Accuracy of Multichannel Intraluminal Impedance and pH Testing for Detection of Gastro-oesophageal Reflux (GOR) in Anaesthetised Dogs

by

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Declaration of Originality

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Topic of work: Comparing methods for detection of gastro-oesophageal reflux in anesthetised dogs

Declaration

1. I understand what plagiarism is and am aware of the University's policy in this regard.

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3. I have not used work previously produced by another student or any other person to hand in as my own.

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List of Abbreviations

%	Percentage
cm	Centimetre(s)
cm H_20	Centimetre(s) water
CRI	Continuous rate infusion
ARRIVE	Animal Research: Reporting of *In Vivo* Experiments
ASA	American Society of Anesthesiologists
AUC	Area under the curve
CRI	Constant rate infusion
GOR	Gastro-oesophageal reflux
GORD	Gastro-oesophageal reflux disease
HCL	Hydrochloric acid
ID	Identification number
IM	Intramuscular
IV	Intravenous
Kg	Kilogram(s)
LED	Light emitting diode
LOS	Lower oesophageal sphincter
LOSP	Lower oesophageal sphincter pressure
NGD	No gastro-oesophageal reflux detected
mg kg^{-1}	Milligram(s) per kilogram
mL	Millilitre(s)
mL kg^{-1} hr	Millilitre(s) per kilogram per hour
mL kg^{-1} minute	Millilitre(s) per kilogram per minute
mg mL^{-1}	Milligram(s) per millilitre
MII	Multiple intraluminal impedance
mm	Millimetre(s)
OVAH	Onderstepoort Veterinary Academic Hospital
pH	Negative log of hydrogen ion concentration
pH-MII	pH-metry with multiple intraluminal impedance
ROC	Receiver operator characteristic

SC	Subcutaneous

Abstract

Abstract

Objective To compare the sensitivity and specificity of pH with multiple intraluminal impedance (pH-MII), pH-metry (pH) alone and multiple intraluminal impedance (MII) alone to direct observation of GOR by endoscopy in anaesthetised dogs. We hypothesized that pH-MII is more sensitive and specific in detecting GOR in anaesthetised dogs compared to pH or MII alone.

Study Design A prospective comparative trial in a live canine model

Animals Thirty-five dogs (22 females, 13 males) of various breeds undergoing elective pelvic limb orthopaedic procedures. The mean (range) mass and age were 31.9 (14.0 to 40.0) kg and 5.6 (0.8 to 12.0) years, respectively.

Methods All dogs were premedicated with medetomidine and morphine, anaesthesia was induced with propofol and maintained on isoflurane in oxygen. A monitoring assembly consisting of an endoscopy camera, endotracheal tube and a disposable flexible pH-MII catheter, was utilized to measure oesophageal pH, MII and directly visualise reflux. Visual reflux was assigned a score (0: none; 3: severe) and pH was recorded on a data capture sheet. Reflux was considered to have occurred whenever oesophageal pH was below 4.0 or above 7.5, device software analyzing MII data detected fluid refluxate or a visual reflux score of 2 or 3 were assigned. ROC analysis was used to determine sensitivity and specificity for each monitoring method to detect GOR. Area under the curve (AUC) was used to discern between an accurate method and non-accurate method (AUC $\leq$ 0.5), a method with poor accuracy (AUC 0.5-0.6), low accuracy (AUC 0.6-0.7), fair accuracy (0.7-0.8), good accuracy (AUC 0.8-0.9) and excellent accuracy (AUC $\geq$ 0.9).

Results Endoscopy identified GOR in 20 dogs (57%), pH-MII in 19 dogs (54%), pH alone in 13 dogs (37%), and MII alone in 12 dogs (24%). As planned, the AUC for the ROC of endoscopy was 1.0 and demonstrated 100% sensitivity and specificity, respectively. AUC analysis for the ROC curve showed fair accuracy for pH-MII and pH alone. pH-MII and pH alone demonstrated a sensitivity and specificity of 69% and 76%, and 71% and 75%, respectively. While MII demonstrated low accuracy with a sensitivity and specificity of 98% and 24%, respectively. Prevalence for detecting GOR events per measured data point was greatest in endoscopy (35%), followed by pH-MII (25%), then pH (21%) with the least detected in MII (7%). pH-MII and pH alone exhibited almost perfect agreement.

Conclusions and clinical relevance pH-MII is a reliable method for detecting GOR and emerges as a promising tool for future research. Endoscopy is reliable and provides the ability to subjectively quantify the volume of reflux, however, lacks the ability to discern the pH of the refluxate. pH alone misses reflux episodes with intermediate pH (4.1-7.4). Incorporation of impedance addresses some of the limitations associated with pH alone and enhances diagnostic accuracy.

Keywords Gastro-oesophageal reflux, dogs, pH, intraluminal impedance, endoscopy

1. Introduction

In dogs and cats, the vomiting reflex is suppressed at stage 3 plane 2 of inhalational anaesthesia, yet, they remain at risk of gastro-oesophageal reflux (GOR) during the maintenance phase of anaesthesia (Adams et al. 2015). Gastro-oesophageal reflux, defined as "the presence of fluids, not reaching the mouth or nose, in the oesophagus" (Fernandez Alasia et al. 2021), is a common occult complication in dogs and cats undergoing general anaesthesia. This phenomenon is typically undetected and involves a transient, retrograde flow of gastric contents into the oesophagus without associated vomiting and passive regurgitation (Ristic et al. 2017; Dugdale et al. 2020). According to Poiseulle's law, the primary driving force of fluid flow through a tube is linked to its pressure gradient. The pathophysiologic mechanism of GOR is believed to result from alterations in barrier pressure, specifically the difference between intragastric pressure and the lower oesophageal sphincter pressure (LOSP), thereby facilitating retrograde flow of gastric contents into the oesophageal lumen (Galatos et al. 2001; Dugdale et al. 2020). The tone of the lower oesophageal sphincter (LOS) is regulated by parasympathetic (vagal nerve) pathways and is crucial in preventing reflux episodes (Raptopoulos & Galatos 1997; Zacuto et al. 2012). Transient LOS relaxation is identified as the primary aetiology of GOR in anaesthetised dogs (Kessing et al. 2011).

Although GOR has been extensively investigated in anaesthetised dogs, the primary method of detection in veterinary medicine has been pH-metry alone (pH) (Galatos & Raptopoulos 1995a; Galatos & Raptopoulos 1995b; Wilson et al. 2005; Johnson 2014; Anagnostou et al. 2015; Savvas et al. 2009; Savvas et al. 2016; Viskjer & Sjöström 2017; Shaver et al. 2017; Lambertini et al. 2020; Appelgrein et al. 2022; Flouraki et al. 2022; Tsompanidou et al. 2022), with limited utilisation of pH-metry with multiple intraluminal impedance (pH-MII) (Zacuto et al. 2012; Tarvin et al. 2016). Numerous diagnostic modalities have been used to detect GOR in anaesthetised dogs; however, there is a lack of comparative studies aimed at determining the most sensitive and specific monitoring method. This paucity in the literature emphasizes the necessity for establishing a well-defined, effective approach for detecting GOR in anaesthetised dogs.

2. Literature Review

2.1 Gastro-oesophageal reflux

GOR can result in significant morbidity and mortality in dogs undergoing general anaesthesia and in severe cases may result in death or euthanasia due to secondary complications (Adamama-Moraitou et al. 2002; Wilson & Walshaw 2004). The incidence of GOR has previously been described to occur in 17.4% – 87.5% of dogs undergoing general anaesthesia (Galatos & Raptopoulos 1995a; Wilson et al. 2005; Lambertini et al. 2020). Passive regurgitation differs from GOR by the observable passive discharge of oesophageal or gastric contents from the oral cavity or nares (Lamata et al. 2012), whereas GOR is typically undetected unless actively monitored (Fernandez Alasia et al. 2021). The incidence of passive regurgitation in dogs under general anaesthesia seems to occur considerably less frequently than GOR with a reported incidence of between 0.42% and 5.5% (Galatos & Raptopoulos 1995a; Wilson et al 2005, Lamata et al. 2012; Savvas et al. 2016). GOR can lead to erosive damage to the oesophageal mucosa, thus is a major cause of postoperative oesophagitis and discomfort in dogs (Wilson 2005; Favarato et al. 2012; Benzimra et al. 2020). Severe cases can result in the formation of scar tissue and subsequent development of strictures (Wilson & Walshaw 2004; Self 2016), making peri-anaesthetic GOR a primary cause of oesophageal strictures in up to 65% of GOR cases in dogs (Galatos et al. 2001; Adamama-Maraitou et al. 2002).

Orad migration of gastro-oesophageal content can lead to aspiration, precipitating pneumonitis and pneumonia, which can be life-threatening (Galatos & Raptopoulos 1995a; Dugdale et al. 2020). Aspiration pneumonia and pneumonitis represent one of the most common causes of death-related complications in human general anaesthesia (Engelhardt & Webster 1999). In the United Kingdom, aspiration of gastric contents has been reported as the second most frequent airway complication in human anaesthesia and has been associated with the highest mortality rate among anaesthesia related complications (Cook et al. 2011). The severity of the pneumonitis can be influenced by multiple factors, including the volume of the refluxate, duration of anaesthesia, resistance to mucosal injury, effectiveness of clearance of gastric contents, pH and composition of the refluxate, as well as the patient's health status (Galatos et al. 2001; Savvas et al. 2009; Wilson & Walshaw 2004; Dugdale et al. 2020).

