated ion channels, of which the $GABA_A$ receptor channel is one of several representatives (Weber et al. 2005). It is recommended to use thiopentone without premedication when assessing laryngeal function, as this preserves arytenoid motion (Jackson et al. 2004). A disadvantage of thiopentone is that it can cause arrhythmias, cardio and respiratory depression (Dennis et al. 2007). Thiopentone results in less suppression of arytenoid motion when compared to propofol, thus allowing more accurate assessment of laryngeal function in dogs (Gross et al. 2002; Jackson et al. 2004; McKiernan et al. 2014).

Propofol (2,6-diisopropylphenol) is a highly lipid soluble alkylphenol derivative that provides rapid and smooth induction of anaesthesia and causes loss of consciousness within 20-40 seconds of administration (Amengual et al. 2013). Adverse effects of propofol include respiratory depression (that may present as hypoventilation or apnoea) and hypotension (Smith et al. 1993; Jackson et al. 2004). Propofol has been associated with weaker arytenoid motion when compared to thiopentone (Gross et al. 2002; Jackson et al. 2004; McKiernan et al. 2014).

Alfaxalone ((3R,5S,8S,9S,10S,13S,14S,17S)-17-acetyl-3-hydroxy-10,13-dimethyl-1,2,3,4,5,6,7,8,9,12,14,15,16,17-tetradecahydrocyclopenta[a]phenanthren-11-one) is a synthetic neuroactive steroid anaesthetic drug known for its wide safety margin (Keates and Whittem 2012). Dose dependent cardiovascular and respiratory effects have been reported,

with supraclinical doses causing increased heart rate, hypotension and hypoventilation (Muir et al. 2008). The mode of action for anaesthesia relies on its ability to interact with the gamma aminobutyric acid (GABA) receptors (Gilron and Coderre 1996; Waelbers et al. 2009). However, limited information is available on its suitability for assessment of laryngeal function; necessitating a need for investigation. Recent studies have indicated that Alfaxalone maintained laryngeal motion similarly to propofol in nonbrachycephalic and brachycephalic dogs (Norgate et al. 2018).

A recent study comparing the effect of thiopentone, propofol and alfaxalone on arytenoid cartilage motion reported no significant difference among these drugs (Smalle et al. 2017). Propofol allowed adequate visualisation of the RG and faster recovery time and was therefore considered by the authors the most suitable drug for aiding laryngeal examination under general anaesthesia (Smalle et al. 2017). The inconsistency in doses and rates of administration of anaesthetic drugs during laryngeal examination in current literature poses a challenge for fair comparison of the effects of these drugs on arytenoid cartilage function. Doses that allow an adequate plane of anaesthesia for laryngeal examination, with minimal adverse effect on laryngeal motion have been recently determined (Smalle et al. 2017) and can aid in development of a standardised anaesthetic regimen for diagnosis of LP.

There is a paucity of information on complementary diagnostic aids that can be utilised to improve accuracy in LP diagnosis. The employment of either chemical or mechanical stimulation to enhance laryngeal reflex responses has been proposed (Miller et al. 2002; Tobias et al. 2004). A chemical stimulant, doxapram hydrochloride, has been used in clinical practice for its respiratory stimulation properties. Doxapram hydrochloride (1-ethyl-4- (2-morpholin-4-ylethyl)- 3,3-diphenyl-pyrrolidin-2-one;hydrochloride) predominantly causes increased respiratory rate and increased intrinsic laryngeal motion. Doxapram hydrochloride stimulates the medullary respiratory centre as well as the carotid body chemoreceptors (increased

sensitivity to blood $pCO_2$) increasing respiratory drive (Evers et al. 1965; Arrioja 2001; Miller et al. 2002). A study performed on 30 dogs reported that doxapram hydrochloride significantly increases respiratory effort and causes an increase in abduction of arytenoid cartilages and laryngeal motion (Miller et al. 2002). A 36% increase in size of RG was observed; confirming the improved abduction ability of the arytenoid cartilages (Miller et al. 2002). A shortcoming of the study by Miller et al. (2002) was that the adverse effects of doxapram hydrochloride were not reported. These potential adverse effects include central nervous system (CNS) excitement, hastened awakening from anaesthesia, tachycardia, hypertension, seizures and paddling (Arrioja 2001). An additional concern is that the study was based on measurements obtained from video-endoscopic images of the RG. These measurements may be inaccurate due to differences in distance from source. The study did not document any observations on the effect of doxapram hydrochloride in dogs that had confirmed LP. A study by Tobias et al. (2004) that evaluated the effects of doxapram hydrochloride in dogs, with normal laryngeal function *versus* dogs with LP, reported that the size of the RG did not increase significantly in normal dogs and that the dogs only displayed greater breathing effort (increased tidal volume) and depth in contrast with the study by Miller et al. (2002). The study by Tobias et al. (2004) reported that the size of the RG, also defined as normalised glottal gap area (NGGA), significantly increased in dogs with confirmed LP.

Miller et al. (2002) used propofol at a dose of 4 mg $kg^{-1}$ for induction and 2 mg $kg^{-1}$ as intermittent supplemental boli for maintenance of general anaesthesia, while Tobias et al. (2004) utilised isoflurane (3-5%) for induction and evaluation of laryngeal function. The differences in observations from these past studies may be attributed to the effects of concurrently-administered drugs. Both of these studies made use of acepromazine and butorphanol, which could have influenced arytenoid motion. After doxapram hydrochloride administration, all dogs with confirmed LP displayed paradoxical motion of the arytenoids

(Tobias et al. 2004). Although an optimal dose of doxapram hydrochloride for enhancement of LP diagnosis has not been established, previous studies cited an intravenous (IV) dose range of 1.1-2.2 mg $kg^{-1}$ IV (Miller et al. 2002; Tobias et al. 2004).

The efficacy of mechanical stimulation as an adjunctive tool in improving assessment of laryngeal function in anaesthetised dogs has not been reported. A study by Poliacek et al. (2008) in cats concluded that mechanical stimulation of the larynx and tracheal mucosa caused increased motion of the laryngeal abductor, posterior *crico-arytenoid* muscle, and laryngeal adductor, *thyroarytenoid*. Anderson et al. (1990) investigated the response of laryngeal receptors to water, pressure, temperature and laryngeal motion in dogs during the breathing cycle and concluded that the receptor fibres in the larynx are not solely sensitive to water but to multiple stimuli. The sensitivity to chemical and mechanical stimulation of the laryngeal and tracheobronchial area, in attempt to cause tussigenic activity, were compared in 22 dogs (Tatar et al. 1994). Myelinated afferent vagal nerve fibres play an important role in mediating mechanically-induced laryngeal cough and expiratory reflex. Mineck et al. (2000) characterised the three-dimensional anatomy of the abductor muscle of the larynx and confirmed that receptors sensitive to mechanical stimulation were present. The need for additional research is evident.

Anaesthetic drugs commonly used for laryngeal examination have been reported to alter the intrinsic laryngeal function. Accurate diagnosis of LP under a light plane of anaesthesia in dogs in order to identify subtle changes in laryngeal function that could be masked by the anaesthetic agents presents a challenge. The lack of spontaneous respiration presents a challenge in the clinical diagnosis of LP. Either direct mechanical stimulation of the arytenoids or administration of a respiratory stimulant can be proposed as a corrective measure.

