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Effects of thiopental, propofol and alfaxalone on laryngeal motion during oral laryngoscopy in healthy dogs

By

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Submitted in partial fulfilment of the requirements for the degree of MMedVet (Surg)(Small Animals) in the Department of Companion Animal Clinical Studies in the Faculty of Veterinary Science, University of Pretoria

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Declaration

I, Tesh M. Smalle, 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 (Surg)(Small Animals).

Signature:

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List of Abbreviations

mg	Milligram
mm	Millimetre
mL	Millilitre
L	Litre
kg	Kilogram
g/L	Gram/litre
µmol/L	Micromol/litre
mg/kg	Milligram per kilogram
mg/kg/minute	Dose rate in milligram per kilogram per minute
”	Inch
%	Percentage
<	Smaller than
>	Greater than
=	Equal to
≤	Smaller or equal to
≥	Greater or equal to
IV	Intravenous
IM	Intramuscular
°C	Temperature in degrees Celsius
°	Angle in degrees
HCl	Hydrochloride
NGGA	Normalised glottal gap area
GABA	Gamma aminobutyric acid
TSP	Total serum protein
PCV	Packed cell volume reported as volume L/L
OTAU	Onderstepoort Teaching Animal Unit
OVAH	Onderstepoort Veterinary Academic hospital
RSA	Republic of South Africa
USA	United States of America

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Summary

Effects of thiopental, propofol and alfaxalone on laryngeal motion during oral laryngoscopy in healthy dogs

By

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Degree: MMedVet (Surg)(Small Animals)

Objective To compare the effects of thiopental, propofol and alfaxalone on arytenoid cartilage motion and establish dose rates to achieve a consistent oral laryngoscopy examination.

Study design Prospective, randomised, blinded crossover study.

Animals Six healthy adult beagle dogs.

Methods Each dog was administered three induction agents in a random order with a one week washout period between treatments. No premedication was used prior to induction of anaesthesia. Thiopental, propofol or alfaxalone were administered at 7.5 mg/kg, 3 mg/kg and 1.5 mg/kg, over 1 minute to effect, for induction of anaesthesia, respectively. If the dog was deemed inadequately anaesthetised then top-up boluses of 1.8 mg/kg, 0.75 mg/kg and

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0.4 mg/kg, respectively, were administered over 10 seconds, repeated every 20 seconds, until an adequate anaesthetic plane had been reached. Continual examination of the larynx, using a laryngoscope, commenced once an adequate anaesthetic depth had been reached until recovery from anaesthesia. The number of arytenoid motions and deep inspiratory efforts (vital breaths) were counted within three time periods and compared over time among treatments. Data were analysed using Friedman test, Mann-Whitney U test, Spearman's rho, linear mixed model with post-hoc pairwise comparison with Tukey correction. Results interpreted at a 5% level of significance.

Results The median (range) induction time was 2.8 (2.0, 3.0), 2.7 (2.0, 3.3) and 2.5 (1.7, 3.3) minutes for thiopental, propofol and alfaxalone, respectively (p = 0.727). The median (range) dose rate required to achieve an adequate depth of anaesthesia was 6.3 (6.0, 6.6), 2.4 (2.4, 2.4) and 1.2 (1.2, 1.2) mg/kg/minute for thiopental, propofol and alfaxalone, respectively. Therefore, the median (range) total dose administered over the induction time was 17.8 (13.2, 18.8), 6.8 (5.3, 8.3) and 3.2 (2.3, 4.1) mg/kg for thiopental, propofol and alfaxalone, respectively. There was no significant difference for the total number of arytenoid motions (p =0.662) or vital breaths (p = 0.789) among induction agents. The median (range) examination times were 14.1 (8.0, 41.8), 5.4 (3.3, 14.8) and 8.5 (3.8, 31.6) minutes for thiopental, propofol and alfaxalone, respectively (p=0.016).

Conclusion and clinical relevance There was no significant difference in the total number of arytenoid motions among the induction agents. However, at the dose rates used in this study, propofol provided adequate conditions for evaluation of the larynx within a shorter examination time which may be advantageous during laryngoscopy in dogs.

Keywords: anaesthetic protocol, canine, induction, laryngeal paralysis, laryngoscopy.

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Justification

Literature review

a) Laryngeal paralysis in dogs

Laryngeal paralysis is the most common disease process involving the larynx of dogs (MacPhail 2014). It is defined as an inability to abduct one or both arytenoid cartilages during inspiration and results from dysfunction of the caudal laryngeal nerve or cricoarytenoideus dorsalis muscle (Kitshoff et al. 2013, Millard & Tobias 2009, Tobias, Jackson & Harvey 2004). Most commonly, this disease process is acquired in middle-aged to older, large-breed dogs. However, a congenital form has been described in Bouvier des Flanders, Siberian Husky, Bull Terrier, Dalmation and Rottweiler dog breeds (Kitshoff et al. 2013, MacPhail 2014, Millard & Tobias 2009). Clinical signs at initial presentation vary, but generally progress as the laryngeal dysfunction becomes more severe. Early in the disease process inspiratory stridor (a harsh vibrating noise during inspiration resulting from turbulent air flow in the larynx) (Blood & Studdert 1999), exercise intolerance and voice change (often manifesting as a high pitched bark) are noticed. This may progress to life-threatening respiratory distress, cyanosis and collapse if not addressed timeously (Kitshoff et al. 2013, MacPhail 2014, Millard & Tobias 2009).

Definitive diagnosis of this condition depends on an accurate laryngeal examination. This may be achieved via direct visualisation of the larynx with a simple laryngoscope, transoral video-endoscopic laryngoscopy, transnasal video-endoscopic laryngoscopy, echolaryngography or computed tomography (CT) (MacPhail 2014, Radlinsky et al. 2009, Stadler et al. 2011). Transnasal video-endoscopic laryngoscopy, echolaryngography and CT avoid the need for heavy sedation/general anaesthesia, but these methods have not been shown to be superior to transoral laryngoscopy. A simple laryngoscope assists with visualisation of the rima glottidis, but retraction of the tongue and application of pressure on the epiglottis with the laryngoscope blade can impair laryngeal function. Thus, many

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clinicians prefer to use transoral video-endoscopic laryngoscopy (Millard & Tobias 2009).

Laryngoscopy, regardless of the method employed, can be confounding due to the influence of sedatives and anaesthetic agents on laryngeal function (MacPhail 2014). Furthermore, the dosages used and rate at which the anaesthetic induction agent is administered could affect the quality of the laryngeal examination. Amengal *et al.* (2013) investigated the quality of anaesthetic induction following rapid intravenous injection of propofol (3 mg/kg over 5 seconds) or alfaxalone (1.5 mg/kg over 5 seconds) approximately 40 minutes after premedication with acepromazine (0.03 mg/kg IM) and pethidine (3 mg/kg IM). They concluded that a rapid administration rate increases the incidence of post-induction apnoea (Amengual et al. 2013). Dugdale *et al.* (2005) investigated the induction dose requirements of thiopental using two different administration rates for induction of anaesthesia. In their study, animals were randomly assigned to receive an intravenous constant rate infusion (using a syringe driver) of 2.5% thiopental at a rate of either 0.1 mL/kg/minute or 0.4 mL/kg/minute, 30–40 minutes after premedication with acepromazine (0.025 mg/kg IM) and pethidine (3.5 mg/kg IM). They found that a slower infusion rate reduced the induction dose requirement, but the quality of induction was inferior with increased excitability (defined as the presence of paddling and vocalisation) during the induction phase (Dugdale et al. 2005). Ideally, laryngoscopy should be performed under a light plane of anaesthesia which is sufficient to relax the muscles of the jaw, but does not inhibit laryngeal reflexes or result in respiratory depression (Jackson et al. 2004, McKeirnan et al. 2014).

b) Anaesthetic protocols used for laryngeal examination

Few studies have been undertaken to determine the most appropriate anaesthetic protocol to allow accurate diagnosis of laryngeal paralysis in dogs. In the first of these studies, the effect of induction of anaesthesia with thiopental (predicted dose of 20 mg/kg, half given as an IV bolus and thereafter titrated to effect over 1 minute), propofol (predicted dose of 6