2.2 Methods for detection of gastro-oesophageal reflux

Although GOR is a frequent complication during general anaesthesia, its transient nature presents challenges for diagnosis. Despite its clinical significance, factors such as cost of equipment, lack of knowledge and awareness, and its transient nature of events likely contribute to the infrequent monitoring of GOR in clinical settings, with monitoring primarily done so for research purposes. However, the absence of monitoring may have adverse consequences on outcomes in dogs (Flouraki et al. 2022). pH monitoring has remained as the primary method for detecting GOR in anaesthetised dogs (Fernandez Alasia et al. 2021). In contrast, in human medicine, pH-MII has emerged as the 'gold standard' technique for monitoring gastro-oesophageal reflux disease (GORD) (Bredenoord 2008; Hojsak et al. 2016; Ristic et al. 2017; Lambertini et al. 2020). pH monitoring alone without intraluminal impedance (MII), has limitations, particularly in detecting reflux episodes of intermediate pH (pH 4-7.5) between gastric and duodenal mixed refluxate, potentially leading to underreporting of GOR episodes (Hila et al. 2007; Zacuto et al. 2012; Anagnostou et al. 2015; Rosen et al. 2018).

Placement of the monitoring devices in dogs involves measuring the length from the mandibular incisors to the level of the 10^{th} rib to ensure correct placement of the catheter (Waterman & Hashim 1991; Wilson et al. 2005; Shaver et al. 2017; Lambertini et al. 2020). A lateral thoracic radiograph or endoscopy can be used to confirm correct placement of the probe

(Ristic et al. 2017; Lambertini et al. 2020). Various diagnostic modalities including endoscopy, videofluroscopy, nuclear scintigraphy, computed tomography, and real-time magnetic resonance imaging, have been explored in both human and veterinary medicine to detect GOR (Favarato et al. 2012; Zhang et al. 2015; Eivers et al. 2019; Grobman et al. 2020; Benzimra et al. 2020; Paran et al. 2023).

2.2.1 pH-metry

Oesophageal pH measurement is achieved by introducing a flexible oesophageal catheter with a pH sensor fixed to the tip that is sensitive to pH fluctuations into the oesophageal lumen and positioned 6 cm rostral to the LOS (Favarato et al. 2011; Zacuto et al. 2012). Prior to use, the probe needs to be calibrated at pH 4.0 and 7.0 using buffer solutions (Johnson 2014). A reflux episode is recorded when there is a pH change of less than 4.0 (indicating acidic gastric reflux) or an increase in pH above 7.5 (suggestive of alkaline biliary reflux) (Wilson et al. 2005; Johnson 2014; Lambertini et al. 2020). The pH of gastric refluxate can range from acidic to alkaline, with intermediate (mixed gastric and duodenal refluxate) pH values also occurring. pH-metry alone may fail to detect reflux events with intermediate pH values (i.e. pH between 4 and 7.5), leading to the underreporting of GOR events (Hila et al. 2007; Zacuto et al. 2012; Anagnostou et al. 2015; Rosen et al. 2018).

2.2.2 pH with multiple intraluminal impedance

pH-MII is a valuable tool to detect GOR in human and veterinary medicine. It has been extensively utilized to monitor GORD in humans (Bredenoord 2008; Hojsak et al. 2016; Ristic et al. 2017; Lambertini et al. 2020); however, has less frequently been used to detect GOR in veterinary medicine (Zacuto et al. 2012; Tarvin et al. 2016). A pH-MII monitoring device typically consists of a flexible catheter with 7 impedance electrodes along with a pH sensor at the probe tip (Ristic et al. 2017). The technique combines measurements of oesophageal pH and fluid movements within the oesophagus, providing valuable information of the nature and composition of the refluxate, distance migrated along the oesophagus, duration and frequency of occurrence (Hojsak et al. 2016). Software analysis of pH-MII data allows differentiation between gas and liquid oesophageal boluses. Similarly to pH-metry, the probe requires calibration using buffer solutions prior to use. With pH-MII, the impedance electrodes detect changes in impedance associated with gas or liquid in the oesophageal lumen, while the pH sensor functions in a similar manner to pH-metry and detects fluctuations in oesophageal pH (Rosen et al. 2018). A GOR event is defined as 50% increase in ohms occurring across 2 consecutive impedance channels in the distal oesophagus for more than 2 seconds (Zacuto et al. 2012). pH values and impedance data are analysed together by computer software. Analysis of the data allows quantification of acidic, weakly-acidic and non-acidic GOR events (Hojsak et al. 2016; Rosen et al 2018).

2.2.3 Endoscopy

Endoscopy makes use of a flexible or rigid slender tube with a camera and internal light source fixed at the distal end to provide real time visual evaluation of the oesophageal lumen. Although less commonly utilised, endoscopy has been shown to be a valuable tool for evaluating GOR and GORD in veterinary and human medicine, respectively (Favarato et al. 2011; Favarato et al. 2012; Shaheen et al. 2012; Hojsak et al. 2016; Kuribayashi et al. 2021). Despite the fact that endoscopy cannot detect the pH of the refluxate, it offers the unique advantage in direct visualisation of the refluxate, providing the opportunity to quantify the refluxate and assess the degree of mucosal changes and injury. In human medicine, endoscopy is predominantly utilised to assess and grade oesophageal mucosal injury and complications associated with GORD, rather than serving as a real-time detection method for GORD events (Shaheen et al. 2012; Kuribayashi et al. 2021).

2.3 Factors affecting the prevalence of gastro-oesophageal reflux

The occurrence of GOR in anaesthetised dogs is influenced by several predisposing factors. The gastric mucosa contains glands responsible for gastric secretions, among which parietal cells play a pivotal role in secretion of hydrochloric acid (HCl) into the gastric juices. HCl secretions are isotonic and typically possess a pH of less than 1 (Herdt 2012). Gastric acidity is recognised to have a significant effect on the LOSP, with highly acidic gastric contents potentially reducing LOS tone, while decreasing acidity can enhance LOS tone (Dugdale et al. 2020).

Pre-anaesthetic fasting duration and feeding of small amounts of specific canned dietary compositions have been shown to decrease gastric acidity and influence the incidence of GOR (Savvas et al. 2009; Savvas et al. 2016; Viskjer & Sjöström 2017). Prolonged fasting durations (greater than 12 hours) has been associated with decreased gastric pH and increased gastric volume and risk of GOR in anaesthetised dogs (Galatos & Raptopoulos 1995b; Savvas et al 2009; Savvas et al. 2016; Viskjer & Sjöström 2017). Savvas et al. (2016) noted that feeding a "light meal" equivalent to half of the daily energy requirements for dogs, 3 hours prior to anaesthesia had a positive impact on incidence of GOR. Conflictingly, Viskjer and Sjöström (2017) found that a "light meal" 3 hours prior to anaesthesia increased the odds of GOR. These conflicting findings may be attributed to the composition and volume of food administered in each of the relevant studies (Viskjer & Sjöström 2017; Savvas et al. 2022). These conflicting findings regarding the impact of pre-anaesthetic meals on GOR incidence underscores the need for further investigations on the impact of food composition and duration of fasting on GOR in anaesthetised dogs.

Additionally, various drugs and the hormone progesterone have been implicated in reducing LOS tone and influence the prevalence and severity of GOR in anaesthetised dogs (Water & Hashim 1992; Raptopoulos & Galatos 1997; Anagnostou et al. 2015). Several anaesthetic drugs, such as propofol and thiopentone, have been shown to elevate the incidence of GOR (Raptopoulos & Galatos 1997). However, propofol has been observed to exert a more profound impact on LOSP and barrier pressure (Water & Hashim 1992), and has been shown to be associated with a significantly higher occurrence of GOR compared to thiopentone (p<0.02) (Raptopoulos & Galatos 1997). Anagnostou et al. (2015) observed that pregnant dogs undergoing general anaesthesia in the second half of pregnancy were more prone to experiencing GOR episodes. This susceptibility was postulated to be linked to progesterone and its influence on reducing gastric pH, as evidenced by studies in rats during late pregnancy and lactation (Lichtenberger & Trier 1979; Takeuchi & Okabe 1984; Vigen et al 2011). The incidence of GOR in pregnant animals may also be attributed to the rise in intrabdominal pressure caused by a gravid uterus, leading to an increase in intragastric pressure.

Opioids perform a fundamental role in providing analgesia during general anaesthesia. However, it is well known that these drugs affect gastrointestinal motility and LOS tone (Sternini et al. 2004; González et al. 2015). Lambertini et al. (2020) observed that dogs premedicated with methadone appeared more likely to experience GOR episodes compared to dogs receiving butorphanol, although the difference was not statistically significant but deemed clinically relevant. Conversely, McFadzean et al (2017) reported that dogs premedicated with butorphanol appeared more prone to GOR episodes compared to those receiving methadone, albeit not statistically significant. Wilson et al. (2005) highlighted that premedication with morphine significantly increases the risk of GOR in dogs during general anaesthesia. In contrast, premedication with meperidine was associated with a 55% reduced risk of developing GOR in dogs under general anaesthesia compared to morphine (Wilson et al. 2007). However, Flouraki et al. (2022) found no significant difference in the incidence of GOR among dogs receiving either morphine, butorphanol or meperidine premedication. The evidence regarding the effect of opioids on GOR prevalence appears conflicting, necessitating further research to determine their impact on GOR. Additionally, acepromazine has been shown to decrease LOS tone (Hall et al. 1975) and increase the risk of GOR (Wilson et al. 2005), whereas premedication with benzodiazepines alone has been shown to decrease the risk of GOR in anaesthetised dogs (Galatos & Raptopoulos 1995b). Notably, in one study, dogs premedicated with midazolam and an opioid exhibited GOR episodes within reported ranges in the literature (Flouraki et al. 2022), suggesting that adding opioids to the premedication regimen may negate the beneficial effects of administering benzodiazepines alone on the risk of GOR.