## Chapter 2: Introduction and study justification

Clinical diagnosis of 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 the breathing cycle. Historical evidence and physical examination findings in conjunction with observations from oral laryngoscopy provide for a higher sensitivity for LP diagnosis (Kitshoff et al. 2013). Laryngeal function can be influenced by the anaesthetic induction drugs causing a loss of laryngeal reflex (MacPhail 2014). Hence the characteristics of an ideal anaesthetic drug include: minimal effect on the laryngeal function, adequate jaw muscle relaxation and circumvention of induction apnoea while enabling ample regular inspiratory efforts. The desired light plane anaesthesia for oral laryngoscopy can be achieved using intravenous anaesthetic induction drugs such as thiopentone, propofol or alfaxalone (Smalle et al. 2017). The effects of the anaesthetic induction drugs are well documented, but there is a lack of consistency in descriptions of ideal dosages for assessment of laryngeal function. There is a paucity of research describing complementary diagnostic aids that may be utilised to improve accuracy in diagnosis of LP. The addition of either chemical or mechanical stimulation to anaesthetic regimens has been proposed. Tobias et al. (2004) investigated the effect of doxapram hydrochloride, as chemical stimulant, on laryngeal function of normal and LP affected dogs. The study utilised neuroleptanalgesia premedication drugs (acepromazine and butorphanol), which may have influenced normal functioning of the *crico-arytenoideus dorsalis* muscle. A study by Miller et al. (2002) examined doxapram hydrochloride's effect in healthy dogs, but also utilised premedication with glycopyrrolate and acepromazine maleate. Thus, making for complicated comparison of the findings between the studies, which creates a need for investigations without the use of anaesthetic premedicants such as neuroleptanalgesic drugs. There is a scarcity of research relating to mechanical stimulation as an additional diagnostic aid in dogs. Stimulation of the larynx and the tracheal mucosa have been proposed

to increase motion of the laryngeal abductor (Poliacek et al. 2008). Mechanical stimulation, including pressure, water, air and temperature on the laryngeal receptors could be considered for future investigations.

## Aims and objectives

The present study compared the effects of chemical and mechanical stimulation on arytenoid cartilage motion in healthy dogs during anaesthetic induction with three drugs (alfaxalone, thiopentone and propofol) at recommended doses for diagnosis of LP in dogs (Smalle et al. 2017). The aim was to determine the anaesthetic drug that had the fewest adverse effects on laryngeal motion, by testing the current recommended dosages, to establish a standardised anaesthetic regimen for diagnosing LP and thereby to minimise factors that may contribute to an inaccurate diagnosis.

## Hypotheses

The primary hypothesis:

$H_0$: There is no difference in response to mechanical stimulation of the larynx among induction drugs: alfaxalone, thiopentone and propofol.

$H_1$: There is a statistical significant difference in response to mechanical stimulation of the larynx among induction drugs: alfaxalone, thiopentone and propofol.

The secondary hypothesis:

$H_0$: There is no difference in response to chemical stimulation of the larynx among induction drugs: alfaxalone, thiopentone and propofol.

$H_1$: There is a statistical significant difference in response to chemical stimulation of the larynx among induction drugs: alfaxalone, thiopentone and propofol.

## Benefits arising from the study

The primary benefit is provision of information that contributes to improving the accuracy of the diagnosis of LP by oral laryngeal examination. Information obtained in this study aided in formulation of a standardised anaesthetic regimen that could minimise errors in diagnosis of LP. Chemical stimulation may aid as a valuable adjunctive diagnostic tool in laryngeal disease and eventually be part of an objective method for use in future comparative research studies.

# Chapter 3: Materials and methods

## Experimental animals

The study was approved by the Animal Ethics Committee of the University of Pretoria (Project number: V106-16). Data collection did not commence until approval by the University of Pretoria's research and animal ethics committee had been granted. Eight healthy castrated male beagle dogs housed and cared for by the University Of Pretoria Biomedical Research Centre (UPBRC) were randomly selected and enrolled into the study. The dogs' median (range) weight and age were 15.2 (12.2-19.8) kg and 14 (13-16) months, respectively. 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 were housed in kennels that were well maintained and cleaned regularly by the UPBRC staff. The dogs were fed twice daily with a diet comprising a premier quality dog food and unrestricted water access outside of the research days. The dogs had access to a communal play area. The dogs were not exposed to any medical treatment or surgical procedures for at least 4 weeks prior to the commencement of the study. The dogs were bred for the purpose of being enrolled in research trials. The duration of the data collection phase of the study was 7 weeks from the 3rd October 2017 – 18th November 2017. All dogs were rehomed successfully after the study. Dogs were selected at random and only enrolled into the study if they met the following criteria:

- clinically healthy with no mechanical or haematological abnormalities
- no history of laryngeal disease
- at least 6 months old
- up to date on recommended vaccinations for the research site

Selected dogs were evaluated under a light plane of general anaesthesia a week prior to commencement of the data collection to ensure that they satisfied the following criteria:

- no signs of pharyngeal or laryngeal pathology
- no evidence of true uni- or bilateral paralysis of the larynx
- no signs of asymmetry of the larynx
- no evidence of paradoxical movement of the arytenoid cartilages

The dogs underwent preanaesthetic physical examinations 24 hours prior to anaesthesia to ensure that they were healthy. The examination consisted of measurement of heart rate, respiratory rate, capillary refill time, mucous membrane colour and rectal temperature. The superficial lymph nodes were palpated for any abnormalities; and habitus was evaluated. After completion of the preanaesthetic physical examination, blood samples were drawn from the jugular vein. The blood was collected into serum and ethylenediaminetetraacetic acid tubes (BD Vacutainer; BD Diagnostics, NJ, USA) for biochemical and haematological analyses, respectively. Haematological and chemical (serum proteins and creatinine) analyses were performed using a haematological (ADVIA 2120 Haematology system; Siemens, ER, Germany) and chemical analyser (Cobas Integra 400 Plus; Roche, BS, Switzerland), respectively. The dogs were enrolled into the study only if the preanaesthetic physical examination, haematological and serum chemistry variables met the following criteria:

- no heart murmur or abnormal lung sounds evident on thoracic auscultation;
- rectal temperature > 37.5°C or < 39.5°C;
- no evidence of blood parasites on blood smear stained with Diff-quick (Kyro-Quick Stain, Kyron Laboratories (Pty) Ltd, South Africa)
- packed cell volume range of 0.35-0.55 L $L^{-1}$;
- total serum protein range of 55-75 g $L^{-1}$;

- serum creatinine of 59-109 µmol $L^{-1}$.

During the study all efforts were made to ensure a good general wellbeing of the dogs. Environmental enrichment was implemented during the study to prevent day-to-day mundane routine and boredom.

## Study design

The study was designed to investigate the effects of chemical and mechanical stimulation on arytenoid abduction activity in healthy dogs that were anaesthetised using three different induction drugs: alfaxalone, thiopentone and propofol. Randomisation of the blinded cross-over experimental trial of treatments (1-6) per dog was done with Winpepi Version 11.20 (see Table 1). The treatment allocations were unknown to the primary investigator (SL) for the duration of the data collection phase of the study. A washout period of 1-week was enforced between treatments, which were as follows:

1. alfaxalone induction with chemical stimulation (Alf-chem);
2. thiopentone induction with chemical stimulation (Thio-chem);
3. propofol induction with chemical stimulation (Prop-chem);
4. alfaxalone induction with mechanical stimulation (Alf-mech);
5. thiopentone induction with mechanical stimulation (Thio-mech);
6. propofol induction with mechanical stimulation (Prop-mech).