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mg/kg administered slowly IV to effect over 1 minute) and diazepam-ketamine (0.5 mg/kg and 10 mg/kg, respectively, mixed in a single syringe and administered slowly IV to effect over 1 minute) on laryngeal function after premedication with butorphanol (0.5 mg/kg IV 5 minutes prior to induction as a mild sedative with minimal respiratory depression) and glycopyrrolate (0.01 mg/kg IV 5 minutes prior to induction as a vagolytic agent) were compared. All three anaesthetic protocols preserved normal laryngeal function in healthy dogs. However, diazepam (mean dose administered 0.3 ± 0.1 mg/kg)-ketamine (mean dose administered 5.6 ± 1.1 mg/kg) resulted in suboptimal visualisation of the rima glottidis due to increased jaw tone, increased swallowing efforts and laryngospasm. They concluded that the use of thiopental (mean dose administered 10.4 ± 1.1 mg/kg) and propofol (mean dose administered 3.6 ± 0.8 mg/kg) allowed optimal visualisation of the rima glottidis for laryngeal examination and could be titrated to effect (Gross et al. 2002). Subsequently, Jackson *et al.* (2004) evaluated the effects of commonly used anaesthetic protocols on laryngeal function. They found that arytenoid motion was significantly greater after thiopental (predicted dose 10-20 mg/kg administered slowly IV to effect; mean dose administered 14 ± 2.3 mg/kg) induction without premedication when compared to: 1) propofol (predicted dose 6-8 mg/kg administered slowly IV to effect; mean dose administered 5.6 ± 1.1 mg/kg) without premedication, 2) ketamine (predicted dose 4-8 mg/kg administered slowly IV to effect; mean dose administered 8.5 ± 2.9 mg/kg) + diazepam (predicted dose 0.2-0.4 mg/kg administered slowly IV to effect; mean dose administered 0.4 ± 0.2 mg/kg), 3) thiopental (predicted dose 8-20 mg/kg administered slowly IV to effect; mean dose administered 9.8 ± 2.1 mg/kg) after acepromazine (0.05 mg/kg IM 20 minutes prior to induction) premedication and 4) propofol (predicted dose 4-6 mg/kg administered slowly IV to effect; mean dose administered 3.7 ± 1.3 mg/kg) after acepromazine (0.05 mg/kg IM 20 minutes prior to induction) premedication. However, no significant difference was found in laryngeal function when acepromazine (0.05 mg/kg IM 20 minutes prior to induction) + butorphanol (0.4 mg/kg

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IM 20 minutes prior to induction) + mask induction with isoflurane and thiopental were compared. They concluded that intravenous thiopental titrated to effect in an unpremedicated patient was the best choice for assessing laryngeal function (Jackson et al. 2004). Due to the recent lack of availability of thiopental in the United States of America, other anaesthetic protocols have been explored (MacPhail 2014). A recent study (McKeirnan et al. 2014), investigated the effect of induction of anaesthesia with propofol (predicted dose 6 mg/kg administered slowly IV to effect over 1 minute; mean dose administered 3.8 ± 1.2 mg/kg) and propofol (predicted dose 2-4 mg/kg IV) + ketamine (colloquially known as Ketofol) (ketamine was given as a calculated bolus of 2 mg/kg IV, followed by propofol administered slowly IV to effect over 1 minute; mean dose administered 2.4 ± 0.7 mg/kg) on laryngeal function after premedication with butorphanol (0.5 mg/kg IM 20 minutes prior to induction) and glycopyrrolate (0.01 mg/kg IM 20 minutes prior to induction). If a patient failed to abduct the arytenoid cartilages, during laryngeal examination, a cotton-tipped applicator was used to stimulate respiration by application of direct pressure to one arytenoid. If the patient became apnoeic (no spontaneous respiration in more than one minute) or respiration could not be manually stimulated then a single dose of doxapram (1 mg/kg IV) was administered. The administration of doxapram has been shown to be useful for differentiating normal dogs from dogs with laryngeal paralysis by increasing respiratory rate and effort (Tobias, Jackson & Harvey 2004). McKeirnan *et al.* (2014) hypothesised that the addition of ketamine, to the anaesthetic protocol, would allow for a reduction in the propofol dose used and therefore decrease respiratory depression and preserve the laryngeal reflexes. They concluded that ketamine was a poor addition to propofol for laryngeal examination as it caused increased respiratory depression and did not allow for a significant reduction in the propofol dose used at induction of anaesthesia (McKeirnan et al. 2014).

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c) Scoring systems used to evaluate laryngeal motion

The studies reporting on the various effects of anaesthetic protocols have used various scoring systems to evaluate the effect of anaesthetic protocols on laryngeal motion (Gross et al. 2002, Jackson et al. 2004, McKeirnan et al. 2014, Nelissen et al. 2012). In all studies the animals were placed in sternal recumbency with the head elevated to the level of normal carriage after induction of anaesthesia. Laryngeal function was examined via a transoral approach using either a simple laryngoscope or video-endoscope.

Some studies used subjective, simple descriptive scoring systems to evaluate laryngeal function. Such systems evaluated **1)** jaw tone on a scale from 0 (absent and easy to open) to 3 (excessive and difficult to open); **2)** breathing score on a scale of 0 (deep respirations, normal respiratory rate, strong attempt) to 3 (no spontaneous respiration); **3)** swallowing as either 0 (absent) or 1 (present); and **4)** incidence of laryngospasm as either 0 (absent) or 1 (present) (Gross et al. 2002, McKeirnan et al. 2014). In the current study, the sum of the scores in each category were defined as the laryngeal exposure score (0 to 8). A low score indicated that the conditions for performing an accurate laryngeal examination were ideal. Additionally, previous studies (Gross et al. 2002, McKeirnan et al. 2014) determined overall exposure of the larynx (exposure score) for evaluation of laryngeal function. This was graded as excellent (mouth easily opened, arytenoid cartilages readily visualised with no swallowing/tongue movement), moderate (some jaw tone, some swallowing/tongue motion during visualisation of arytenoid cartilages), or poor (mouth difficult to open, arytenoid cartilages difficult to visualise due to swallowing/tongue movement). Finally, laryngeal function was scored using a simple descriptive scoring system where function was designated as either normal (abduction during inhalation) or abnormal (no abduction during inhalation or paradoxical movement).

More objective measures to score laryngeal function have also been described where a video-endoscope was inserted transorally, over the tip of the epiglottis, to a point where

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the entire rima glottidis was visible on the monitor. Laryngoscopy was video-recorded (digitised) for the first 15 seconds after induction (which included 3 breaths) and the last 15 seconds immediately before recovery. Still images of maximal inspiration and expiration from 3 separate breaths upon induction and recovery were selected. Height and area measurement from each set of still images were averaged and normalised (normalised glottal gap area) to account for any variations in patient size and distance between the laryngeal ostium and the tip of the laryngoscope. The range of arytenoid motion was defined as the change in normalised glottal gap area (NGGA) (Jackson et al. 2004, Nelissen et al. 2012).

In horses, recurrent laryngeal neuropathy describes a disease that can manifest as laryngeal paresis/paralysis. Clinically, it manifests as an abnormal inspiratory sound during exercise (referred to as “roaring” or “whistling”). Diagnosis depends on an accurate laryngeal examination using trans-nasal videoendoscopy in a standing horse with as little restraint (physical and chemical) as is necessary for the safety of the horse and personnel. A number of scoring systems are available and have been validated. As per the conclusions of the Havemeyer Workshop they score the laryngeal function from grade I to grade IV. Grade I indicates that all arytenoid cartilage movements are synchronous and symmetrical and full arytenoid cartilage abduction can be achieved and maintained. Grade IV indicates that there is complete immobility of the arytenoid cartilage and vocal fold (Robinson 2004). In the current study, the laryngeal function score specifically looked at grading the quality of arytenoid abduction activity and was adapted, for dogs, from the Havemeyer grading system used in horses. A score of 0A to 3B was assigned where a low score was desirable and indicated that all arytenoid cartilage movements were synchronous, symmetrical and full arytenoid cartilage abduction could be achieved and maintained.