The positioning of dogs during surgical procedures has been shown to influence the incidence of GOR. Specifically, positioning in dorsal recumbency, as opposed to lateral or sternal recumbency, has been found to decrease LOSP and is correlated with a higher incidence of GOR (Waterman et al. 1995; Viskjer & Sjöström 2017). However, conflicting results exist, Galatos and Raptopolous (1995a) and Flouraki et al. (2022) reported no significant influence of recumbency on the incidence of GOR in anaesthetised dogs. Moreover, it has been noted that dogs undergoing abdominal surgery are at greater risk of GOR to those undergoing non-abdominal surgical procedures (Galatos & Raptopolous 1995a). This association is likely attributed to an elevation in intragastric pressure during manipulation of abdominal viscera (Galatos & Raptopolous 1995a) and is supported by studies indicating that an increase in intragastric pressure above 10 cmH_2O increases the risk of GOR (Nimmo 1984; Hardy 1988). Additionally, Lamata et al. (2012) reported that dogs undergoing orthopedic surgeries also face an increased risk of passive regurgitation, and Galatos & Raptopolous (1995a) indicated that a prolonged duration of anaesthesia further increases the risk of GOR. Lamata et al. (2012) noted that dogs undergoing orthopedic procedures often required pre-surgical radiographs, which likely result in changes in body position and associated alterations in intra-abdominal pressure, therby increasing the risk of GOR and regurgitation.

While not statistically significant, Galatos and Raptopolous (1995a) noted a trend of increasing likelihood of GOR in older dogs. However, other studies, such as that conducted by Flouraki et al. (2022), did not find age to be associated with an increased incidence of GOR. Contrastingly, Viskjer and Sjöström (2017) reported that younger dogs were more prone to experience GOR episodes. Anagnostou et al. (2015) noted that large sized, deep chested breeds are at higher risk of GOR episodes compared to small-sized, barrel-chested dogs. Some authors have suggested that brachycephalic breeds may be at higher risk of GOR (Poncet et al. 2005); however, Shaver et al. (2017) found no significant difference in GOR incidence between non-brachycephalic and brachycephalic breeds. Additionally, increasing body weight has previously been identified as a risk factor for GOR (Shaver et al. 2017), with heavier dogs showing a significant increase risk during anaesthesia (Shaver et al. 2017). Lamata et al. (2012) also observed a significant increase in passive regurgitation with increasing weight in dogs during general anaesthesia. Contrary to these above findings, Galatos and Raptopoulous (1995a), Torrente et al. (2017) and Flouraki et al. (2022) failed to find an association between increasing weight or body condition score and GOR.

2.4 Interventions and treatment

To prevent the occurrence of regurgitation and aspiration into the airway during endotracheal intubation, a technique similar to a Sellick’s manoeuvre, such as performed in human medicine, can be performed (Self 2016). Cricoid pressure, a common technique utilised in human anaesthesia, has been shown to decrease the incidence of aspiration after reflux has occurred (Chaney & Brady 2023). However, to the author’s knowledge, there is a paucity of literature evaluating the efficacy of Sellick’s manoeuvre in dogs.

While complete prevention of GOR may not be possible; attentive monitoring is imperative to detect if oropharyngeal reflux has occurred, necessitating brisk implementation of interventional procedures to mitigate the potentially harmful impact of GOR (Adams et al. 2015). In cases of passive regurgitation, the dog's head should be elevated with swift measures taken to hastily secure the airway with an appropriately sized cuffed endotracheal tube (Self 2016). The cuff should be inflated to 25 cmH_20, and the endotracheal tube securely fixed to the patient to prevent easy dislodgement. Subsequently, the dog's head should be tilted downward below the level of the cardia to facilitate drainage of gastric contents and prevent accumulation of gastric contents around the airway (Adams et al. 2015; Self 2016).

Gastric content should be removed from the oral cavity using clean swabs followed by gentle suction of the oral cavity, oropharynx and cranial oesophagus using a surgical suction machine or syringe attached to a feeding tube (Adams et al. 2015; Self 2016; Dugdale et al. 2020). Ensuring the cuff of the endotracheal tube is inflated, lavage of the oral cavity and oesophagus with saline or tap water is recommended in order to neutralise the pH (Adams et al. 2015; Figuerido & Green 2015; Self 2016; Dugdale et al. 2020). Additionally, bicarbonate can be instilled post-lavage to further elevate oesophageal pH, thereby negating the detrimental effects of an acidic environment on the oesophageal mucosa (Wilson & Evans 2007). Lotti et al. (2022) found that large volumes of tap water were only mildly effective at raising oesophageal pH after strongly acidic GOR episodes, whereas instilling 20 mL of bicarbonate (1% - 2%) solution was more effective in increasing oesophageal lumen pH to above 4. Therefore, it is recommended to lavage the oesophagus with a bicarbonate solution when treating strongly acidic reflux episodes. If gastric content is noticed in the nares, prompt suction and lavage is necessary to prevent choanal stricture formation (Self 2016).

During recovery from anaesthesia, thorough examination of the oropharynx is recommended to ensure clearance of as much gastric content as possible before extubation.

The dogs should be placed in sternal recumbency for recovery with the head elevated above the cardia, the nose positioned down and extubated with the cuff partially inflated (Adams et al. 2015). Animals that experienced regurgitation should receive treatment with alkalinizing agents or proton pump inhibitors such as sucralfate and omeprazole to prevent oesophagitis and should be placed on adequate pain control (Han 2003; Self 2016). Postoperative monitoring is crucial to detect complications of GOR such as oesophagitis, pneumonitis's and aspiration pneumonia.

Effective prevention of GOR in anaesthetised dogs remains contentious. Maropitant has been shown to be effective in preventing emesis but not GOR episodes in dogs receiving morphine or hydromorphone (Johnson 2014). Wilson et al. (2006) observed that administering a higher dose of metoclopramide at 1 mg kg^{-1} followed by a constant rate infusion (CRI) at 1 mg kg^{-1} hour resulted in a notable 54% reduction in GOR episodes compared to administration of lower metoclopramide doses. Conversely, Favarato et al. (2012) reported that high dose metoclopramide at 1 mg kg^{-1} followed by a constant rate infusion (CRI) at 1 mg kg^{-1} hour in conjunction with ranitidine at 2 mg kg^{-1} was ineffective in reducing GOR episodes.

In one study, a single bolus of omeprazole at 1 mg kg^{-1} administered four hours before surgery successfully reduced the incidence of GOR in anaesthetised dogs (Panti et al. 2009). In contrast, Lotti et al. (2021) found that the use of a single dose of omeprazole at 1 mg kg^{-1} administered three hours prior to surgery failed to reduce the incidence of GOR. However, they observed that administering two oral doses, one in the evening and another three hours before surgery, significantly reduced the incidence of GOR in anaesthetised dogs. In another study, Zacuto et al. found that administering two intravenous (IV) doses of omeprazole at 1 mg kg^{-1}, given 12-18 hours and 1-1.5 hours before surgery, did not effectively reduce the frequency of GOR episodes. However, when omeprazole was administered in combination with cisapride at

1 mg kg^{-1}, dogs experienced a significantly lower number of GOR episodes (Zacuto et al. 2012).

2.5 Inference and outlines

Conflicting findings exist among studies concerning predisposing factors and preventative strategies of GOR in anaesthetised dogs. A multitude of factors can influence the incidence of GOR, and the lack of standardisation across studies may offer an explanation for the observed discrepancies between them (Savvas et al. 2022). Variables such as premedication protocols, induction drugs, fasting durations, breed and conformation differences, body weight and condition variations, surgical procedures, and monitoring methods amongst others were inconsistently applied across these investigations, potentially leading to a myriad of outcomes. Therefore, there is a need for more robust research to better refine our understanding of predisposing factors associated with GOR in anaesthetised dogs. Additionally, the impact of different monitoring methods on GOR outcomes remains to be determined.

Gastro-oesophageal reflux is a frequent complication in dogs undergoing general anaesthesia. Ineffective monitoring may lead to detrimental implications. To the author's knowledge, there is a lack of comparative studies aimed at determining which monitoring method is most sensitive and specific to detect GOR in dogs. There are still significant gaps in our understanding of GOR and how we can mitigate its occurrence. Without the adoption of a universally accepted 'gold standard monitoring method' in dogs, we cannot accurately explain GOR's incidence or assess the effectiveness of treatment or preventative measures. Therefore, it is imperative to establish and validate a reliable and effective method for the detection of GOR so we can accurately determine the occurrence of GOR in anaesthetised animals in both future research and clinical practice.

2.5.1 Aims and objectives

The aim of the study was to compare different methods for the detection of GOR in anaesthetised dogs. Three methods, pH, pH-MII and MII were compared to endoscopy. The objective of this trial was to compare the binomial outcome (yes/no) of pH, pH-MII and MII to direct observation of GOR by endoscopy in anaesthetised dogs.

2.5.2 Hypothesis

We hypothesized that pH-MII was more sensitive and specific in the detection of the occurrence of GOR in anaesthetised dogs compared to pH or MII.