**Table 1:** Randomised treatment schedule generated by the program (Winpepi Version 11.20).

| Treatment | Week 1: | Week 2: | Week 3: | Week 4: | Week 5: | Week 6: |
|---|---|---|---|---|---|---|
| Subject 1 | 2 | 1 | 3 | 6 | 5 | 4 |
| Subject 2 | 3 | 6 | 2 | 1 | 5 | 4 |
| Subject 3 | 3 | 5 | 2 | 1 | 4 | 6 |
| Subject 4 | 3 | 2 | 5 | 6 | 1 | 4 |
| Subject 5 | 2 | 6 | 4 | 5 | 3 | 1 |
| Subject 6 | 6 | 1 | 3 | 2 | 5 | 4 |
| Subject 7 | 6 | 2 | 3 | 4 | 5 | 1 |
| Subject 8 | 2 | 5 | 6 | 3 | 4 | 1 |

## Experimental procedure

The experimental procedure is described in four phases: 1) preanaesthetic, 2) anaesthetic induction, 3) laryngeal examination and stimulation and 4) postanaesthetic period.

### Phase 1: Preanaesthetic

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. A resting period of 1 hour was enforced to decrease excitement levels and stress prior to the anaesthetic procedure. 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 a floor scale (JS series; Jadever Weightec Inc. ON, Canada) that was verified and calibrated according to the South African National Standards (SANS) 1649 and the Trade Metrology Act 77 of 1973. Identification of each dog was verified using a microchip Pocket Reader EX™ (Identipet, South Africa) compatible with Identipet microchips.

**Phase 2: Anaesthetic induction**

The anaesthetic induction drugs were drawn up into 20 mL syringes (B. Braun; B. Braun Medical (PTY) Ltd, RSA) and a low volume extension set (Extension set REF 011-C150; Poly Medicure Ltd/ICU medical SA, RSA) was attached to each syringe. Each extension set was covered with black general purpose electrical trunking material to obscure visibility and blind the primary investigator (SL) of the treatment (Fig. 1). Each anaesthetic induction drug was administered from a separate electronic syringe driver (B. Braun Perfusor® Space; B. Braun Medical (PTY) Ltd, RSA). The predetermined volume was set for each patient individually according to bodyweight and administered over the pre-defined induction period. To further blind the primary investigator's view, the syringe drivers were positioned outside the investigators field of view and obscured by a blanket screen.

**Figure 1**: Electronic syringe driver systems used to administer the anaesthetic induction drugs. Note the black general purpose electrical trunking material used to cover each extension set to obscure visibility.

The dogs were positioned on the procedure table 10 minutes prior to induction and a 21 Gauge IV cannula (Jelco: Smiths Medical, UK) was aseptically inserted percutaneously into the lateral saphenous vein. The saphenous vein was preferred over the cephalic vein due to ease of

concealing the induction drug from the primary investigator (SL) who was positioned at the cranial end of the dog ready to assess effects of the induction drugs on laryngeal function. A stopper with an injection port 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:

- alfaxalone (Alfaxan®-CD RTU 1%; Jurox (Pty) Ltd/Kyron laboratories (Pty) Ltd, RSA); induction bolus 1.5 mg $kg^{-1}$; supplemental bolus 0.4 mg $kg^{-1}$.
- thiopentone (Thiopentone Sodium Fresenius 0.5 g. 20 $mL^{-1}$; Fresenius Kabi South Africa (Pty) Ltd, RSA); induction bolus 7 mg $kg^{-1}$; supplemental bolus 1.8 mg $kg^{-1}$.
- propofol (Fresenius Propoven 1% (50mL); Fresenius Kabi South Africa (Pty) Ltd, RSA); induction bolus 3 mg $kg^{-1}$; supplemental bolus 0.75 mg $kg^{-1}$.

The dosages of the induction drugs (induction and supplemental bolus) were as per the study by Smalle et al. (2017). The calculated induction bolus was administered over a 60 second period intravenously using a syringe driver and allowed to take effect for 10 seconds before assessment of anaesthetic depth. The anaesthetic depth was determined by evaluating jaw tone and palpebral reflexes while the syringe driver was set on standby mode. Adequate depth of anaesthesia was defined by 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 lateral palpebral reflex and a regular breathing pattern. If the dog was deemed inadequately anaesthetised, a supplemental bolus was administered over a 10-second long period followed by a further 10-second long 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. To allow for improved visibility of the RG, the

dogs were placed in sternal recumbency and the maxilla was hooked over a padded rectangular frame (Fig. 2). Positioning of the dog was done gently and swiftly to minimise external stimulation of the dog.

**Figure 2**: Positioning of dog for oral laryngoscopy in sternal recumbency with the maxilla hooked over a padded frame.

**Phase 3: Laryngeal examination and stimulation**

Laryngeal examination was performed by the primary investigator. Continual visual examination of the larynx was achieved by using an illuminated laryngoscope (Macintosh size 3, Satin™ Fiber Optic Macintosh Laryngoscope Blades, American Diagnostic Corporation, NY, USA) placed ventral to the epiglottis into the vallecula at the base of the tongue. The laryngoscope blade tip was directed ventrally, to expose the RG and then the laryngeal evaluation, by the primary investigator, commenced. A tongue depressor was used to raise the soft palate as an additional way to improve visualisation of the larynx. Placing excessive pressure on the base of the tongue with the laryngoscope blade and any form of mechanical

stimulation of the arytenoid cartilages could have influenced results and was avoided. The onset of inspiration was verbally communicated so as to assist the primary investigator (SL) in ascertaining whether arytenoid abduction and inspiration were coordinated. Chemical stimulation was performed at a fixed time period; 2 minutes after the induction end-point. The examination end-point was considered reached once chewing or conscious swallowing attempts were observed. The chemical stimulant used, doxapram hydrochloride (Dopram Fresenius® 20mg.mL$^{-1}$; Fresenius Kabi Manufacturing SA (Pty) Ltd, RSA), was administered IV at a dose of 2.5 mg.kg$^{-1}$, over 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, utilising a cotton bud. The primary investigator (SL) made every attempt to apply consistent pressure for each mechanical stimulation event. Examination of the larynx continued until the examination end-point had been reached.

A well-stocked anaesthesia crash (emergency) cart was available during treatments for purposes of appropriate and prompt intervention in the event of an emergency. The contents in the crash cart included endotracheal tubes and emergency drugs including atropine (Atropine 0.5mg mL$^{-1}$, Bayer (Pty) Ltd, RSA ) and adrenaline (Adrenaline 0.1%, Kyron Prescription CC, RSA). Isotonic crystalloid fluids were available. The dogs were continuously monitored for any signs signalling need for emergency intervention. Rescue intervention would have been undertaken if the dog displayed one or more of the following:

1. excitement and paddling that could result in injury of dog during induction;
2. prolonged apnoea; the absence of spontaneous ventilation for longer than 60 seconds;
3. desaturation of haemoglobin; that was defined as cyanosis of mucous membranes;
4. bradycardia; defined as a heart rate of less than 60 beats min$^{-1}$;
5. cardiopulmonary arrest or failure of the heart to contract effectively.

Potential rescue interventions included:

1. achievement of a deeper plane of anaesthesia by administration of 25% of initial anaesthetic induction bolus;
2. endotracheal intubation and mechanical ventilation;
3. oxygen support administered with a flow-by nasogastric feeding tube at 3 L minute$^{-1}$ and/or endotracheal intubation and mechanical ventilation if required;
4. administration of atropine intravenously at 0.04 mg kg$^{-1}$;
5. cerebral cardiopulmonary resuscitation.

**Phase 4: Postanaesthetic period**

Once the laryngeal examination end-point had been reached, the dogs were moved to a warm, dry and padded area; and were allowed to recover under observation. Recovery from anaesthesia was defined as the ability to walk without signs of ataxia, full consciousness of surroundings and ability to maintain normal homeostasis. A postanaesthetic physical examination was performed on each dog and IV cannulas removed. The dogs were returned to their enclosures once they were considered fully recovered from the effects of the general anaesthetic drugs.