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d) Use of alfaxalone as an induction agent for laryngeal examination

The authors of this clinical trial propose the use of alfaxalone for induction of anaesthesia in dogs where oral laryngoscopy is to be performed. Alfaxalone is a synthetic neuroactive steroid that acts as a gamma aminobutyric acid (GABA) receptor agonist to produce anaesthesia and muscle relaxation (Amengual et al. 2013). Studies in healthy dogs have shown that alfaxalone produced good short-term anaesthesia in unpremedicated patients with minimal cardiorespiratory depression at the recommended induction dose range (Muir et al. 2008). The most common adverse effect documented with its use was apnoea which was related to the total dose and rate of intravenous administration (Amengual et al. 2013, Muir et al. 2008). A recent study concluded that propofol was more likely to induce apnoea in dogs when compared to alfaxalone. The study reported that the median dose which induced apnoea was higher for alfaxalone (10 mg/kg IV, i.e. 5 times the recommended induction dose) than for propofol (13 mg/kg IV, i.e. twice the recommended induction dose) in unpremedicated dogs (Keates & Whittem 2012). Therefore aflaxalone poses distinct advantages in patients where laryngeal examination is to be performed. Predicted induction doses in unpremedicated and premedicated dogs are 4 mg/kg and 2 mg/kg, respectively (Muir et al. 2008). Nelissen *et al.* (2012) investigated the effect of induction of anaesthesia with alfaxalone (administered to effect at a rate of 2 mg/kg/min IV), propofol (administered to effect at a rate of 5 mg/kg/min IV) and midazolam (0.1 mg/kg IV) + ketamine (as a bolus at 2 mg/kg IV, then incremental doses at 1 mg/kg IV to effect) on laryngeal function in healthy cats after premedication with methadone (0.2 mg/kg IM 45 minutes prior to induction). They concluded that no significant difference in arytenoid movement was observed between the three anaesthetic protocols when used for induction of anaesthesia (Nelissen et al. 2012). When considering this, together with high therapeutic index and minimal respiratory depression of alfaxalone further investigation of this drug as an induction agent for laryngeal examination in dogs is justified.

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Outcome of the literature review

Based on the reviewed literature there are several areas that need to be further studied:

- Investigation of alfaxalone as an induction agent where laryngeal examination is to be performed for diagnostic purposes in dogs.
- Subjective and objective evaluation of the effect of alfaxalone on the abduction activity of the arytenoid cartilages and number of movements during oral laryngeal examination in dogs in comparison to more conventional induction agents thiopental and propofol.
- Establishment of appropriate dose rates for the induction agents to achieve an accurate, standardised laryngeal examination by oral laryngoscopy.

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Introduction

Laryngeal paralysis is the most common acquired disease process involving the larynx of middle to old-aged large-breed dogs (MacPhail 2014). In one study, laryngeal examination was performed in 250 dogs receiving general anaesthesia. They found that 25% of the experimental animals had some degree of laryngeal paresis. It was strongly associated with age, with animals less than 6 years of age being unlikely to show signs of laryngeal dysfunction (Broome, Burbidge & Pfeiffer 2000).

Diagnosis based on history, signalment and clinical signs has a high sensitivity (90%). However, confirmation via an accurate laryngeal examination is recommended (Kitshoff et al. 2013). In private veterinary practice, this is generally performed via simple transoral laryngoscopy under a light plane of anaesthesia (Millard & Tobias 2009). Examination by oral laryngoscopy can be complicated by the influence of anaesthetic agents on laryngeal function (MacPhail 2014). The ideal anaesthetic agent for oral laryngoscopy should circumvent induction apnoea and provide adequate jaw muscle relaxation, ample regular inspiratory efforts and normal functioning of the cricoarytenoideus dorsalis muscle.

Thiopental, an ultra-short-acting thio-barbiturate, is frequently used as an anaesthetic agent during laryngeal examination. When administered without premedication thiopental shows significantly greater arytenoid motion before recovery when compared to propofol in dogs (Jackson et al. 2004). Propofol (2,6-di-isopropylphenol), an alkyl-phenol, when administered shows adequate exposure of the larynx, but exhibits weaker arytenoid motion compared to thiopental in dogs (Gross et al. 2002, Jackson et al. 2004, McKeirnan et al. 2014). Also, propofol is more likely to induce apnoea in dogs when compared to alfaxalone, a synthetic neuro-active steroid anaesthetic (Amengual et al. 2013, Keates & Whittem 2012). There is a paucity of literature on alfaxalone use for oral laryngeal examination in dogs. Furthermore, there is a lack of consistency in the thiopental and propofol dosages used and the rate of administration to achieve an adequate depth of anaesthesia to allow for oral

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laryngeal examination in dogs.

Aims and objectives

This study aimed to investigate the effect of three anaesthetic induction agents on laryngeal function in healthy unpremedicated dogs. The specific goals of the present study were the following:

- Comparing abduction activity of the arytenoid cartilages after induction of anaesthesia with thiopental, propofol and alfaxalone by counting the number of arytenoid motions over time and using a subjective composite scoring system of laryngeal function;
- Determination of appropriate dose rates to achieve an accurate laryngeal examination using simple transoral laryngoscopy. An appropriate dose rate for laryngeal examination is defined as a rate which achieves the resultant anaesthetic plane that maintains the laryngeal and medial palpebral reflexes, with regular inspiratory efforts while allowing the mouth to be easily opened without conscious swallowing attempts.

Hypothesis

We hypothesised that there would be no significant difference between the total number of arytenoid motions over time during thiopental, propofol or alfaxalone induction and recovery in unpremedicated healthy adult dogs.

Benefits arising from the project

To the investigators' knowledge, this was the first study to document the effect of induction of anaesthesia with alfaxalone on laryngeal function in healthy dogs. This was achieved by comparing it to induction with thiopental and propofol in unpremedicated healthy dogs. In addition, this study was the first to report appropriate dose rates to achieve an accurate laryngeal examination using simple transoral laryngoscopy. Establishment of such dose rates allows a more objective comparison and helps standardise a research model.

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Materials and methods

Experimental animals

The study was approved by the University of Pretoria's research and animal ethics committees prior to commencement (Project number: V004-15). Six adult Beagle dogs (three males and three females), weighing a median (range) of 12.6 (11.1-14.3) kg and 69.2 (22-82) months of age, were enrolled into the blinded, crossover experimental trial.

These animals were sourced from the Onderstepoort Teaching Animal Unit (OTAU). They were purpose-bred and primarily used for undergraduate student training at the Onderstepoort campus, Faculty of Veterinary Science, University of Pretoria. The management of these animals was undertaken by OTAU with them being responsible for their feeding, housing and ensuring their general well-being. An advisory committee, affiliated with OTAU, was responsible for decision making with regards to the use of these Beagles for research purposes. The OTAU advisory committee gave written consent for the participation of these animals in this study.

These animals were rested from all student training obligations for one week prior to data collection and 4 weeks following data collection. The data collection phase of the study spanned from the 13th May 2015 to the 2nd June 2015.

The dogs had to meet the following selection criteria before being enrolled into the study:

1. Aged between eighteen months and seven years of age
2. Non-pregnant
3. Full vaccination history
4. No nasal discharge
5. No clinical signs of upper respiratory tract disease

Within four days of commencement of data collection, basic blood tests were performed along with a thorough physical examination and blood smear evaluation. The

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dogs had to meet the following criteria to allow continued participation in the study:

1. No heart murmur/abnormal lung sounds evident on thoracic auscultation
2. Rectal temperature > 37.5°C or < 39.5°C
3. No evidence of blood parasites on blood smear stained with Diff-quick
4. Packed cell volume ranging between 0.35-0.55 L/L
5. Total serum protein ranging between 55-75 g/L (using refractometry)
6. Serum creatinine between 59-109 µmol/L

Within seventy two hours prior to each subsequent anaesthetic protocol a thorough physical examination, basic blood tests and blood smear evaluation were performed. The dogs had to meet the same criteria as outlined above (serum creatinine evaluation was not repeated) to allow continued participation in the study.

On the day of data collection the dogs had to meet the following criteria (evaluated under a light plane of general anaesthesia) to allow continued participation in the study:

1. No signs of laryngeal asymmetry
2. No signs of pharyngeal or laryngeal pathology
3. No evidence of true uni- or bilateral laryngeal paralysis
4. No evidence of paradoxical movement of the arytenoid cartillages

Study design

The study was designed as a prospective, randomised, blinded cross-over experimental trial used to investigate the effect of three different anaesthetic induction agents on arytenoid abduction activity in healthy dogs. Six adult beagle dogs were rotated through three treatments with a one week washout period between treatments. Treatments were as follows:

1. Alfaxalone induction
2. Thiopental induction

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3. Propofol induction

Randomisation of treatments were achieved using a web based random assignment (www.randomization.com). Each data collection day allowed for two dogs to be assigned to one of the three treatments. The dogs were randomly rotated through the treatments over three separate data collection days until each dog had been exposed to each treatment once (Addendum: Randomisation of treatments).