3. Material and Methods

3.1 Animal housing

The study population was selected from dogs scheduled for elective pelvic limb orthopedic procedures admitted to the Onderstepoort Veterinary Academic Hospital (OVAH). Before acceptance into the study, informed consent (Appendix i) from owners was obtained. Inclusion criteria were a body mass between 10 and 40 kg, physiologic variables, and blood work (creatinine, hematocrit, and total serum protein) results within normal reference intervals, and an American Society of Anesthesiologists (ASA) physical classification of I or II. Dogs with a history of respiratory or gastrointestinal disease were excluded from participation in the study. Additionally, dogs that received medications that may reduce the risk of GOR or increase LOS tone were excluded from the study. The dogs were admitted to the Small Animal Surgery Clinic the day of the procedure and were housed in the small animal surgery ward in large dog kennels with comfortable bedding during the pre-anaesthetic period. After the surgery, the dogs were recovered in the high care ward and they remained there for at least 2 days postoperatively under 24-hour monitoring and care performed by students, qualified nurses and veterinarians. Ethics approval for the prospective comparative trial was obtained from the Research (REC204-21; Appendix iii) and Animal Ethics Committees of the University of Pretoria (REC204-21; Appendix ii).

3.2 Study design

A prospective comparative clinical trial (without treatment interventions) was conducted in a live canine model, comprising 35 dogs. This study was reported using Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines 2.0 (Appendix iv). The sampling of the population of dogs admitted for pelvic limb procedures was opportunistic.

3.3 Sample size

The sample size was calculated using commercially available software (MedCalc Statistical Software, Version 19.5; MedCalc Software Ltd; Ostend, Belgium) where a comparison of receiver operating characteristic (ROC) curves equation was used with the following assumptions: alpha 0.05; beta 0.20; area under the curve (AUC) 1: 0.85; AUC 2: 0.75; equal positive and negative correlations (0.93); and a negative to positive ratio of 0.5.

3.4 Experimental procedures

At least one hour prior to each use of the probes, the pH electrode was calibrated in buffer solutions of pH 4.0 and 7.0 (Buffer solution, Given Imaging; Vietnam) (Fig 3.1a). After calibration, the monitoring devices used to detect GOR were assembled (assembly) in-and-around a 8.5 mm internal diameter polyvinyl chloride endotracheal tube as follows: an endoscopy camera (6-LED Wifi-Endoscope Cam, Sanoxy; USA) was threaded through the inside of the endotracheal tube until the tip was positioned at the level of the tube bevel and then a single layer of 25 mm electrical insulation tape was wrapped around the assembly to form a liquid-tight seal. Then, a disposable flexible pH-MII catheter (VersaFlexZ, Given Imaging; Vietnam) was affixed to the side of the endotracheal tube-camera construct using narrow strips of insulation tape, positioning the pH sampling tip 10 mm beyond the camera (Fig 3.1b).

Throughout the study duration and preceding induction, measures were implemented to avoid conditions that could potentially impact data recordings and subsequent results. Such precautions consisted of minimizing movements and adjustments of body position during surgical preparation and to avoid inadvertent increases in intra-abdominal pressure, ensuring correct placement of the monitoring devices to avoid inadvertent placement into the stomach, and preventing accidental dislodgement or removal of devices.

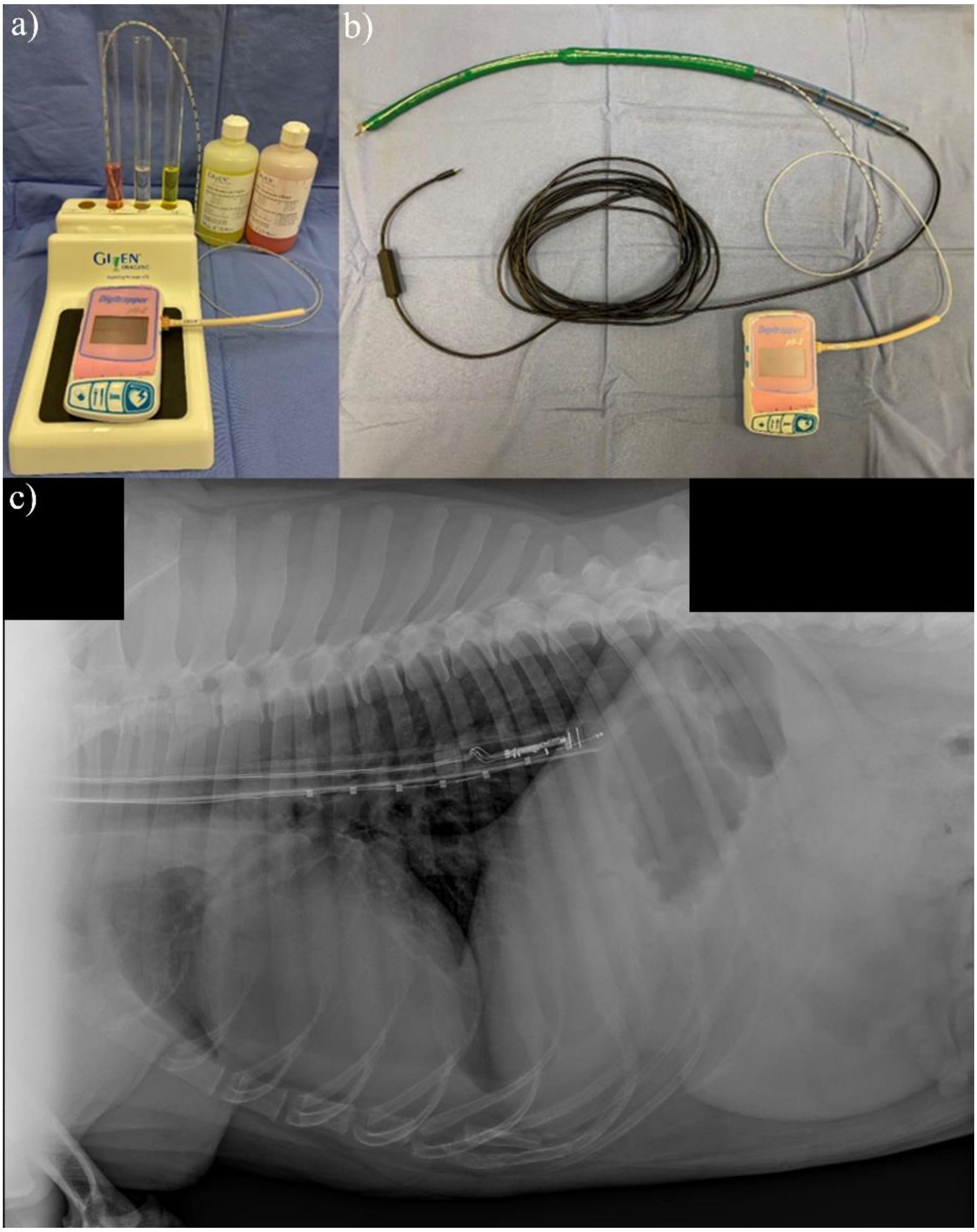

Figure 3.1. (a) Calibration of pH electrode in buffer solutions of pH 4.0 and 7.0. (b) Gastro-oesophageal reflux (GOR) monitoring assembly consisting of an 8.5 mm internal diameter polyvinyl chloride endotracheal tube, endoscopy camera, disposable flexible pH-impedance (pH-MII) catheter affixed using 25 mm electrical insulation tape. (c) Lateral thoracic radiograph of a dog enrolled in the study used to determine correct placement of the assembly at the level of the 10th rib.

Prior to induction, food was withheld for 6 to 12 hours, while *ad libitum* access to water was permitted until 2 hours before premedication. All dogs were premedicated with a combination of medetomidine (Domitor, 1 mg mL^{-1}, Zoetis, South Africa) at 0.01 mg kg^{-1} and morphine (morphine, 10 mg mL^{-1}; Fresenius-Kabi, South Africa) at 0.3 mg kg^{-1} drawn up in separate syringes but then mixed into one syringe for a single intramuscular (IM) administration into the quadriceps muscle group. After 30 minutes, a cephalic vein as aseptically cannulated using a 20G, over-the-needle, IV catheter (Jelco; Smiths Medical, UK). Anaesthetic induction ensued with propofol (Propofol 1% Fresenius Injection, 10 mg mL^{-1}; Intramed, South Africa) administered IV, titrated to effect in order to achieve tracheal intubation. Endotracheal intubation was facilitated with the use of an illuminated laryngoscope utilising a cuffed polyvinyl chloride endotracheal tube (KRUUSE PVC Endotracheal tube with cuff; KRUUSE; Denmark).

Subsequently, the dogs were connected to a semi-closed, rebreathing system equipped with a precision vaporizer (Ohmeda Isotec 5; BOC Health Care; UK). The vaporizer dial was adjusted to between 2.0% to 2.5% and an initial fresh gas flow rate set to 100 mL kg^{-1} minute to maintain general anaesthesia using isoflurane (Isofor; Safeline Pharmaceuticals; South Africa) in oxygen. After 10 minutes, the fresh gas flow rate was adjusted to 50 mL kg^{-1} minute. The dogs were placed in lateral recumbency, with the non-affected pelvic limb positioned on the dependent side, and thorax and abdomen positioned atop a digital radiography (DR) detector plate (VIVIX-S, VIEWORKS Co. Ltd.; Korea). Lactated Ringer's solution (Ringers Lactate Solution, Fresenius-Kabi, South Africa) was administered IV throughout the anaesthetic at a rate of 5 mL kg^{-1} $hour^{-1}$ using an electronic infusion pump (MedCaptain HP60, MedCaptain Medical Technology Co. Ltd.; China).

Following induction, after the dogs were deemed stable and adequately anaesthetised the endoscope was then linked to a laptop computer (Lenovo E50, Lenovo; China) to provide real-time video analysis. To ensure correct placement of the assembly, a measurement was taken from the cranial aspect of the maxillary incisors to the level of the 10th rib, as previously described by Waterman & Hashim (1991), Wilson et al. (2005) and Shaver et al. (2017). The measured length was demarcated on the assembly with tape to guide the depth of advancement into the oesophagus. During advancing, the endoscopy video was monitored for any visual reflux and to ensure that no accidental advancement into the stomach occurred. The primary investigator performed the placement and positioning of the assembly to ensure consistency with placement. A lateral radiograph of the thorax, using a portable x-ray generator (ULTRA 9020BT Diagnostic X-ray unit, Ecoray Co. Ltd.; South Korea), was then performed to verify correct positioning of the assembly. The radiograph confirmed that the tip of the catheter was positioned at the level of the 10th rib (Fig 3.1c). Importantly, the placement of the assembly was guided by endoscopy, conducted in a manner to avoid causing injury to the oesophagus and its sequalae to any of the dogs used in the study.