### Data Capture

The **induction time** (defined as the time from the start of administration of the induction bolus until the dog was deemed adequately anaesthetised for laryngeal examination to commence) was recorded. The incidence of excitement was recorded for each dog. During the induction period, the number of supplemental boli required were recorded. The total dose (mg kg$^{-1}$) of anaesthetic induction drug administered was calculated retrospectively. The **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 3 recorded time periods:

- **Time period 1:** defined as the period from start of laryngeal examination (or end of induction period) until 2 minutes.
- **Time period 2:** commenced at the end of Time period 1 and spanned 3 minutes or until early examination end-point was reached. During this time period mechanical or chemical stimulation was applied. Time period 3 was not recorded if examination end-point was reached during time period 2.
- **Time period 3:** commenced at the end of Time period 2 and continued until the examination-end point had been reached.

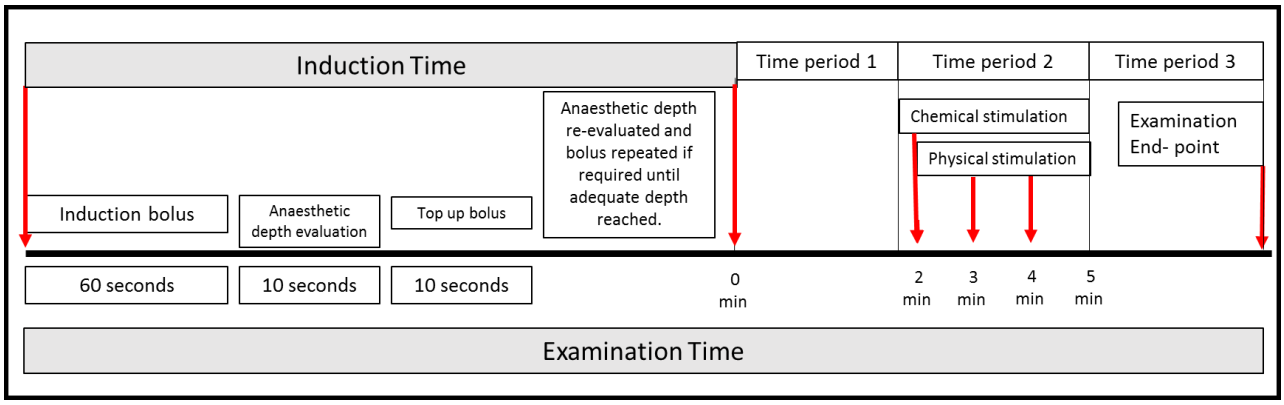


**Figure 3**: Diagrammatic representation of the time scale during oral laryngoscopy in dogs anaesthetised with thiopentone, propofol and alfaxalone followed by chemical or mechanical stimulation of the larynx.

The total number of arytenoid abductions and vital breaths (deep inspiratory efforts) were recorded per time period. It was important to note if vital breaths were synchronised with arytenoid motions. **Vital breaths** were defined as a maximum inspiratory effort with increased abdominal component on inspiration. Two aspects of laryngeal motion were scored subjectively. The first was a laryngeal exposure score consisting of a breathing, jaw tone, swallowing and laryngospasm score, defined in Table 2. This composite scoring system was used to subjectively evaluate the ability to visualise the RG and determine the quality of

arytenoid abduction activity, during oral laryngoscopy in dogs anaesthetised with thiopentone, propofol and alfaxalone. This scoring system was adapted from Smalle et al. (2017) and was used to ensure repeatability and standardisation for comparison among trials.

**Table 2:** Adapted composite scoring system (Smalle et al. 2017) used to subjectively evaluate the ability to visualise the *rima glottidis* during oral laryngoscopy in dogs anaesthetised with alfaxalone, thiopentone and propofol followed by chemical or mechanical stimulation of the larynx.

| **Laryngeal exposure score (0 best score; 8 worst score)** | | |
|---|---|---|
| a) 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 |
| b) 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 |
| c) Swallowing | | |
| | Score | Definition |
| | 0 | Absent |
| | 1 | Present |
| d) Laryngospasm | | |
| | Score | Definition |
| | 0 | Absent |
| | 1 | Present |

The second scoring system evaluated the characteristics and quality of arytenoid abduction by using a laryngeal function scoring system (Smalle et al. 2017), see Table 3. This scoring system is similar to the subjective scoring systems used by Gross et al. (2002) and McKiernan et al.

(2014). This scoring system is focused on grading the abduction activity quality and was originally adapted for dogs by Smalle et al. (2017) from the Havemeyer grading system used in horses (Robinson 2004).

**Table 3:** Adapted composite scoring system (Smalle et al. 2017) used to subjectively evaluate the quality of arytenoid abduction activity during oral laryngoscopy in dogs anaesthetised with alfaxalone, thiopentone and propofol 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<br>i) Right<br>ii) Left |
| | | 7: Bilateral |

Different laryngeal exposure score components were assessed by the anaesthetist and primary investigator. The data detailed in Table 4 were recorded for the three time periods.

**Table 4:** Allocation of laryngeal exposure score components recorded by the primary investigator (SL) and anaesthetist.

| Allocation | Time period 1 | Time period 2 | Time period 3 |
|---|---|---|---|
| Anaesthetist | Breathing score | Breathing score | Breathing score |
| Primary investigator | Jaw tone Score | Jaw tone Score | Jaw tone Score |
| | Swallowing reflex | Swallowing reflex | Swallowing reflex |
| | Laryngospasm | Laryngospasm | Laryngospasm |
| | Laryngeal function Score | Laryngeal function Score | Laryngeal function Score |

Consistent evaluation of arytenoid movement was achieved by defining terminology used to describe arytenoid motion by Smalle et al. (2017), detailed in Table 5.

**Table 5:** Definitions of arytenoid activity used to describe arytenoid movement observed during oral laryngoscopy in dogs anaesthetised with alfaxalone, thiopentone and propofol followed by chemical or mechanical stimulation of the larynx.

| Term | Definition |
|---|---|
| Abduction | Movement of the corniculate process of the arytenoid cartilage away from the midline of the *rima glottidis*. |
| Adduction | Movement of the corniculate process of the arytenoid cartilage toward the midline of the *rima glottidis*. |
| Full abduction | Most of the corniculate process of the arytenoid cartilage lies diagonally from dorsomedial to ventrolateral (>30° to the midline of the *rima glottidis*). |
| Asymmetry | A difference in position of the right and left corniculate processes relative to the midline of the *rima glottidis*. |
| Asynchrony | Movement of the corniculate processes occurs at different times. This can include twitching, shivering and delayed or biphasic movement of one arytenoid. |

The data were captured manually on data sheets during the study and remained the responsibility of the primary investigator. The data capture sheets were filed in duplicate. The

file contained all data pertaining to the study and dogs. All data collected during treatments were manually stored. The captured data were transferred to an electronic copy on an Excel spreadsheet. Data were saved on the supervisor's computer as well as the computer of the primary investigator (SL) to ensure safekeeping.

### 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 pair-wise 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 $p < 0.05$.

# Chapter 4: Results

## Examination time

The median (range) examination times (Table 6) were 8.97 (7.7 - 10.02), 3.75 (3.16 - 4.17) and 4.02 (3.55 - 4.82) minutes for Alf-chem, Thio-chem, and Prop-chem respectively, while the examination times for Alf-mech, Thio-mech, and Prop-mech were 10.86 (9.80 -11.55), 12.15 (5.99 - 18.45) and 4.98 (4.08 - 6.80) minutes, respectively. Examination time was statistically longer for Alf-chem compared to both Thio-chem ($p < 0.01$) and Prop-chem ($p = 0.04$). Examination time was statistically longer for Alf-mech and Thio-mech compared to Prop-mech ($p = 0.01$). Examination time was significantly longer for Alf-mech in comparison to Thio-chem ($p < 0.01$) and Prop-chem ($p = 0.01$).