Experimental procedures

The experimental procedure was divided into different stages, namely the a) pilot study and b) study. The study was divided into i) pre-anaesthetic, ii) anaesthetic induction, iii) laryngeal examination and iv) post-anaesthetic periods.

a) Pilot study

Two separate pilot studies (15th December 2014 and 20th January 2015) were conducted prior to commencement of the data collection. These pilot studies aimed to determine if the induction dose was appropriate for the purpose of laryngeal examination. The ideal induction for oral laryngoscopy was defined as a calm induction to the point where the mouth could safely and easily be opened without vocalisation, paddling or conscious swallowing attempts. In addition, the pilot studies allowed the authors of this clinical trial to familiarise themselves with the experimental procedures, thus ensuring optimal and accurate data collection.

Four beagle dogs aged between 26-82 months were enrolled in the pilot studies. The dogs were collected from OTAU on the afternoon prior to each pilot study. At this point a thorough physical examination and blood smear evaluation were performed. The dogs were placed in designated cages in the small animal surgery ward, at the Onderstepoort Veterinary Academic Hospital (OVAH). An eight-to-twelve hour fasting from food (with free access to water) was performed prior to each pilot study.

On the morning of the data collection, the dogs were transferred to designated cages

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in the induction area of the theatre complex at least one hour prior to induction of anaesthesia. Prior to the induction protocol, the induction agents were drawn up into a 20 mL syringe and a low-volume extension set (Extension set Ref 011-C150, Poly Medicure Ltd/ICU medical SA, Johannesburg, RSA) was attached with a needle (1" 20 Gauge) at the end. An additional 150% of each induction agent was drawn up into each syringe to account for individual variations in anaesthetic requirements. Each induction agent was administered using a separate electronic syringe driver (B. Braun Perfusor® Space, B. Braun Medical (Pty) Ltd, Johannesburg, RSA). The pre-determined volume (based on recommended induction doses in unpremedicated dogs) was set individually for each patient and administered over a predefined induction and top-up bolus time period. Each dog was randomly selected and placed on the procedure table and an intravenous catheter was placed, by the anaesthetist, in the lateral saphenous vein approximately 5-10 minutes prior to induction. A stopper with an injection port was secured to the catheter, through which the induction agent could be administered, flushing (2 mL lactated Ringer's solution) after each bolus.

The anaesthetic protocols were as follows:

Pilot study 1

- Dog 1: Thiopental 2.5%; induction bolus 15 mg/kg; top-up bolus 3.7 mg/kg.
- Dog 2: Thiopental 2.5%; induction bolus 10 mg/kg; top-up bolus 2.5 mg/kg.
- Dog 3: Propofol 1%; induction bolus 6 mg/kg; top-up bolus 1.5 mg/kg.
- Dog 4: Alfaxalone 1%; induction bolus 3 mg/kg; top-up bolus 0.8 mg/kg.

A **top-up bolus** was defined as 25% of the predefined induction bolus and it was administered, following the induction bolus, to achieve the desired anaesthetic depth for laryngeal examination. The induction bolus was administered over 1 minute and top-up boluses over 10 seconds. A waiting period of 10 seconds, to allow for drug effect, was observed after each bolus.

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After the initial induction bolus had been administered it was concluded that the anaesthetic depth was excessive for accurate laryngeal examination. A second pilot study was necessary where the induction bolus could be reduced and the induction agent carefully titrated to an appropriate depth of anaesthesia.

For pilot study 2, the same experimental procedure was followed as outlined for pilot study 1. The pre-determined volume (based on 50% of the recommended induction doses in unpremedicated dogs) was set individually for each patient and administered over a predefined induction and top-up bolus time period. Each dog was randomly selected and placed on the procedure table for the experimental procedure.

Pilot study 2

- Dog 1: Thiopental 2.5%; induction bolus 7.5 mg/kg; top-up bolus 1.8 mg/kg.
- Dog 2: Propofol 1%; induction bolus 3 mg/kg; top-up bolus 0.75 mg/kg.
- Dog 3: Alfaxalone 1%; induction bolus 1.5 mg/kg; top-up bolus 0.4 mg/kg.
- Dog 4: Not induced.

These induction and top-up boluses allowed the anaesthetic agent to be carefully titrated to a depth of anaesthesia appropriate for laryngeal examination. These dosages were carried over into the study experimental procedure.

b) Study

i. Pre-anaesthetic period

Within seventy two hours prior to data collection, the six Beagles were collected from OTAU and a thorough physical examination, basic blood tests and blood smear evaluation were performed. In the event that this was not performed on the day prior to data collection, the dogs were returned to their designated OTAU enclosures.

The six Beagles were collected from OTAU on the afternoon prior to the morning of data collection. The dogs were placed in designated cages in the small animal surgery ward, at the OVAH.

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An eight-to-twelve hour fasting period (with free access to water) was performed prior to data collection to ensure that no food was present in the animal's stomach immediately prior to induction of anaesthesia.

On the day of data collection, the first two dogs were randomly selected by a final year veterinary science student and taken through to the OVAH theatre complex. They were placed in designated cages in the induction area one hour prior to induction of anaesthesia. This allowed sufficient time to decrease the excitement levels prior to anaesthesia. The first animal assigned to be anaesthetised was randomly chosen (out of the two) by an assistant. This animal was handled as stated in "Anaesthetic induction period" point b ii) below, thus, leaving its cage in the induction area unoccupied, and whereupon the next animal was transferred from the small animal surgery ward to the theatre complex. This rotational flow of dogs into and out of the theatre complex was repeated until all six experimental animals had participated in the data collection for that day.

Prior to the experimental procedure, the induction agents were drawn up into a 20 mL syringe and a low-volume extension set (Extension set Ref 011-C150, Poly Medicure Ltd/ICU medical SA, Johannesburg, RSA) was attached with a needle (1" 20 Gauge) at the end. An additional 150% of each induction agent was drawn up into each syringe to account for individual variations in anaesthetic requirements. The extension set was covered with 25 mm adhesive tape to disguise the contents (Photo 1). Each induction agent was administered using a separate electronic syringe driver (B. Braun Perfusor® Space, B. Braun Medical (Pty) Ltd, Johannesburg, RSA). The pre-determined volume (based on pilot studies undertaken prior to commencement of

Photo 1. Low volume extension set covered with adhesive tape

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experimental procedure) of the induction bolus and top-up boluses were set individually for each patient based on the bodyweight. The administration time of the induction bolus and top-up boluses was standardised. Syringe drivers were stacked behind a pre-hung blanket ("blanket screen") to obscure the laryngeal evaluator's view (Photo 2).

Photo 2. Syringe drivers stacked behind pre-hung blanket

ii. Anaesthetic induction period

The dog was placed on the procedure table (Photo 3) and an intravenous catheter was placed, by the anaesthetist, in the lateral saphenous vein approximately 5-10 minutes prior to induction. A stopper with an injection port was secured to the catheter, through which the induction agent could be administered, flushing after each bolus. Before induction of anaesthesia, each dog was subjectively assessed as being excited (active on table; wrestling physical restraint) or not.

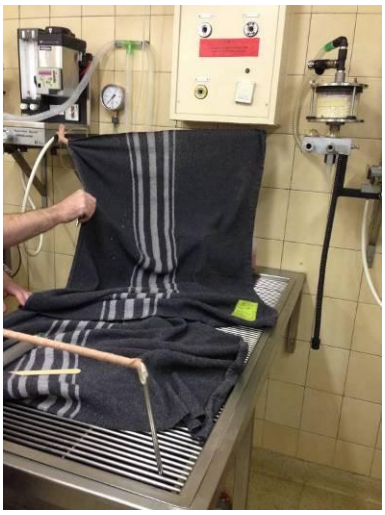

Photo 3. Procedure table with "blanket screen" and padded anaesthetic frame in position

The **induction end-point** was defined as an anaesthetic depth that allowed the jaw to be opened easily with a regular breathing pattern and normal visual thoraco-abdominal respiratory effort, without chewing or conscious swallowing attempts and absence of the lateral palpebral reflex. Conscious swallowing was defined as swallowing with head avoidance movement away from laryngeal examiner. Each dog was administered three induction agents, in a random order, with at least a one week washout period between treatments, as follows:

- Thiopental 2.5% (Thiopental Sodium Fresenius 0.5 g/20 mL; Fresenius Kabi South Africa (Pty) Ltd; Midrand, RSA); induction bolus 7.5 mg/kg; top-up bolus 1.8 mg/kg.