Following confirmation of the correct positioning of the assembly it was secured to the dog's maxilla using 25 mm ribbon gauze (Cutisoft Gauze; BSN Medical, Germany), positioned just caudal to the maxillary canines to mitigate inadvertent displacement during data collection. Subsequently, the pH-MII catheter was connected to its respective portable data recording and monitoring device (Digitrapper, Medtronic; South Africa), and the endoscope provided real-time visual analysis of the distal oesophagus. Continuous monitoring of pH and MII values were recorded every second via the data monitoring device, which was later uploaded and stored on a laptop computer. These data sets were viewed for each dog using proprietary software (Reflux Software 6.1, Medtronic; South Africa). Throughout the initial 20 minutes of the surgical preparation with the anaesthetized dog in the induction room, pH values and visual reflux score (Table 3.1) were recorded every minute on a data capture sheet (Appendix v). Thereafter, values were recorded at 5-minute intervals up to the 45-minute mark. The initiation of the lower oesophageal pH, impedance monitoring, and endoscopy occurred within 5 minutes of induction, with the placement of the assembly designated as time 0.

Table 3.1. Visual reflux score used to grade refluxate within the distal oesophagus in anaesthetised dogs with an endoscope placed to the level of the tenth rib.

Visual Reflux Score			
Score	Classification	Description	Picture
0	None	No reflux visible on camera	 
1	Mild	Small amount of fluid visibly lining the oesophageal wall; however, oesophageal wall still easily visible. No evidence of pooling of gastroduodenal content in the lumen.	 
2	Moderate	Pooling of a small amount of gastroduodenal content on dependent surface in the oesophageal lumen. Some oesophageal wall still visible where there is no GOR content.	 
3	Severe	Near to complete obliteration of camera view with reflux content in the oesophageal lumen.	 

During the dog's preparation for surgery and data collection, an assistant monitored vital parameters, including heart rate, respiratory rate, mucous membrane colour, capillary refill time, peripheral pulses, jaw tone, eye position to monitor and adjust depth of anaesthesia, if required. These variables were systematically recorded on a monitoring sheet at 5-minute intervals. All dogs received the same perioperative drug therapy which was preoperative meloxicam (0.2 mg kg^{-1}, subcutaneously (SC); Metacam, 5 mg mL^{-1}; Boehringer Ingelheim, South Africa), cefazolin (20 mg kg^{-1}, IV; Zefkol, Acino Pharma (Pty) Ltd; Namibia) at 20 mg kg^{-1}, and various locoregional blocks of the pelvic limb using bupivacaine (0.1 mL kg^{-1} perineural injection, Macaine, 5 mg mL^{-1}; Adcock Ingram Critical Care (Pty) Ltd; South Africa). All dogs received postoperative analgesia in the form of IV morphine (0.3 mg kg^{-1} every 4 hours, IV; 10 mg mL^{-1}; Fresenius-Kabi, South Africa) and meloxicam (0.1 mg kg^{-1} daily; SC). After 45 minutes, the dogs were moved to a surgical theatre.

Dogs that manifested GOR episodes were given omeprazole (1 mg kg^{-1}, IV; Nexipraz, 8 mg mL^{-1}; Ranbaxy Pharmaceuticals (Pty) Ltd, South Africa) every 12 hours and sucralfate (0.5 g dog^{-1} in dogs less than 20 kg and 1 g dog^{-1} in dogs greater than 20 kg orally; Ulsanic, 200 mg mL^{-1}; Aspen Pharmacare, South Africa) administered once daily for 5 days. For dogs that exhibited passive regurgitation, their oral cavity was rinsed and swabbed dry and oesophagus lavaged with saline prior to termination of general anaesthesia and tracheal extubation. These interventions aimed to mitigate the incidence of aspiration and minimize oesophageal stricture formation.

4. Data Analysis

A dichotomous outcome (yes/no) was assigned for each method used to monitor GOR episodes at each timepoint. A 'yes' was assigned for pH method when the distal oesophageal pH was below 4.0 (indicative of acidic reflux) or above 7.5 (indicative of biliary reflux) for a duration of at least 30 seconds (Wilson et al. 2005; Johnson 2014; Lambertini et al. 2020). The device software was used to analyze the MII data to assign a 'yes' for liquid only reflux which was determined as a decrease in impedance value from the baseline value. For pH-MII a 'yes' was assigned when either pH alone or MII alone were already assigned 'yes'. For the endoscopy method, a visual reflux score of 2 or 3 were assigned a 'yes'. Statistical analysis was performed using commercially available software (MedCalc Statistical Software, Version 19.5; MedCalc Software Ltd; Ostend, Belgium).

4.1 Part A

For each GOR monitoring method, receiver operator curve (ROC) analysis (Delong et al. 1988 method of analysis) was used to determine sensitivity and specificity for detecting GOR. Each data point for pH, pH-MII and MII, was used and plotted against the true outcome detected by the endoscopy method. Additionally, data points for pH and pH-MII was plotted against the true outcome detected by the pH method. Area under the curve (AUC) was used to discern between an accurate method and non-accurate method (AUC $\leq$ 0.5), a method with poor accuracy (AUC 0.5-0.6), low accuracy (AUC 0.6-0.7), fair accuracy (0.7-0.8), good accuracy (AUC 0.8-0.9) and excellent accuracy (AUC $\geq$ 0.9) (Nahm 2022; Swets 1988).

4.2 Part B

The agreement between endoscopy 'yes' and pH, pH-MII and MII 'yes' was compared using inter-rater kappa agreement, respectively. Inter-rater kappa agreement was used to analyse the extent that each method assigned the same 'yes/no' value for each data collection point, thereby, determine method reliability. Inter-rater agreement between pH and pH-MII as well as pH and MII was also analysed. For all tests, where applicable, a significance was interpreted as a p-value < 0.05.

5. Results

The mean (range) mass and age of the dogs (22 female; 13 male) of various breeds were 31.9 (14.0 to 40.0) kg and 5.6 (0.8 to 12.0) years, respectively. No dogs were excluded as a result of the exclusion criteria. Endoscopy identified GOR events in 20 dogs, constituting 57% of the study population, while pH-MII monitoring detected GOR events in 19 dogs, representing 54% of the total dogs enrolled in the study. Whereas pH and MII identified GOR events in 13 (37%) and 12 (34%) dogs, respectively. Notably, of the 19 dogs identified by the pH-MII monitoring method, 7 were detected by pH, 6 MII, and 6 by both pH and MII (Table 5.1). Among the 13 dogs identified by the pH method, 12 exhibited acidic reflux (pH below 4), whereas 1 experienced alkaline reflux (pH above 7.5). During the study, endoscopy view was obstructed, whether temporarily or permanent by gastric content, in 10 of the 35 dogs.

Table 5.1 Outcome of gastro-oesophageal reflux events detected over a 45 minute period using 4 different methods of detection in 35 anaesthetised dogs positioned in lateral recumbency directly after induction.

Dog ID	Endoscopy	pH alone	pH-MII	MII alone
1	Yes	Acidic Reflux	Yes	NGD
2	Yes	NGD	NGD	NGD
3	NGD	NGD	NGD	NGD
4	Yes	NGD	NGD	NGD
5	Yes	Acidic Reflux	Yes	NGD
6	NGD	NGD	NGD	NGD
7	NGD	NGD	Yes	Yes
8	NGD	NGD	NGD	NGD
9	NGD *	NGD	NGD	NGD
10	Yes	Acidic Reflux	Yes	NGD
11	Yes	NGD	Yes	Yes
12	Yes †	Acidic Reflux	Yes	NGD
13	NGD	NGD	NGD	NGD
14	NGD	NGD	NGD	NGD
15	NGD	NGD	NGD	NGD
16	Yes †	Acidic Reflux	Yes	Yes
17	Yes *	NGD	NGD	NGD
18	Yes †	NGD	NGD	NGD
19	Yes *	Alkaline Reflux	Yes	NGD
20	Yes †	Acidic Reflux	Yes	Yes
21	Yes †	NGD	Yes	Yes
22	NGD	NGD	NGD	NGD
23	NGD	NGD	NGD	NGD
24	Yes	Acidic Reflux	Yes	Yes
25	Yes	Acidic Reflux	Yes	NGD
26	NGD †	NGD	NGD	NGD
27	Yes	NGD	Yes	Yes
28	Yes	Acidic Reflux	Yes	Yes
29	Yes	NGD	Yes	Yes
30	NGD	NGD	NGD	NGD
31	NGD	NGD	Yes	Yes
32	Yes	Acidic Reflux	Yes	Yes
33	Yes	Acidic Reflux	Yes	NGD
34	NGD	NGD	NGD	NGD
35	NGD †	Acidic Reflux	Yes	Yes
Total	20	13	19	12
Percentage	57 %	37%	54%	34%

Identification number (ID), pH with intraluminal impedance (pH-MII), impedance (MII), No gastro-oesophageal reflux detected (NGD), Acidic reflux is classified as gastro-oesophageal pH below 4, alkaline reflux classified as gastro-oesophageal pH above 7.5, endoscopy view temporarily (*) or permanently (†) obstructed during study.