## Number of supplemental boli:

The number of supplemental boli required during Alf-chem, Thio-chem, and Prop-chem were 3 (3 - 6), 4 (3 - 5) and 4 (4 - 5) boli, respectively. The number of supplemental boli required during Alf-mech, Thio- mech, and Prop-mech were 4 (3 - 4), 4 (4 - 4) and 4 (3 - 4) boli, respectively. The number of supplemental boli was statistically higher during Thio-mech induction compared to Alf-chem ($p = 0.02$). No differences were observed among other treatments.

## Vital breaths:

No difference was observed among treatments for number of vital breaths recorded during the 3 different time periods as shown in Table 6.

## Arytenoid motions:

The number of arytenoid motions recorded during Alf-chem, Thio-chem, and Prop-chem were 36 (26 - 53), 67 (35 - 168) and 59 (50 - 148) motions, respectively for time period 2. The

number of arytenoid motions recorded during Alf-mech, Thio-mech, and Prop-mech were 1 (0 - 2), 2 (1 - 3) and 2 (1 - 8) motions, respectively for time period 2. The number of arytenoid motions was statistically higher during Thio-chem compared to Alf-mech ($p < 0.01$), Thio-mech ($p = 0.01$) and Prop-mech ($p = 0.01$), respectively for time period 2. The number of arytenoid motions was statistically higher during Prop-chem compared to Alf-mech ($p < 0.01$), Thio-mech ($p < 0.01$) and Prop-mech ($p < 0.01$), respectively for time period 2. Both Thio-chem and Prop-chem conditions demonstrated significantly higher number of arytenoid motions compared with mechanical stimulation for all induction drugs. The number of arytenoid motions recorded during Alf-chem, Thio-chem, and Prop-chem were 0 (0 - 0), 1 (0 - 1) and 2 (1 - 5) motions, respectively for time period 1. The number of arytenoid motions recorded during Alf-mech, Thio-mech, and Prop-mech were 0 (0 - 0), 1 (0 - 2) and 1 (0 - 2) motions, respectively for time period 1. The number of arytenoid motions was statistically higher during time period 2 compared to time period 1 ($p < 0.01$) for Thio-chem. The number of arytenoid motions was statistically higher during time period 2 compared to time period 1 ($p < 0.01$) for Prop-chem.

**Laryngeal exposure score:**

The ability to visualise the larynx and arytenoid cartilages comprises a composite score consisting of four variables namely laryngeal evaluation jaw tone score, swallowing score, laryngospasm score and laryngeal evaluation breath score. No differences were observed among treatments (1-6) or time periods (T1, T2, T3) within a treatment for jaw tone, laryngospasm or breath scores as seen in Table 7. No difference was observed among treatments (1-6) for swallowing score. However, the swallowing score was significantly higher during time period 2 compared to time period 1 ($p = 0.02$) for Thio-chem.

**Laryngeal Function score:**

The laryngeal function score observed during Alf-chem, Thio-chem, Prop-chem, Alf-mech, Thio- mech, and Prop-mech were all 7 (7 - 7), respectively for time period 1 (Table 8 and Fig. 7). The laryngeal function score observed during Alf-chem, Thio-chem, Prop-chem, Alf-mech, Thio- mech, and Prop-mech were 3 (2 - 5), 2 (2 - 3), 3 (2 - 3), 6 (2 - 7), 5 (2 - 6), 4 (3 - 5), respectively for time period 2. The laryngeal function score recorded during Alf-chem, Thio-chem, Prop-chem, Alf-mech, Thio- mech, and Prop-mech were 4 (3 - 5), 3 (3 - 3), 3 (3 - 3), 5 (3 - 5), 3 (2 - 3), 5 (4 - 6), respectively for time period 3.

The laryngeal function score was significantly higher during time period 1 compared to time period 2 for Alf-chem ($p < 0.01$), Thio-chem ($p < 0.01$) and Prop-chem ($p < 0.01$). The laryngeal function score was significantly higher during time period 1 compared to time period 3 for Alf-chem ($p = 0.04$) and both Alf-mech ($p = 0.04$) and Thio-mech ($p < 0.01$).

**Paradoxical motions score:**

No difference was observed among treatments or time periods for paradoxical motion score.

**Table 6:** The median (range) examination time, number of supplemental boli, vital breath score and arytenoid motions observed for Time period 1, Time period 2 and Time period 3 during oral laryngoscopy in eight beagle dogs anaesthetised with alfaxalone, thiopentone and propofol intravenously.

| | | | Number of vital breaths | | | Number of arytenoid motions | | |
|---|---|---|---|---|---|---|---|---|
| **Treatment** | **Examination time (min)** | **Number supplemental boli** | **T1** | **T2** | **T3** | **T1** | **T2** | **T3** |
| **Alf-chem** | 8,97 (7,7-10,02)[b,c] | 3 (3-3)[e] | 0 (0-0) | 16 (1-39) | 2 (1-6) | 0 (0-0) | 36 (26-53) | 46 (6-114) |
| **Thio-chem** | 3,75 (3,16-4,17)[a,d] | 4 (3-5) | 1 (0-2) | 3 (0-17) | 0 (0-0) | 1 (0-1)[#] | 67 (35-168)[d,e,f*] | 170 (170-170) |
| **Prop-chem** | 4,02 (3,55-4,82)[a,d] | 4 (4-5) | 1 (0-1) | 8 (2-39) | 2 (1-2) | 2 (1-5)[#] | 59 (50-148)[d,e,f*] | 3 (2-3) |
| **Alf-mech** | 10,86 (9,80-11,55)[b,c,f] | 4 (3-4) | 0 (0-0) | 0 (0-1) | 2 (1-4) | 0 (0-0) | 1 (0-2)[b,c] | 2 (1-25) |
| **Thio-mech** | 12,15 (5,99-18,45) | 4 (4-4)[a] | 1 (0-1) | 3 (2-3) | 7 (5-8) | 1 (0-2) | 2 (1-3)[b,c] | 11 (6-59) |
| **Prop-mech** | 4,98 (4,08-6,80)[d] | 4 (3-4) | 1 (0-1) | 2 (1-8) | 1 (1-6) | 1 (0-2) | 2 (1-8)[b,c] | 1 (1-1) |

[a]) Significant statistical difference ($p < 0.05$) compared to Alf-chem
[b]) Significant statistical difference ($p < 0.05$) compared to Thio-chem
[c]) Significant statistical difference ($p < 0.05$) compared to Prop-chem
[d]) Significant statistical difference ($p < 0.05$) compared to Alf-mech
[e]) Significant statistical difference ($p < 0.05$) compared to Thio-mech
[f]) Significant statistical difference ($p < 0.05$) compared to Prop-mech

*) Statistically significant difference ($p < 0.05$) compared to Time period 1
#) Statistically significant difference ($p < 0.05$) compared to Time period 2

Alfaxalone: induction bolus 1.5 mg $kg^{-1}$; supplemental bolus 0.4 mg $kg^{-1}$
Thiopentone: induction bolus 7 mg $kg^{-1}$; supplemental bolus 1.8 mg $kg^{-1}$
Propofol: induction bolus 3 mg $kg^{-1}$; supplemental bolus 0.75 mg $kg^{-1}$

**Table 7:** Laryngeal examination variables median (range) observed for Time period 1, Time period 2 and Time period 3 during oral laryngoscopy in eight beagle dogs anaesthetised with alfaxalone, thiopentone and propofol intravenously.