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- Propofol 1% (Fresenius Propoven 1% (50 mL); Fresenius Kabi South Africa (Pty) Ltd; Midrand, RSA); induction bolus 3 mg/kg; top-up bolus 0.75 mg/kg.
- Alfaxalone 1% (Alfaxan®-CD RTU; Jurox (Pty) Ltd/Kyron laboratories (Pty) Ltd; Johannesburg, RSA); induction bolus 1.5 mg/kg; top-up bolus 0.4 mg/kg.

The induction bolus was administered intravenously, via a syringe driver, over one minute to effect. If, at any point during this first minute, the dog could no longer lift its head, the syringe driver was placed on standby (stopping infusion of the induction bolus) and reflexes (palpebral and withdrawal after toe pinch) and jaw tone were assessed after waiting 10 seconds. If the dog was still conscious following administration of the full induction bolus over one minute, a 10 second interval was given before checking the reflexes and jaw tone.

If, after any of the two situations above, the dog was deemed too light, a top-up bolus (25% bolus of the calculated induction bolus) was administered over a 10 second period and the reflexes and jaw tone were re-evaluated after waiting a further 10 seconds. This sequence continued until the induction end-point was achieved. Once achieved, the induction sequence was stopped. The dog was positioned in sternal recumbency with the maxilla hooked over a padded anaesthetic frame of an adequate height to open the mouth easily (Photo 4).

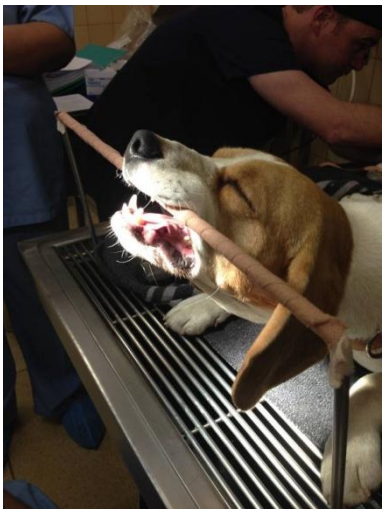

Photo 4. Positioning of animal for oral laryngoscopy

Rescue interventions taken during the experimental procedure

Access to a well-stocked anaesthesia crash cart was readily available. Among other items, the crash cart had a range of endotracheal tubes, emergency drugs (including atropine and adrenalin), isotonic crystalloid fluids and an ambu-bag. Immediate action was taken if the dog had one or more of the following:

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1. Prolonged apnoea defined as the absence of spontaneous ventilation (either small, shallow breaths with a weak attempt or vital breaths with maximal inspiratory effort and an increased abdominal component on inspiration) for longer than 1 minute.
2. Desaturation of haemaglobin defined as cyanosis of mucous membranes.
3. Excitement and paddling during induction.
4. Bradycardia defined as a heart rate of less than 60 beats per minute.
5. Cardiopulmonary arrest defined as failure of the heart to contract effectively or at all as evidenced by absence of normal heart sounds on thoracic auscultation, no palpable peripheral pulses and an absence of spontaneous ventilation.

The following actions were to be taken, respectively:

1. Endotracheal intubation and mechanical ventilation.
2. Oxygen support provided via flow-by nasogastric feeding tube at 3 L/minute and/or endotracheal intubation and mechanical ventilation if required.
3. Administer 25% of initial induction bolus to induce a deeper plane of anaesthesia.
4. Atropine (0.04 mg/kg IV).
5. Begin Cerebral Cardiopulmonary Resuscitation immediately, as per the RECOVER published guidelines (Fletcher et al. 2012).

iii. Laryngeal examination

Continual examination of the larynx commenced once an adequate anaesthetic depth had been reached. Laryngeal examination was performed by a single individual (the primary investigator) who was blinded to the anaesthetic protocol used.

Laryngeal examination was performed using simple transoral laryngoscopy. The animal was positioned in sternal recumbency with the maxilla hooked over a padded anaesthetic frame of an adequate height to open the mouth easily (Photo 4). The mouth

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was opened and the distal tip of an illuminated laryngoscope blade (size 3 Macintosh) was placed ventral to the epiglottis into the vallecula at the base of the tongue, and directed ventrally to expose the rima glottidis. A tongue depressor was positioned to lift the soft-palate and improve the visualisation of the rima glottidis (photo 5). Care was taken to avoid placing excessive pressure on the base of the tongue with the laryngoscope blade. Excessive traction of the tongue and any manual stimulation of the arytenoid cartilages was also avoided.

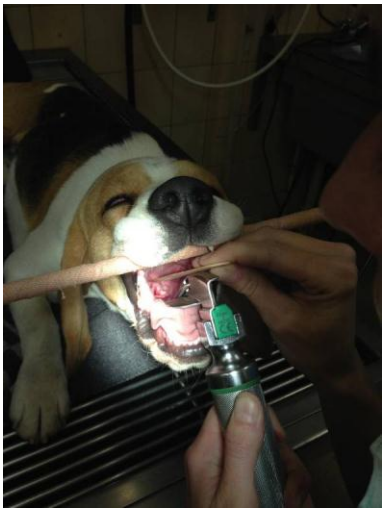

Photo 5. Exposure of rima glottidis during oral laryngoscopy examination

The primary investigator's ability to assess whether arytenoid abduction activity was co-ordinated with breathing was facilitated by the anaesthetist verbally indicating the onset of inspiration for each breath. Examination of the larynx continued until the dog recovered from anaesthesia. The **examination end-point** was defined as the point where further restraint, of the dog, would result in injury to the dog, equipment or personnel.

iv. Post-anaesthetic period

Upon recovery from anaesthesia, the patient was moved to a heated recovery room in the OVAH theatre complex. A final year veterinary science student monitored the animal's recovery by recording respiratory rate, heart rate and temperature every 5 minutes until the animal could lift its head. If the animal was intubated, then the final year veterinary science student deflated the cuff of the endotracheal tube and extubated the animal when it regained its swallowing reflex.

The animals were returned to their designated OTAU enclosures by late afternoon on the day of data collection once they had recovered fully from anaesthesia. Full recovery from anaesthesia was defined as an animal that was fully conscious of its environment, able to stand unassisted, walk without signs of ataxia and able maintain normal homeostasis.

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Data capture

Each dog was administered three induction agents, over the data collection phase of the study, with at least a one week washout period between treatments.

a) Anaesthesia related data

The time from the start of administering the induction bolus until commencement of laryngeal examination was recorded and classified as the induction time. The presence of excitement (yes, no) and number of top-up boluses required to reach an adequate depth of anaesthesia were recorded. The total dose administered over the induction time was calculated for each induction agent and converted to a dose rate (mg/kg/minute) for each dog. The time from the start of administering the induction bolus until the examination end-point was classified as the **examination time**. Data was captured if any rescue interventions were necessary during the experimental procedure.

b) Laryngeal examination data

The period of laryngeal examination was divided into three time periods, namely: **Time 1** (Induction) – time from starting laryngeal examination to 2 minutes following commencement of examination; **Time 2** (early recovery) – from 2 minutes following commencement of laryngeal examination to 5 minutes or examination end-point, whichever came first; and **Time 3** (late recovery) – from 5 minutes following commencement of laryngeal examination until examination end-point. The total number of arytenoid abductions and number of vital breaths were recorded per time period of examination. A **vital breath** was defined as a maximal inspiratory effort with an increased abdominal component on inspiration.

A similar subjective scoring system as described by Gross *et al.* (2002) and McKeirnan *et al.* (2014) was used for evaluation of the laryngeal examination. Two aspects of the laryngeal examination were subjectively scored, namely: 1) laryngeal exposure score, and 2) laryngeal function score (Table 1). The first scoring system determined the ability to visualise the rima glottidis during laryngeal examination. The second scoring system

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specifically looked at grading the quality of arytenoid abduction activity and was adapted, for dogs, from the Havemeyer grading system used in horses (Robinson 2004).

Table 1. Composite scoring system used to subjectively evaluate the ability to visualise the rima glottidis and determine the quality of arytenoid abduction activity during oral laryngoscopy in dogs anaesthetised with thiopental, propofol and alfaxalone.