5.1 Part A

As planned, the AUC for the ROC of endoscopy was 1.0 and demonstrated 100% sensitivity and specificity, respectively. By using endoscopy as the true diagnostic outcome, pH and pH-MII both showed a fair accuracy in discerning GOR events (Fig 5.1). Notably, MII demonstrated a low accuracy in discerning GOR events (Table 5.2). Prevalence for detecting GOR events per measured data point was greatest in endoscopy (35%), followed by pH-MII (25%), then pH (21%) with the least detected in MII (7%).

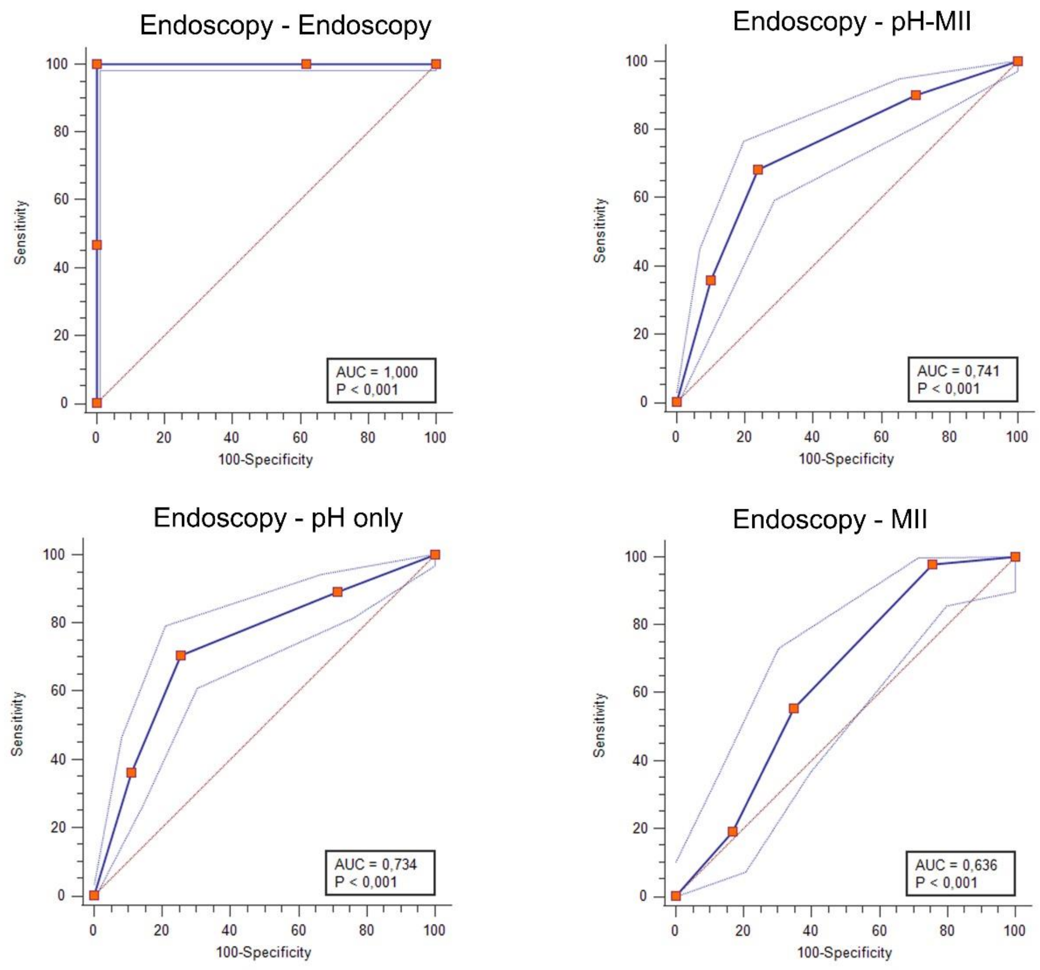

Figure 5.1. Receiver operator curve (ROC) graphs used to determine sensitivity and specificity between the different monitoring methods for detecting gastro-oesophageal reflux (GOR) in 35 anaesthetised dogs. The true outcome detected by the endoscopy method (a) was used and plotted against each data point for pH-impedance (pH-MII) (b), pH alone (c) and impedance (MII) alone (d). 95% confidence interval (CI) lines for sensitivity and specificity were included in ROC curve graphs b, c, and d. Area under the curve (AUC) was used to discern between an accurate method and non-accurate method (AUC ≤ 0.5), a method with poor accuracy (AUC 0.5-0.6), low accuracy (AUC 0.6-0.7), fair accuracy (0.7-0.8), good accuracy (AUC 0.8-0.9) and excellent accuracy (AUC ≥ 0.9) (Nahm 2022; Swets 1988).

When using pH outcomes as the true diagnostic outcome, pH showed an excellent test accuracy. The sensitivity and specificity of pH for discerning GOR prevalence in dogs with a pH less than 4 were 94% and 99%, respectively. Whereas the sensitivity and specificity for pH to discern GOR prevalence in dogs with a pH greater than 7 were 94% and 12%, respectively. Similarly, comparing pH as the true diagnostic outcome to pH-MII showed excellent test accuracy in discerning GOR events (Table 5.2). When comparing detection rates of measured data points between pH as the true diagnostic outcome with pH and pH-MII, GOR events were detected in 25% and 28%, respectively.

5.2 Part B

Inter-rater kappa agreement analysis revealed fair agreement between endoscopy and pH, as well as endoscopy and pH-MII (Table 5.3). Conversely, there was none to slight agreement between endoscopy and MII. Almost perfect agreement was observed between pH and pH-MII. In contrast, there was none to slight agreement between pH and MII.

Table 5.2 Sensitivity and specificity for detecting gastro-oesophageal reflux (GOR) in 35 dogs anaesthetised with isoflurane in oxygen in lateral recumbency for 45 minutes. Each data point for pH alone, pH-MII and MII alone, was used and plotted against the true outcome detected by the endoscopy method. Additionally, data points for pH alone and pH-MII was plotted against the true outcome detected by the pH alone method.

Variable	Prev %	ROC AUC	ROC 95 % CI	*p*	Sen (%)	Spe (%)
Endoscopy – endoscopy	35	1.00	0.99-1.00	<0.0001	100	100
Endoscopy - pH	21	0.73	0.70 – 0.76	<0.0001	71	75
Endoscopy - pH-MII	25	0.74	0.71 – 0.77	<0.0001	69	76
Endoscopy - MII	7	0.64	0.60 – 0.67	<0.0001	98	24
pH – pH	25	0.94	0.93 – 0.96	<0.0001	94	100
pH – pH-MII	28	0.90	0.88 – 0.92	<0.0001	83	100

pH with intraluminal impedance (pH-MII), impedance (MII), prevalence (Prev), percentage (%), area under the curve (AUC), confidence interval (CI), significance level (*p*), Sensitivity (Sen), specificity (Spe).

Table 5.3 Statistical analysis using Inter-rater Kappa agreement was used to analyse the extent of agreement between endoscopy true outcome to pH only, pH-MII and MII only for detecting gastro-oesophageal reflux (GOR) in 35 dogs anaesthetised with isoflurane in oxygen in lateral recumbency for 45 minutes. Inter-rater Kappa agreement was used to analyse the extent that each method assigned the same 'yes/no' value for each data collection point, thereby, determine method reliability. Inter-rater agreement between pH-only and pH-MII as well as pH and MII only was also analysed.

Variable	Weighted Kappa	Standard error	κ 95 % CI
Endoscopy – pH alone	0.36	0.035	0.29 – 0.43
Endoscopy – pH-MII	0.39	0.035	0.31 – 0.46
Endoscopy – MII alone	0.07	0.025	0.02 – 0.12
pH – pH-MII	0.91	0.02	0.88 – 0.94
pH – MII alone	0.11	0.032	0.06 – 0.18

pH with intraluminal impedance (pH-MII), impedance (MII), confidence interval (CI)

6. Discussion

The prevalence of GOR during general anaesthesia in dogs has previously been reported with a varying incidence ranging from 17.4 % to 87.5% (Galatos & Raptopoulos 1995a, Wilson et al. 2005; Lambertini et al. 2020; Paran et al. 2023). Our study, focused on dogs undergoing general anaesthesia for elective pelvic limb surgery, revealed that GOR occurred in a considerable percentage of dogs, consistent with reported ranges. We noticed that endoscopy detected the most GOR events in these dogs followed closely by the pH-MII method. Whereas pH alone and MII alone had a lower detection rate of GOR events. This observation indicated that endoscopy and pH-MII were more sensitive at detecting GOR under anaesthesia in dogs.

The application of pH-MII has been rarely used in veterinary medicine. To date, Zacuto et al. (2012) and Tarvin et al. (2016), represent the sole contributors to studies using the pH-MII method for detecting GOR in anaesthetised dogs to our knowledge. The substantial variability in reported GOR prevalence among anaesthetised dogs in previous investigations is broad and prompts scrutiny regarding the accuracy of the current preferred methods of detection and adoption of a standardized technique across studies. There are a vast number of described predisposing risk factors for the occurrence of GOR in anaesthetised dogs, which include: administration of certain anaesthetic drugs, body positioning, type of food and pre-operative fasting times, deep-chested breeds, body weight, pregnancy, increased intra-abdominal pressure, abdominal surgery, orthopedic surgery, and older dogs (Galatos & Raptopoulos 1995a; Galatos & Raptopoulos 1995b; Raptopoulos & Galatos 1997; Wilson et al. 2005; Savvas et al. 2009; Lamata et al. 2012, Anagnoustou et al. 2015; Anagnoustou et al. 2017; Dugdale et al. 2020; Flouraki et al. 2022). In a comprehensive review, Savvas et al. (2022) summarized several factors influencing GOR development in dogs during general anaesthesia. These factors may potentially be the reason to the observed variations between previous studies. However, the review did not discuss the potential role of detection methods or techniques utilized as probable contributing factor to the variability in reported prevalence.