| Treatment | Jaw tone score | | | Swallowing score | | |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T1 | T2 | T3 |
| **Alf-chem** | 0 (0-0) | 1 (1-1) | 1 (1-2) | 0 (0-0) | 0 (0-0) | 0 (0-1) |
| **Thio-chem** | 1 (1-1) | 2 (2-2) | 2 (2-2) | 0 (0-0)# | 1 (1-1)* | 1 (1-1) |
| **Prop-chem** | 1 (0-1) | 2 (1-2) | 1 (1-2) | 0 (0-0) | 0 (0-1) | 1 (0-1) |
| **Alf-mech** | 1 (0-1) | 1 (0-1) | 1 (1-2) | 0 (0-0) | 0 (0-0) | 0 (0-1) |
| **Thio-mech** | 0 (0-0) | 1 (1-1) | 1 (1-1) | 0 (0-0) | 0 (0-0) | 0 (0-1) |
| **Prop-mech** | 1 (0-1) | 2 (1 -2) | 1 (1-1) | 0 (0-1) | 1 (1-1) | 1 (1-1) |

*) Statistically significant difference ($p < 0.05$) compared to Time period 1

#) Statistically significant difference ($p < 0.05$) compared to Time period 2

| | | |
|---|---|---|
| Alfaxalone: | induction bolus 1.5 mg $kg^{-1}$; | supplemental bolus 0.4 mg $kg^{-1}$ |
| Thiopentone: | induction bolus 7 mg $kg^{-1}$; | supplemental bolus 1.8 mg $kg^{-1}$ |
| Propofol: | induction bolus 3 mg $kg^{-1}$; | supplemental bolus 0.75 mg $kg^{-1}$ |

**Table 8:** Laryngeal function score and Paradoxical motion median (range) observed for Time period 1, Time period 2 and Time period 3 during oral laryngoscopy in eight beagle dogs anaesthetised with alfaxalone, thiopentone and propofol intravenously.

| | Laryngeal function score | | |
|---|---|---|---|
| **Treatment** | **T1** | **T2** | **T3** |
| **Alf-chem** | 7 (7-7)$^{\#\alpha}$ | 3 (2-5)* | 4 (3-5)* |
| **Thio-chem** | 7 (7-7)$^{\#}$ | 2 (2-3)* | 3 (3-3) |
| **Prop-chem** | 7 (6-7)$^{\#}$ | 3 (2-3)* | 3 (3-3) |
| **Alf-mech** | 7 (7-7)$^{\alpha}$ | 6 (2-7) | 5 (3-5)* |
| **Thio-mech** | 7 (7-7)$^{\alpha}$ | 5 (2-6) | 3 (2-3)* |
| **Prop-mech** | 7 (6-7) | 4 (3-5) | 5 (4-6) |

*) Statistically significant difference ($p < 0.05$) compared to Time period 1
#) Statistically significant difference ($p < 0.05$) compared to Time period 2
α) Statistically significant difference ($p < 0.05$) compared to Time period 3

| | | |
|---|---|---|
| Alfaxalone: | induction bolus 1.5 mg kg$^{-1}$; | supplemental bolus 0.4 mg kg$^{-1}$ |
| Thiopentone: | induction bolus 7 mg kg$^{-1}$; | supplemental bolus 1.8 mg kg$^{-1}$ |
| Propofol: | induction bolus 3 mg kg$^{-1}$; | supplemental bolus 0.75 mg kg$^{-1}$ |

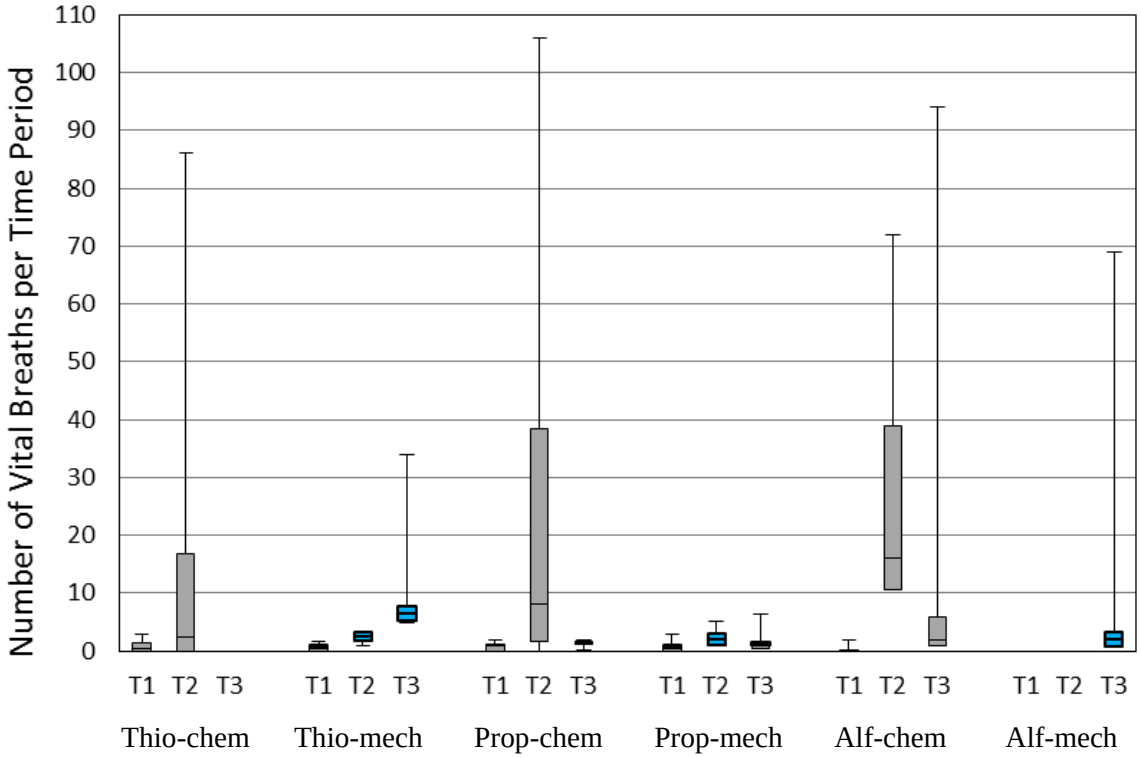


**Figure 4**: Boxplot number of vital breaths *versus* anaesthetic drug over Time period 1(T1), Time period 2 (T2) and Time period 3 (T3) during chemical and mechanical stimulation during oral laryngoscopy in eight beagle dogs anaesthetised with alfaxalone, thiopentone and propofol intravenously.