Definitions of laryngeal examination responses

1. 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

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2. Laryngeal function score (0A best score; 3B worst score)

Category Score	Description	Subcategory
0	All arytenoid cartilage movements are synchronous, staccato and symmetrical and full arytenoid cartilage abduction can be achieved and maintained.	A: Arytenoid abduction activity is noted with every inspiratory effort.
		B: Arytenoid abduction activity is not noted with every inspiratory effort.
1	All arytenoid cartilage movements are synchronous and symmetrical. Full abduction of the arytenoid cartilages is not achieved.	A: Moderate abduction of arytenoid cartilages (15-30° to the midline of the *rima glottidis*).
		B: Sluggish abduction of arytenoid cartilages (<15° to the midline of the *rima glottidis*).
2	Arytenoid cartilage movements are asynchronous and/or larynx is asymmetrical at times but full arytenoid cartilage abduction can be achieved and maintained	A: Transient asynchrony, flutter or delayed movements are seen.
		B: 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.
3	Complete immobility of the arytenoid cartilages and vocal fold.	A: Unilateral i) Right ii) Left
		B: Bilateral

3. Paradoxical motion of arytenoid cartilages

Score	Definition
0	Absent
1	Present

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The laryngeal exposure score components were assigned to designated people as follows: the anaesthetist assigned breathing score at 2 minutes and 5 minutes after commencement of the laryngeal examination, and at the examination end-point. Normal respiratory rate was set at ≥ 10 breaths per minute, moderate rate at < 10 and > 4 breaths per minute and slow rate at ≤ 4 breaths per minute. The primary investigator assigned the jaw tone score and determined the presence/absence of swallowing attempts and laryngospasm for each animal at 2 minutes and 5 minutes after commencement of the laryngeal examination, and at the examination end-point. At the same three time points, the primary investigator also allocated a laryngeal function score. At recovery, the total laryngeal exposure scores and laryngeal function scores were captured on data collection forms by the primary investigator.

The terminology used to define the simple transoral laryngoscopic appearance of the arytenoid abduction activity were defined in Table 2 (Robinson 2004).

Table 2. Definitions of arytenoid activity used to describe arytenoid movement observed during oral laryngoscopy in dogs anaesthetised with thiopental, propofol and alfaxalone.

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.

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Data collection

The primary investigator was responsible for manually collecting all data during the study. The written data were captured on various data capture sheets and filed within a research file containing the data of all the experimental animals who participated in this study. This file with all its data capture sheets will be stored for a period of five years in the OVAH, section of anaesthesiology. The captured data were stored electronically on an excel spreadsheet specifically designed for the study. A copy was stored on the primary investigator's and supervisor's computers for safe keeping and storage. Once the final data capture excel document was completed then copies were saved onto five compact discs and distributed to all the co-investigators of the section of anaesthesiology and small animal surgery for safe keeping.

Statistical analysis

Data were assessed for normality through the plotting of histograms, calculation of descriptive statistics, and the Anderson-Darling test for normality. Data were subsequently described using median (range) and boxplots due to apparent lack of normality. Laryngeal exposure score and laryngeal function score outcomes were counted within each induction agent and time period and reported in a frequency table. The number of arytenoid motions and vital breaths were rank-transformed and then compared among treatments using a linear mixed model (fixed factors: induction agent and time period; random factor: dog) with the p values from the multiple pairwise post-hoc comparisons adjusted using Tukey's correction. The total number of swallowing attempts, top-up boluses required and the induction and examination times were compared among induction agents using Friedman tests. The number of top-up boluses administered to excited dogs was compared to the number administered in not excited dogs using a Mann-Whitney U test. Spearman's rho was used to estimate correlations between the number of vital breaths and number of arytenoid movements. Data were analysed using commercially available software (MiniTab

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17.1.0; MiniTab Incorporated; Pennsylvania, USA) and results interpreted at the 5% level of significance.

Ethical considerations

The major ethical consideration of this study was to ensure that purpose-bred animals were used responsibly for research projects. Thus, no collection of data commenced until approval by the University of Pretoria's research and animal ethics committees had been received. Furthermore, it was important to discuss (via email correspondence and verbally) the research project with the members of the OTAU advisory committee to allow them to give informed consent for the use of the OTAU Beagles in this experimental procedure.

In the event of disease, death or loss of a dog, the primary investigator had to inform OTAU within as short a period as reasonably possible. In the event of death during the study process a post-mortem examination was to be conducted to determine the cause of death. No such events were encountered during the data collection phase of the study.

People who participated in the study were not obliged or forced. They participated by invitation only. The participants did not receive any financial compensation.

None of the procedures or drugs used during the study were considered harmful to dogs or humans.

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Results

The median (range) number of top-up boluses required for thiopental, propofol and alfaxalone were 5.0 (3.0, 6.0), 5.0 (3.0, 7.0) and 5.0 (2.0, 7.0) boluses, respectively (p = 0.554). Also, the number of top-up boluses used in excited dogs were 6.0 (5.0, 7.0) and not significantly different to unexcited dogs who required 5.0 (2.0, 7.0) boluses (p = 0.1032). The total number of unconscious swallowing attempts median (range) for thiopental, propofol and alfaxalone were 0 (0.0, 1.0), 1 (0.0, 1.0) and 0 (0.0, 1.0), respectively (p = 0.096).

The median (range) induction time was 2.8 (2.0, 3.0), 2.6 (2.0, 3.3) and 2.5 (1.7, 3.3) minutes for thiopental, propofol and alfaxalone, respectively (p = 0.727). The median (range) dose rate required to achieve induction of adequate depth of anaesthesia to conduct an accurate simple transoral laryngeal examination was 6.3 (6.0, 6.6), 2.4 (2.4, 2.4) and 1.2 (1.2, 1.2) mg/kg/minute for thiopental, propofol and alfaxalone, respectively. Therefore, the median (range) total dose administered over the induction time was 17.8 (13.2, 18.8), 6.8 (5.3, 8.3) and 3.2 (2.3, 4.1) mg/kg for thiopental, propofol and alfaxalone, respectively.

The number of arytenoid motions (Figure 1a; p = 0.019) and vital breaths (Figure 1b; p = 0.044) observed among the induction agents over the three time periods differed significantly (interaction: induction agent x time period). However, when comparing the induction agent effect alone, there was no significant difference for the total number of arytenoid motions (p = 0.662) and vital breaths (p = 0.786) among the induction agents. The number of arytenoid motions demonstrated a moderate positive correlation to the number of vital breaths observed (r_s = 0.550; p < 0.001).

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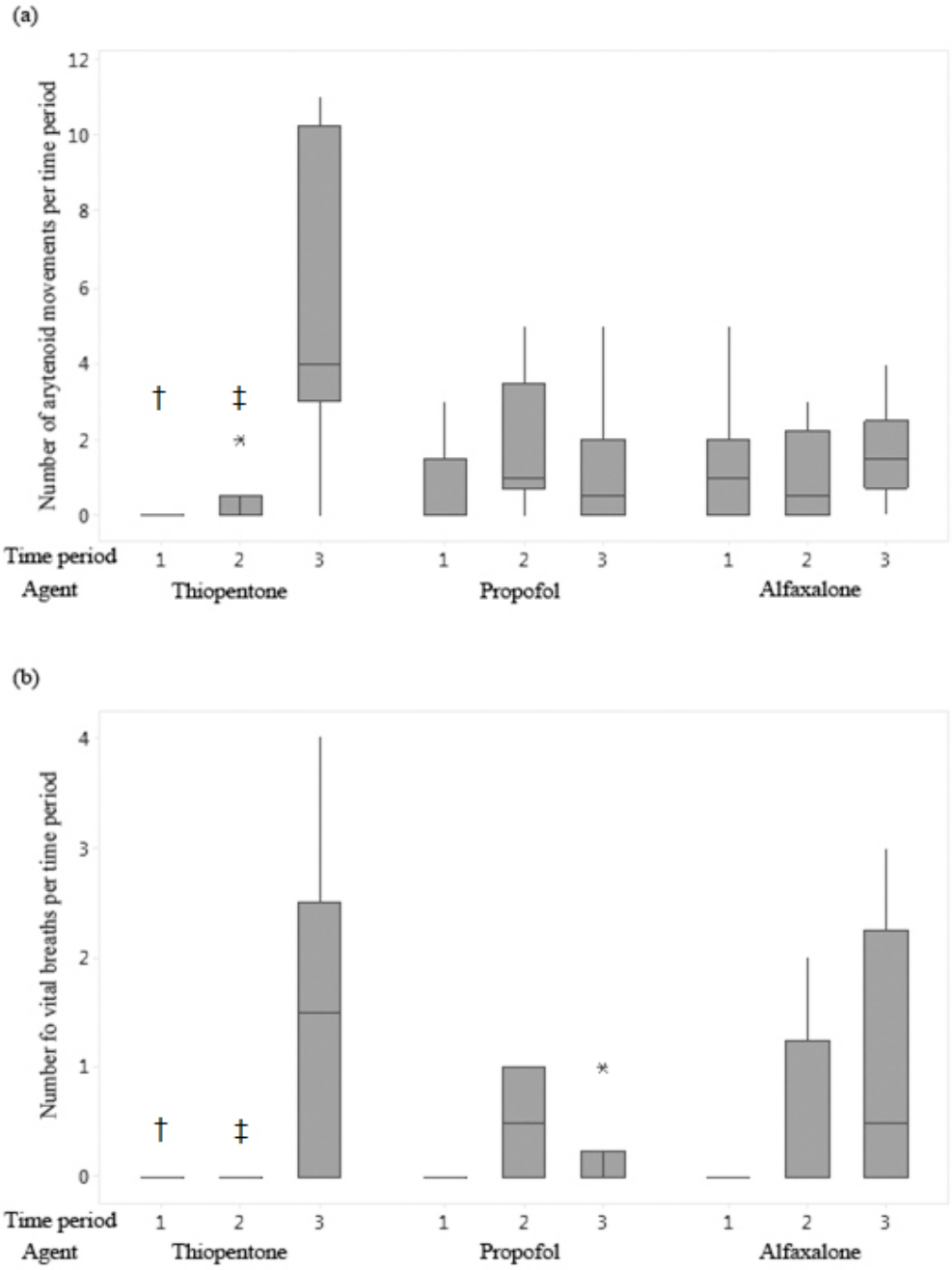