We noted that out of the 19 dogs detected by pH-MII, approximately a third of dogs were exclusively detected by pH only and MII alone, respectively, where a third of dogs were detected by both monitoring modalities. We also observed slightly higher detection rates in each measured data point in pH-MII when compared to pH alone and MII alone. This observation highlights the complementary nature of pH-metry and intraluminal impedance, suggesting that when one method failed to detect GOR, the other was successful in identifying it and vice versa. The limitations of pH-metry alone, which misses reflux episodes with intermediate pH values (pH 4.0 - 7.5) (Hila et al. 2007; Zacuto et al. 2012; Anagnostou et al. 2015; Rosen et al. 2018), provide a possible explanation for these findings, resulting in a potential underreporting of the frequency of GOR events. Similarly, in a study utilising endoscopy and pH monitoring to detect GOR episodes in anaesthetised dogs, pH-metry missed 50% of reflux episodes identified by endoscopy (Favarato et al. 2011). Based on this summation, our findings support the notion that endoscopy and pH-MII appear as superior modalities for identifying GOR events during anaesthesia in dogs. The incorporation of impedance addresses some of the limitations associated with pH alone and improve detection rates. This finding aligns with previous studies advocating for the use of pH-MII in human medicine (Bredenoord 2008; Francavilla et al. 2010; Hojsak et al. 2016; Kizilkan et al. 2016; Ristic et al. 2017; Lambertini et al. 2020) and supports its potential utility in veterinary anaesthesia.

Despite being a frequent complication during general anaesthesia, the transient nature of GOR poses a diagnostic challenge. Due to the substantial cost of equipment and challenges encountered by its monitoring, GOR is infrequently monitored in clinical practice and primarily done so for research purposes. Over the years, pH monitoring has remained the primary method for detecting the prevalence of GOR in anaesthetised dogs. In humans, pH-MII has become the gold standard technique for monitoring gastro-eosophageal reflux disease (Bredenoord 2008; Hojsak et al. 2016; Ristic et al. 2017; Lambertini et al. 2020). It has been reported that using pH-metry alone could miss up to 40.0% - 52.3% of GORD episodes in children and infants (Hojsak et al. 2016; Ristic et al. 2017). Francavilla et al. (2010) and Kizilkan et al. (2016) both noted that the concurrent use of MII with conventional pH-metry provided a more sensitive diagnostic method to detect gastroesophageal reflux disease in human paediatric patients when compared to pH alone. Similarly, we noticed that pH-MII surpasses pH alone in identifying GOR events.

Examining the data point detection rates, we noted endoscopy outperformed pH alone, pH-MII and MII alone. In one study, 42.86% of GOR episodes in healthy anaesthetised female dogs were detected by endoscopy only and missed by pH-metry alone (Favarato et al 2012). Additionally, Favarato et al. (2011) reported the detection of GOR in 4 out of 30 healthy anaesthetised dogs using endoscopy. Interestingly, out of the 4 dogs, pH monitoring identified GOR in only 2 dogs, corroborating our findings indicating that endoscopy may detect more reflux episodes. This disparity suggests that endoscopy possess heightened accuracy in detecting intermittent GOR events. However, the diagnostic capability of pH and MII alone may have been influenced by the assembly's construct, potentially impacting efficacy if the catheter was situated on the non-dependent side of the oesophagus. Further investigations are warranted to investigate the potential influence of the assembly's construct on the effectiveness of this pH-MII alone.

Although pH-MII demonstrated marginal superiority in discerning GOR events at each data point compared to pH alone, the disparity in our results were not as significant as reported by Hojsak et al. (2016) in which pH alone did not recognise GORD in 52.3% children compared to pH-MII. We hypothesized that the lower gastric pH in dogs may contribute to this discrepancy. Existing literature indicates that fasted gastric pH in humans (Dressman et al. 1990; Russel et al. 1993) is comparable to that in dogs (Sagawa et al. 2009; Younberg et al. 1985). Notably, observed postprandial gastric pH in humans is higher when compared to that of dogs which demonstrated a decrease in gastric pH. The GORD studies in humans were on awake children and infants over 24 hours duration in which meals were consumed. This discrepancy in postprandial events potentially explain why pH alone detected more GOR events in our dogs compared to human studies. Despite its limitations, our findings suggest that pH remains an accurate and reliable method for detecting GOR in anaesthetised dogs. The cost of pH-MII may be a limiting factor for its use in detecting GOR in dogs in both clinical and research settings, making pH monitoring an acceptable alternative.

The analysis of inter-rater kappa agreement provided insights into the reliability among the distinct diagnostic methods utilized in this study. Fair agreement was observed between endoscopy and pH alone with similar findings between endoscopy and pH-MII, suggesting that pH-MII is a reliable alternative to endoscopy. Furthermore, almost perfect agreement between pH alone as the true outcome and pH-MII monitoring suggest a potential synergy between these methods. MII alone showed none to slight agreement with pH true outcome, indicating potential limitations and an unreliability in measure as a standalone method for GOR detection. The observed patterns of agreement, emphasize the benefits of combing pH and MII monitoring techniques, thereby, improving diagnostic accuracy and reliability in detecting GOR events.

Endoscopy presented inherent challenges and limitation, being labour-intensive, time-consuming and requiring constant direct supervision detracting the investigator from other tasks. Additionally, endoscopy equipment can be cumbersome, fragile and expensive. pH-MII equipment is equally, if not more, costly compared to pH alone and may be a hindering factor for its use in veterinary studies. Despite providing the ability to quantify the volume of refluxate, endoscopy lacks the ability to discern the pH of the refluxate. Evidence has shown that a more alkaline mixed refluxate, with both gastric and duodenal enzymes, work synergistically to cause a more profound inflammatory injury of the oesophageal mucosa than with an acidic gastric or alkaline bile reflux alone (Nehra et al. 1999; Galatos et al. 2001; Oh et al. 2006; Favarato 2012). By providing a pH value, pH alone and pH-MII have the benefit to inform the investigator of the intensity and type of refluxate is present. In our study, only one dog exhibited alkaline reflux, while the remaining cases demonstrated acidic reflux. These results align with previous research, where reports of alkaline reflux were infrequent or not reported at all in dogs (Galatos & Raptopoulos 1995a; Galatos & Raptopoulos 1995b; Raptopoulos & Galatos 1997; Wilson et al. 2005; Panti et al. 2009; Savvas et al. 2016; Flouraki et al. 2022). Conversely, our results diverged from those reported by Favarato et al. (2012) and Lambertini et al. (2020), both of which observed a more balanced distribution of acidic and alkaline reflux in dogs.

Additionally, assuming endoscopy as the reference standard, introduces potential biases, considering human error and over-interpretation challenges. A potential source of inconsistency may have been due to possible over-interpretation of GOR events, in instances where large volumes of mucus or foamy saliva may be mistaken for a GOR event. pH-MII possess a distinct advantage in which it possesses software that excludes gas reflux from analysis. Some dogs were discerned to have experienced GOR events, however, this was not subsequently detected by any of the other modalities and vice versa. Discrepancies in detection rates may be attributed to the construct of the assembly, with the potential for missed GOR events if the catheter is positioned on the non-dependent side of the oesophagus. This may result in pH alone, pH-MII and MII-alone failure to detect GOR events. Additionally, endoscopy only evaluates the oesophagus at a fixed point within its length. If the fluid bolus is orad to the endoscope, the GOR event will likely therefore be missed by endoscopy. A flexible tipped endoscope may overcome this limitation. In 10 of the 35 dogs, there was obstruction of the camera view, potentially affecting the accuracy of GOR identification. Future research should explore methods to mitigate such obstructions. Unfortunately, the use of air or liquid bolus to clean the lens cannot be used as this can result in false interpretation of a liquid bolus or affect lower oesophageal tone by introducing air in the oesophagus.

A review on GOR in anaesthetised dogs noted several inconsistencies between studies that could potentially influence results and method accuracy. In some of the previous investigations, correct positioning of the probe was not confirmed, there was a lack of consensus on pH cut-off values for GOR, and calibration of the equipment was often inadequate (Fernandez Alasia et al. 2021). To mitigate these inconsistencies, correct probe placement at the level of the 10th rib was confirmed using thoracic radiographs, explicit definition of gastric pH cut-off values were assumed prior to commencing data collection and calibration of equipment was performed before each used. By confirming placement at the level of the 10th rib, we can ensure that the catheter tip is located between 2.0 cm and 7.5 cm rostral to the LOS (Waterman and Hashim 1991). Incorrect position can result in false interpretations and influence the accuracy of our results. By adopting these standardized procedures, we aimed to enhance reliability and comparability of GOR measurement in our study.

Notable limitations to our study include the unknown influence the semi-rigid assembly had on the oesophagus and LOS and the occurrence of GOR. Every effort was made to minimize this perceived influence. There were partial and completely obstructed endoscopy views in 10 of the dogs. We considered direct observation as the indicator of the true outcome of GOR for the ROC analysis, and we were confident that a visual reflux score of 2 or 3 would be a true 'yes' for GOR. However, the assignment of the score was subjective and the ROC analysis using endoscopy as the true outcome needs to be interpreted with this caveat in mind.