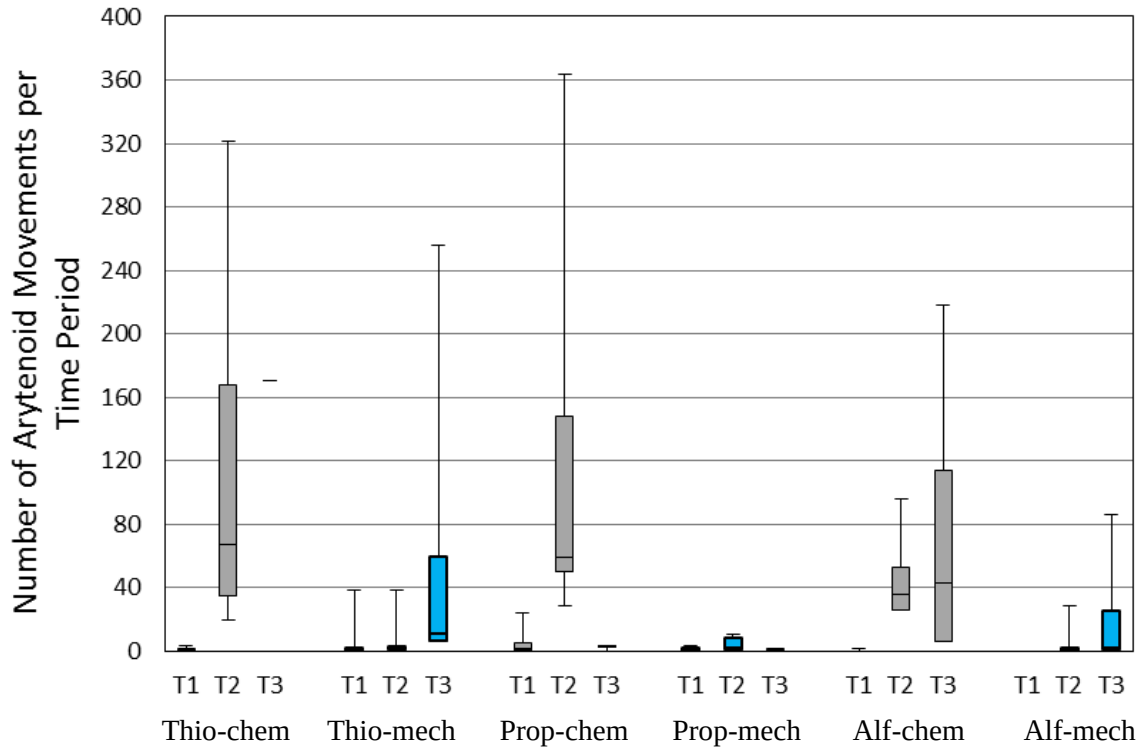


**Figure 5:** Boxplot number of arytenoid movements *versus* anaesthetic drug over Time period 1(T1), Time period 2 (T2) and Time period 3 (T3) during chemical and mechanical stimulation during intravenous oral laryngoscopy in eight beagle dogs anaesthetised with alfaxalone, thiopentone and propofol intravenously.

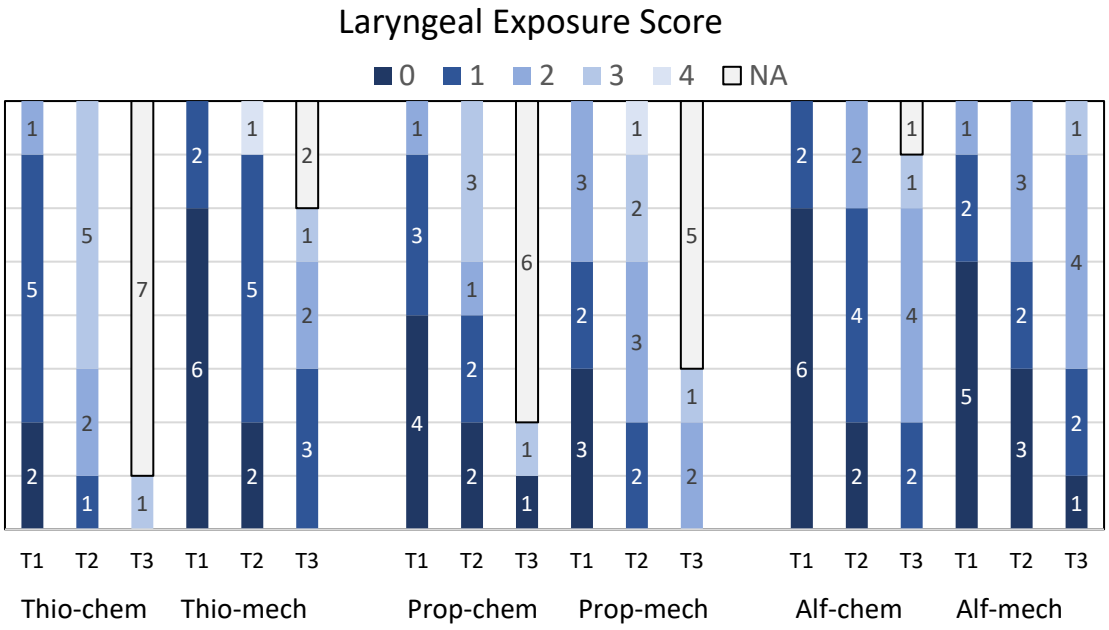


**Figure 6:** Frequency bar chart summarising the laryngeal exposure scores for chemical and mechanical stimulation. The figure indicates the number of dogs that can be assigned to a specific score each treatment over Time period 1(T1), Time period 2 (T2) and Time period 3 (T3).

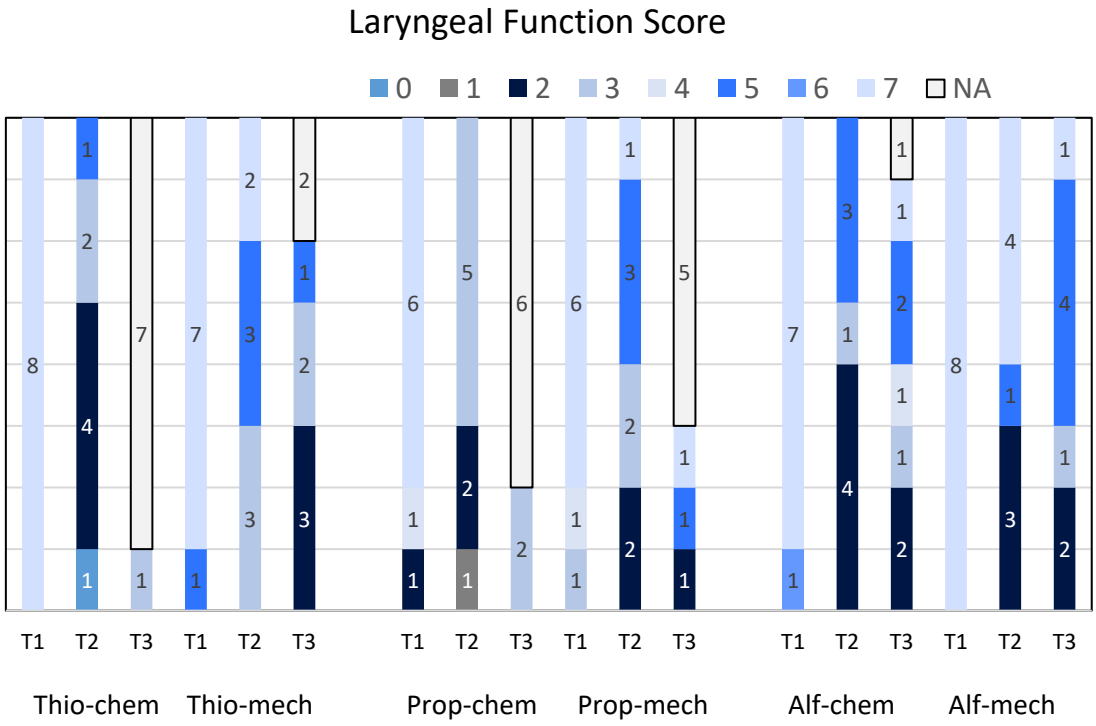


**Figure 7:** Frequency bar chart summarising the laryngeal function scores for chemical and mechanical stimulation. The figure indicates the number of dogs that can be assigned to a specific score each treatment over Time period 1(T1), Time period 2 (T2) and Time period 3 (T3).