Figure 1. Boxplot number of arytenoid motions (a) and number of vital breaths (b) vs. anaesthetic agent and time period observed in beagle dogs undergoing oral laryngoscopic examination. Where, † thiopental Time 1 (time from starting examination to 2 minutes) significantly different from Time 3 (from 5 minutes after starting examination until examination end-point); ‡ thiopental Time 2 (from 2 to 5 minutes after starting examination) significantly different from Time 3.

The * refers to outliers in the specified time periods.

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The laryngeal exposure score was consistently lower for thiopental induced dogs over the duration of the entire examination which translates into better examination conditions to detect arytenoid motion (Table 3). However, the laryngeal function score was consistently higher for thiopental, which demonstrated more partial abduction and asynchronous arytenoid activity over time. Furthermore, propofol and alfaxalone demonstrated consistently higher laryngeal exposure scores (more difficult to visualise and examine the rima glottidis), yet the conditions were adequate for diagnostic purposes. Propofol and alfaxalone demonstrated lower laryngeal function scores which translated into visualising more full abduction and synchronous arytenoid activity over time in comparison to thiopental.

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Table 3. Frequency table summarising the laryngeal exposure score and laryngeal function score outcomes among thiopental, propofol and alfaxalone induced dogs undergoing an oral laryngoscopy examination, over Time 1 (time from starting examination to 2 minutes); Time 2 (from 2 to 5 minutes after starting examination); and Time 3 (from 5 minutes after starting examination until examination end-point).

Laryngeal exposure score									
Score	0	1	2	3	4	5	6	7	8
Thiopental									
Time 1			3	3					
Time 2	1		2	3					
Time 3			1	4		1			
Propofol									
Time 1			3	2			1		
Time 2				2	4				
Time 3				1	4	1			
Alfaxalone									
Time 1			2	3		1			
Time 2			2	3	1				
Time 3			3	1	1		1		

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Table 3 continued…

Laryngeal Function Score								
Score	0		1		2		3	
	A	B	A	B	A	B	A	B
Thiopental								
Time 1								6
Time 2						1		5
Time 3		2				3		1
Propofol								
Time 1		1				1		4
Time 2		3				2		1
Time 3		2				2		2
Alfaxalone								
Time 1		2		1		1		2
Time 2		2				1		3
Time 3		3				2		1

The examination times were significantly different among all treatments (p = 0.016) with the median (range) for thiopental, propofol and alfaxalone being 14.1 (8.0, 41.8), 5.4 (3.3, 14.8) and 8.5 (3.8, 31.6) minutes to examination end-point, respectively.

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Discussion

Diagnosis of laryngeal paralysis relies on an accurate laryngeal examination under a light plane of anaesthesia combined with historical and physical examination findings. The ideal anaesthetic plane should facilitate retraction of the jaws so that the laryngoscope can be inserted and the rima glottidis visualised, but should maintain the laryngeal reflexes and cause minimal respiratory depression (McKeirnan et al. 2014). In this study, each induction agent was carefully titrated to reliably achieve evaluation of the rima glottidis under a light plane of anaesthesia. Despite the total number of arytenoid motions over total time not differing among the induction agents, there was a significant difference in the number of arytenoid motions observed at the three time periods. This finding highlights important characteristic differences among the induction agents to achieve a light plane of anaesthesia and preserve arytenoid function.

Textbook doses of thiopental (12-30 mg/kg), propofol (± 6.5 mg/kg) and alfaxalone (2-3 mg/kg) for induction of general anaesthesia in a healthy unpremedicated dog are recommended to be administered intravenously over a one minute period or until a predefined desired effect (Berry 2015, Clarke, Trim & Hall 2014, Dugdale 2010, Waelbers, Vermoere & Polis 2009). The most common desired effect is to achieve hypnosis and tracheal intubation, however, this desired effect would have been too deep to allow examination of arytenoid cartilage abduction activity. Previous studies using thiopental and/or propofol for oral laryngeal examination report total doses ranging from 10.4 to 14.0 and 3.6 to 5.6 mg/kg, respectively (Gross et al. 2002, Jackson et al. 2004, McKeirnan et al. 2014). The total doses reported in the present study were similar or less than textbook ranges yet comparable to previous studies (Gross et al. 2002, Jackson et al. 2004, McKeirnan et al. 2014, Nelissen et al. 2012) and adequate to reliably achieve oral laryngeal examination. The rate of administration is an important factor whereby a rapid administration rate tends to increase the incidence of apnoea (Amengual et al. 2013). This

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would adversely affect an accurate laryngeal examination. Recommended dose rates to achieve light anaesthesia for laryngeal examination in dogs have not been documented. The narrow difference between the reported induction time medians (range) suggests that equi-effect drug rates were achieved amongst the induction agents and the reported dose rates can be recommended for future studies.

Thiopental induction allowed excellent conditions for visualisation of the rima glottidis, but resulted in sluggish arytenoid motion in the early stages of laryngeal examination when compared to propofol and alfaxalone. This was similar to a previous study where thiopental (14.0 ± 2.26 mg/kg) demonstrated more predictable arytenoid motion in late recovery compared to propofol (5.6 ± 1.14 mg/kg) in their unpremedicated treatment groups (Jackson et al. 2004). Yet, this contrasted findings reported by Gross et al (2002) where thiopental (10.4 ± 1.1 mg/kg) demonstrated excellent exposure and arytenoid movement, comparable to propofol (3.6 ± 0.8 mg/kg). The contrasting findings are likely due to the total dose administered. Gross et al (2002) did make use of an intravenous butorphanol (0.5 mg/kg) and glycopyrrolate (0.01 mg/kg) premedication five minutes prior to induction which could explain the lower total doses reported. Furthermore, the rate of administration was not clearly described in either study therefore making final comparison difficult (Gross et al. 2002, Jackson et al. 2004). Dugdale et al (2005) reported that a slow infusion rate (2.5 mg/kg/minute) of thiopental resulted in a lower total dose (7.5 mg/kg) to achieve induction of general anaesthesia and tracheal intubation compared to a faster infusion rate of 10 mg/kg/minute (11.0 mg/kg). This observation could explain the high reported total dose of thiopental in the present study where a moderately fast infusion rate was used. Dugdale et al. (2005) reported that the slow infusion rate had a higher incident of excitement compared to the faster rate during thiopental induction. This may be unfavourable under conditions where laryngeal examination is being performed to confirm a suspected laryngeal paralysis. In such animals, excitement during the induction phase may lead to dyspnoea and

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potentially life threatening complications. No severe excitement (paddling and whining) was noticed in the present study, therefore a slightly lower thiopental infusion rate (5 mg/kg/minute) than the one recommended here may provide adequate visualisation and shorter examination times without undesirable induction excitement. The paucity in the literature detailing similar studies on laryngeal examination in dogs precludes further in-depth interpretation and comparison of our total dose and dose rate results.