## Chapter 5: 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-chem and Prop-chem compared to Alf-mech, Thio-mech 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 (Evers et al. 1965; Arrioja 2001; Miller et al. 2002). 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 due to increased inspiratory effort. This renders chemical stimulation with doxapram more beneficial

than mechanical stimulation as an aid to arytenoid motion evaluation during the 2 to 5-minute period (time period 2 of the present study) after induction using both thiopentone and propofol induction agents. Doxapram was administered at a dosage of 2.5mg $kg^{-1}$ IV, which is higher than previous studies that used 1.1 mg $kg^{-1}$ IV 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 to 5 minute period after induction) over time period 1 (period from start of laryngeal examination until 2 minutes) when utilising any of the anaesthetic induction agents investigated herein. The time period of 2 to 5 minutes after anaesthetic induction was identified as the optimal time period for laryngeal evaluation due to the higher number of arytenoid motions, vital breaths and desirable arytenoid function scores observed within some treatments. The number of arytenoid motions were statistically higher during this time compared to the earlier time period soon after induction for Thio-chem and Prop-chem. The laryngeal function score was significantly higher during early end-induction time, and was characterised by complete immobility of arytenoid cartilage and vocal folds for all induction agents combined with chemical stimulation compared to the time period of 2 to 5 minutes after anaesthetic induction. Laryngeal function scores after chemical stimulation in time period of 2 to 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 were higher during time period of 2 to 5 minutes after anaesthetic induction compared to early end-induction time period and the recovery period (time period 3 of present study) under chemical stimulation for all induction agents. We, therefore, recommend that laryngeal function evaluation be performed during time period of 2 to 5 minutes after anaesthetic induction when utilising 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 (2011).

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 due to 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 anaesthetised 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 a negligible depressive effect on the respiratory system compared to the other induction agents (Gilron and Coderre 1996, Ferré et al. 2006, Muir et al. 2008).

Examination times were longer under alfaxalone anaesthesia compared to propofol and agrees with the study by Maney et al. (2013) where a single bolus of alfaxalone resulted in a longer recovery time compare to a single bolus of propofol. Previous studies by Ferré 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 differences in examination time reported in this study. 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 anaesthetised 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 by 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 (60 seconds with utilisation of a syringe driver) 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.

Doxapram hydrochloride has been reported to cause various side effects including CNS 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. Only healthy, non-premeditated dogs were utilised for the study, which may be a shortcoming and warrants a need for further investigation on dogs confirmed to have LP. The small sample size (n=8) could be a potential limitation of the present study, however, the limitation was remedied by the implementation of a randomised cross-over trial. The number of dogs used in the present study compares favourably to numbers ranging between 6 and 8 used in similar previous studies (Smalle et al. 2017, Gross et al. 2002).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, while 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.

## Chapter 6: 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.

## Chapter 7: References

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## Addenda

AEC certificate - Project number: V106-16

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
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# Animal Ethics Committee

| | |
|---|---|
| PROJECT TITLE | **Effects of chemical and mechanical stimulation on laryngeal motion during anaesthetic induction with alfaxalone, thiopentone or propofol** |
| PROJECT NUMBER | **V106-16** |
| RESEARCHER/PRINCIPAL INVESTIGATOR | **Dr. S Labuschagne** |

| | |
|---|---|
| STUDENT NUMBER (where applicable) | **UP_2505 6159** |
| DISSERTATION/THESIS SUBMITTED FOR | **MSc** |

| | | |
|---|---|---|
| ANIMAL SPECIES | **Canine** | |
| NUMBER OF ANIMALS | **8** | |
| Approval period to use animals for research/testing purposes | | **August 2016 –August 2017** |
| SUPERVISOR | **Prof. B Dzikiti** | |

*KINDLY NOTE:*

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

| | |
|---|---|
| **APPROVED** | Date 29 August 2016 |
| CHAIRMAN: UP Animal Ethics Committee | Signature |

S4285-15

Data capturing sheet

| | | |
|---|---|---|
| **Patient Name:** | | **Date:** |
| **Microchip number:** | | |
| **Experimental animal number:** | | |
| **Species:** | **Breed:** | **Weight:** |
| **Sex:** | **Age:** | **Pregnant: Yes/No** |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Excitement** | **Yes** | **No** | **Chem** | | **Mech** | | |
| **Induction time** | | | **Number of Top up boluses** | | | | |
| **Examination time** | | | **Total dose** | | | | |

| | Time 1<br>0-2 min | | | Time 2<br>2-5 min | | | Time 3<br>5 min to end point | | |
|---|---|---|---|---|---|---|---|---|---|
| Number of arytenoid motions | | | | | | | | | |
| Number of Vital breaths | | | | | | | | | |
| Breathing score | 0 | 1 | | 0 | 1 | | 0 | 1 | |
| | 2 | 3 | | 2 | 3 | | 2 | 3 | |
| Jaw tone score | 0 | 1 | | 0 | 1 | | 0 | 1 | |
| | 2 | 3 | | 2 | 3 | | 2 | 3 | |
| Swallowing | 0 | 1 | | 0 | 1 | | 0 | 1 | |
| Laryngospasm | 0 | 1 | | 0 | 1 | | 0 | 1 | |
| Laryngeal function score | 0 | 1 | A | 0 | 1 | A | 0 | 1 | A |
| | 2 | 3 | B | 2 | 3 | B | 2 | 3 | B |
| Paradoxical motion | 0 | 1 | | 0 | 1 | | 0 | 1 | |

**Data capture sheet:** Effects of chemical and mechanical stimulation on laryngeal motion during anaesthetic induction with alfaxalone, thiopentone or propofol. If found please contact Dr Sandra Labuscagne (072 959 7629)

Inclusion criteria for experimental animals

| | | | |
|---|---|---|---|
| **Patient Name:** | | | **Date:** |
| **Microchip number:** | | | |
| **Experimental animal number:** | | | |
| **Species:** | **Breed:** | **Weight:** | |
| **Sex:** | **Age:** | **Pregnant: Yes/No** | |
| **Vaccination history:** | | | |
| **Temperature:** | **Pulse:** | **Respiratory rate:** | |
| **PCV (L $L^{-1}$):** | **TSP (g $L^{-1}$):** | **Serum creatinine (µmol $L^{-1}$):** | |

| **Basic physical examination:** | **Yes** | **No** |
|---|---|---|
| Nasal discharge | | |
| Rectal temperature < 37.5ºC | | |
| Rectal temperature > 39.5ºC | | |
| Heart murmur/abnormal lung sounds on thoracic auscultation | | |
| Clinical signs of upper respiratory disease | | |
| **Blood smear evaluation:** | | |
| *Babesia* spp. Identified | | |
| Comments: | | |
| **Blood tests:** | | |
| PCV less than 0.35 | | |
| PCV more than 0.55 | | |
| TSP less than 55 g $L^{-1}$ | | |
| TSP more than 75 g $L^{-1}$ | | |
| Serum creatinine less than 59 µmol $L^{-1}$ | | |
| Serum creatinine more than 109 µmol $L^{-1}$ | | |
| **N.B. Answering "yes" in any of the above questions excludes the dog from further participation in the study.** | | |

**Data capture sheet:** Effects of chemical and mechanical stimulation on laryngeal motion during anaesthetic induction with alfaxalone, thiopentone or propofol. If found please contact Dr Sandra Labuscagne (072 959 7629)

## Reporting

Publications and presentations:

Labuscagne, S., Zeiler, G.E., Dzikiti, T.B. 'Effects of chemical and mechanical stimulation on laryngeal motion during anaesthetic induction with alfaxalone, thiopentone or propofol in healthy dogs', Veterinary Anaesthesia and Analgesia. Status: Submitted 21 December 2018 and accepted online. https://doi.org/10.1016/j.vaa.2018.12.010

Labuscagne, S. 'Effects of chemical and mechanical stimulation on laryngeal motion during anaesthetic induction with alfaxalone, thiopentone or propofol in healthy dogs', Faculty day 2017, Faculty of Veterinary Science, University of Pretoria.