It is important to consider the use of a subjective scoring system for laryngeal function determination in this study. More objective methods such as electromyography and change in NGGA have been investigated for evaluation of arytenoid abduction activity. Laryngeal electromyography is recognised as a valuable diagnostic tool in human medicine, but its routine use is uncommon. It requires technical equipment as well as specialised knowledge of methodology and interpretation. Furthermore, its validity and clinical application is still questionable within human medicine (Volk et al. 2012). Change in NGGA has been applied in few studies investigating the effect of anaesthetic protocols on laryngeal examination in healthy animals (Jackson et al. 2004, Nelissen et al. 2012). Nelissen *et al* (2012) found that it was more sensitive at detecting laryngeal motion in cats than subjective methods. In that study, change in NGGA confirmed laryngeal motion in all study animals, however, a subjective scoring system revealed no arytenoid abduction activity in 6 of these animals (n = 35) (Nelissen et al. 2012). To allow the assessment NGGA, specialised equipment (videoendoscope) is necessary for data capture. Furthermore, specific image processing software is required for final calculation of change in NGGA. The authors of this study did not have access to the required equipment, expertise and/or software to allow this objective scoring system to be used. Thus it was decided to use subjective methods to evaluate the effect of the anaesthetic protocols on laryngeal motion. In clinical practice diagnosis of laryngeal paralysis is dependent on a subjective laryngeal examination. It was the intent of the authors that the study conditions should closely resemble the realities of clinical practice.

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Using a subjective scoring system it was also possible for arytenoid abduction activity to be evaluated in conjunction with respiratory cycle and vital breaths (McKeirnan et al. 2014). This scoring system has not been validated, but was adopted from previous studies and adjusted using the grading system for laryngeal function in unsedated horses (Gross et al. 2002, McKeirnan et al. 2014, Robinson 2004).

The examination times were significantly different among all treatments with the median examination time of thiopental and alfaxalone being longer than that of propofol. This implies that time period three would be longer for thiopental when compared to that of propofol and may account for the higher number of arytenoid motions and vital breaths observed during this time period. It is important to note that all the animals that participated in this study were of a similar body condition score (Boveri, Brearley & Dugdale 2013), thus obviating the effect of body condition on the pharmacodynamics of each induction agent. The longer examination times are most likely due to variations in drug metabolism where thiopental and alfaxalone are more dependent on hepatic metabolism compared to propofol (Waelbers, Vermoere & Polis 2009). Hence, for the full benefit of thiopental to be reached, the laryngeal examination would have to be extended to recovery from anaesthesia. Which, in the current study, might take up to 42 minutes from initiation of the induction sequence. This is not always practical as it implies that the clinician should perform a more time-consuming laryngeal examination and it also makes surgery under the same anaesthetic event less desirable.

A limitation of this study would be the small sample size (n = 6). This limitation was obviated by performing a randomised, cross-over trial. However, our sample size compared favourably with previous prospective, randomised, blinded, cross-over trials where six (Jackson et al. 2004) and eight (Gross et al. 2002) animals were used.

A further deficiency of this study would be the omission of doxapram from our experimental design. Doxapram is a general central nervous stimulant that results in a

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transient increase in respiratory rate and tidal volume by its excitatory effect on the inspiratory and expiratory centers of the medulla. Its administration, during laryngeal examination, allows better differentiation between normal dogs and those with laryngeal paralysis (Tobias, Jackson & Harvey 2004). Its administration was purposefully excluded from this study, as the experimental design was such that the effect of thiopental, propofol and alfaxalone on laryngeal motion could be investigated by removing as many variables as possible. Further research is warranted to investigate the effects of Doxapram using a similar esperimental design.

Follow-up studies, using a similar experimental design and induction dose rates as given in this study, are indicated to determine the effects of premedication on arytenoid motion. Furthermore, the effects of mechanical and chemical stimulation of arytenoid motion should also be investigated.

Conclusion

The ideal anaesthetic protocol for laryngeal function evaluation should allow adequate depth of anaesthesia for visualisation of the rima glottidis, cause minimal respiratory depression, maintain laryngeal reflexes and allow the evaluation to be performed in as short a time period as possible. It can be concluded that there was no significant difference in the total number of arytenoid motions among the induction agents. However, at the dose rates used in this study, propofol provided adequate conditions for evaluation of the larynx within a shorter examination time which may be advantageous during laryngoscopy in dogs.

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Addenda

Inclusion criteria for experimental animals

Patient Name:		**Date:**
Microchip number:		
Experimental animal number:		
Species:	**Breed:**	**Weight:**
Sex:	**Age:**	**Pregnant: Yes/No**
Vaccination history:	Full vaccination history	Vaccinations not up-to-date
Temperature:	**Pulse:**	**Respiratory rate:**
PCV (L/L):	**TSP (g/L):**	**Serum creatinine (µmol/L):**

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		
TSP more than 75 g/L		
Serum creatinine less than 59µmol/L		
Serum creatinine more than 109µmol/L		
N.B. Answering "yes" in any of the above questions excludes the animal from further participation in the study.		

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Randomisation of treatments

A Randomization Plan from http://www.randomization.com

1. **Elvis**

Propofol

Thiopental

Alfaxalone

2. **Watson**

Thiopental

Alfaxalone

Propofol

3. **Einstein**

Alfaxalone

Propofol

Thiopental

4. **Minke**

Propofol

Alfaxalone

Thiopental

5. **Cornelie**

Alfaxalone

Thiopental

Propofol

6. **Kalika**

Thiopental

Propofol

Alfaxalone

6 subjects randomized into 1 block.

To reproduce this plan, use the seed 19446.

Randomization plan created on 5/12/2015, 1:43:49 PM.

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Composite scoring system used to subjectively evaluate laryngeal function

Patient Name:	**Date:**
Microchip number:	
Experimental animal number:	

1. Laryngeal exposure score (0-8)

	Score	**Definition**
a) Breathing score		
	0	Deep respirations, normal respiratory rate (≥10 breaths per minute), strong attempt
	1	Moderate respirations, respiratory rate (<10 and >4 breaths per minute) and attempt
	2	Shallow respiration, slow respiratory rate (≤4 breaths per minute), weak attempt
	3	No spontaneous respiration
b) Jaw tone score		
	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		
	0	Absent
	1	Present
d) Laryngospasm		
	0	Absent
	1	Present

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2. Laryngeal function score (0-3)			
Score	**Description**	**Subcategory**	
0	All arytenoid cartilage movements are synchronous, staccato and symmetrical and full arytenoid cartilage abduction can be achieved and maintained.	A: Arytenoid abduction activity is noted with every inspiratory effort.	
		B: Arytenoid abduction activity is not noted with every inspiratory effort.	
1	All arytenoid cartilage movements are synchronous and symmetrical. Full abduction of the arytenoid cartilages is not achieved.	A: Moderate abduction of arytenoid cartilages (15-30° to the midline of the *rima glottidis*).	
		B: Sluggish abduction of arytenoid cartilages (<15° to the midline of the *rima glottidis*).	
2	Arytenoid cartilage movements are asynchronous and/or larynx is asymmetrical at times but full arytenoid cartilage abduction can be achieved and maintained	A: Transient asynchrony, flutter or delayed movements are seen.	
		B: 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.	
3	Complete immobility of the arytenoid cartilages and vocal fold.	A: Unilateral	i) Right
			i) Left
		B: Bilateral	
4. Paradoxical motion of arytenoid cartilages			
0	Absent		
1	Present		
Comments:			

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Reporting

a) Publications

- Smalle, T.M., Hartman, M.J., Bester, L., Buck, R.K., Fosgate, G.T., Zeiler, G.E. 'Effects of thiopentone, propofol and alfaxalone on laryngeal motion during oral laryngoscopy in healthy dogs', *Veterinary Anaesthesia and Analgesia.* Submitted: 02/02/2016. Status: Accepted and in Press.

b) Oral presentations

- Smalle, T.M., 'Effects of thiopentone, propofol and alfaxalone on laryngeal motion during oral laryngoscopy in healthy dogs', European College of Veterinary Surgeons Congress, Lisbon, Portugal. Presentation date: 09/07/2016 15:45 GMT +1. Status: Presented.
- Smalle, T.M., 'Effects of thiopentone, propofol and alfaxalone on laryngeal motion during oral laryngoscopy in healthy dogs', Faculty day, Faculty of Veterinary Science, University of Pretoria, Pretoria. Presentation date: 25/08/2016 08:45 GMT. Status: Presented.

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