7. Conclusion

The high incidence of GOR in our study emphasizes the clinical relevance of GOR in anaesthetised dogs undergoing elective orthopedic procedures, highlighting the risk posed by lack of monitoring. It is therefore paramount to identify a reliable and standardize a method to use for future research in the field. pH-MII is rapid, provides real-time interpretative analysis with data recorded continuously and rendered into an interpretative graphic, does not require continuous laborious monitoring, is robust and is more reliable than pH alone. As previously mentioned, pH-metry alone is faced with significant limitations and the observed discrepancy in detection rates emphasize the importance of employing complementary monitoring techniques to enhance the accuracy of GOR diagnosis during anaesthesia in dogs.

This study is the first to compare the accuracy between endoscopy, pH alone and pH-MII to detect GOR in anaesthetised dogs. In light of the absence of a universal consensus on a “gold standard” method for detecting GOR in anaesthetised dogs, it is evident that there is a need for establishing a well-defined effective technique to detect the occurrence of GOR in anaesthetised dogs. Further investigations are required to determine the ‘gold standard’ method in veterinary medicine and further validate the efficacy of combined pH with MII across a larger and more diverse cohort of anaesthetised dogs.

In conclusion, our findings indicated that pH-MII is a reliable method for detection of GOR that is rapid to use and not prone to operator error or bias. The combination of pH with MII offers improved sensitivity compared to singular techniques. While pH alone remains highly accurate and may be a more cost-effective method of monitoring GOR in dogs, we recommend that future research should use pH-MII when investigating GOR in anaesthetised dogs.

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Appendices

Appendix i

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

CONSENT TO PARTICIPATE IN RESEARCH

Establishing a Gold Standard Method for Detection of Gastro-oesophageal Reflux in Anaesthetised Dogs

You are asked to allow your animal to be included in a research study conducted by Dr CJ Blignaut, Prof GE Zeiler, Dr AR Kadwa and Dr E Basson, from the department of Companion Animal Clinical Studies at Onderstepoort Academic Veterinary Hospital. This study is in fulfilment of a postgraduate MMedVet (anaesthesiology) mini dissertation. Your animal's participation in this study is entirely voluntary. Please read the information below and ask questions about anything you do not understand, before deciding whether or not to include your animal in this study.

You have been asked to participate in this study because your dog fits the criteria in fulfilment of this study. Exclusion criteria is dogs weighing <10kgs or >40kgs, abnormal clinical exam or blood results, dogs with a history of coughing or gastrointestinal disease (oesophagitis, IBD, lymphangiectasia, food sensitivities, GERD), dogs diagnosed with hiatal hernias and dogs that have been vomiting/regurgitating for the last 24 hours prior to the surgery.

- **PURPOSE OF THE STUDY**

Gastro-oesophageal reflux (GOR) is a common complication in dogs and cats undergoing general anaesthesia. GOR is typically a transient, backward flow of gastric contents into the oesophagus that is not associated with vomiting during general anaesthesia. GOR's occurrence can be associated with significant complications under general anaesthesia. Up to 87.5% of dogs undergoing general anaesthetic experience a GOR episode, which is a significant statistic. Due to its transient nature and the expense and difficulty to obtain the equipment, GOR is very rarely monitored in the clinical setting and only done so for research purposes.

To date, there has been no gold standard method described for the detection of GOR in dogs undergoing general anaesthetic. Until a gold standard method is defined, future research will be unreliable and inaccurate. The purpose of this study is to establish a gold standard technique to identify GOR so future research is more reliable and with that, it will help us to better understand the mechanism of GOR's occurrence and how we can prevent it. This study will also provide the opportunity to describe a new method of detecting GOR that has never been described before in human or veterinary medicine.

- **PROCEDURES**

It is important to understand, that by participating animals participating in this study will be sampled form dogs presenting to the small animal surgery department for elective hindlimb orthopedic surgery. The research will in no way interfere with the surgeon performing the surgery nor will it impact the final outcome of the surgery.

Pre-study Period

1. Before use, the pH electrodes will be calibrated.

ETHICS APPROVAL NUMBER: Initial_____ Page 1 of 4

2. Each dog will receive a standard intramuscular premedication and induction. They will be maintained on isoflurane (inhalation) in oxygen throughout the procedure.
3. The three probes will be fixed to the outer surface of an ET tube.
4. After general anaesthetic induction, the patient will be positioned in lateral recumbency and a clinical exam will be performed to maker sure they are okay.
5. Before placing the probes, a measurement will be performed in order to ensure accurate placement of the probe.
6. The oesophageal probes then be gently introduced into the oesophagus to approximately the 10th rib by one of the trained and experienced invatigators (CJB or GEZ).
7. An endoscope will then be advanced through the ET tube to allow inspection of the lower oesophageal sphincter.
8. A x-ray will be used to confirm correct placement of the probe.
9. Data will be recorded at 10-minute intervals for a total of 60 minutes from induction (30 minutes prep table, 30 minutes theatre).
10. After 60 minutes of recording, the oesophageal probes and oesophageal ET tube along with the endoscope will be gently removed and the oral cavity inspected for any regurgitation.

- **POTENTIAL RISKS AND DISCOMFORTS**

There are very little risks or discomforts involved with patients participating in this study. Every possible risk has been assessed and measures have been put in place in order to mitigate these risks. However unlikely, the risk for participation in this study include minor oral or oesophageal mucosal trauma/abrasions (unlikely), regurgitation (very unlikely), oesophagitis (unlikely), oesophageal tear (extremely unlikely), aspiration pneumonia (extremely unlikely).

If the animal participating in the study is deemed an anaesthetic risk or at risk with participation in the study, they will be excluded from the study and the pet owner will be contacted immediately. In the event of a significant reflux episode or regurgitation the patient will be treated appropriately to prevent any post-anaesthetic complications. All dogs which regurgitated during the procedure will be monitored 48 hours postoperatively in order to identify any possible complications.

In the event injury resulting from participation in this research project, the investigator do not provide any insurance for animals participating in this research study, nor will they provide compensation for any injury sustained as a result of participation in this research study, except as required by law.

- **POTENTIAL BENEFITS TO SUBJECTS AND/OR TO SOCIETY**

Until today, there has been no gold standard method described for detection in GOR in anaesthetised animals in veterinary medicine.

This study will allow us to possibly describe a gold standard method of detection of GOR in dogs. Furthermore, a novel method, chloride concentration monitoring may be a cost effective and reliable method of monitoring GOR in the clinical setting. Describing a gold standard method will benefit future GOR related research and allow us to more accurately detect GOR episodes.

- **CONFIDENTIALITY**

In accordance with the POPI Act, any information that is obtained in connection with this study and that can be identified with you or your animal will remain confidential and will be disclosed only with your permission or as required by law. Confidentiality will be maintained by means of assigning individual numbers to the participating animals.

Only the primary investigator, supervisor and co-supervisor of the study will have access to the data gathered from this study. The study will be aimed to be published in a journal, however, there will be no identifying details of yourself or your pet in the published article.

- **PARTICIPATION AND WITHDRAWAL**

You can choose whether or not to be in this study. If you volunteer to be in this study, you may withdraw at any time without consequences of any kind or loss of benefits to which you are otherwise entitled. You may also refuse to answer any questions you do not want to answer. There is no penalty if you withdraw from the study and you will not lose any benefits to which you are otherwise entitled.

The investigator may withdraw your animal from this research if circumstances arise which warrant doing so. If there has been any vomiting after the premedication or if there has been any history of gastrointestinal disease or regurgitation episodes within 24 hours prior to the surgery, the animal will be excluded from the study. If your animal has any concurrent diseases that will put them at risk or which may impact the study, they will be withdrawn from the study and the owner will be notified immediately.

- **IDENTIFICATION OF INVESTIGATORS**

If you have any questions or concerns about this research, please contact Dr Christiaan Blignaut (Companion Animal Clinical Studies, University of Pretoria), christiaan.blignaut@up.ac.za, or Prof Gareth Zeiler (Companion Animal Clinical Studies, University of Pretoria), gareth.zeiler@up.ac.za.

- **RIGHTS OF RESEARCH SUBJECTS**

The University of Pretoria Animal Ethics Review Board has reviewed my request to conduct this project.

I understand the procedures described above. My questions have been answered to my satisfaction, and I agree for my animal to participate in this study.

____
Printed Name of Subject

________________________________ ____________________
Signature of Subject Date

________________________________ ____________________
Signature of Witness Date

Appendix ii

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Animal Ethics Committee

8 July 2022

Approval Certificate
New Application

AEC Reference No.: REC204-21
Title: Establishing a gold standard method for detection of gastro-oesophageal reflux in anaesthetised dogs
Researcher: Dr CJ Blignaut
Student's Supervisor: Prof GE Zeiler

Dear Dr CJ Blignaut,

The **New Application** as supported by documents received between 2022-05-12 and 2022-06-27 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2022-06-27.

Please note the following about your ethics approval:

1. The use of species is approved:

Species	Number
Dogs – Various breeds (privately owned)	65

2. Ethics Approval is valid for 1 year and needs to be renewed annually by 2023-07-08.
3. Please remember to use your protocol number (REC204-21) on any documents or correspondence with the AEC regarding your research.
4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
5. **All incidents** must be reported by the PI by email to Ms Marleze Rheeder (AEC Coordinator) within 3 days, and must be subsequently submitted electronically on the application system within 14 days.
6. The committee also requests that you record major procedures undertaken during your study for own-archiving, using any available digital recording system that captures in adequate quality, as it may be required if the committee needs to evaluate a complaint. However, if the committee has monitored the procedure previously or if it is generally can be considered routine, such recording will not be required.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

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Fakulteit Veeartsenykunde
Lefapha la Diseanse tša Bongakadiruiwa

Appendix iii

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Research Ethics Committee

27 June 2024

LETTER OF APPROVAL

Ethics Reference No	**REC204-21**
Protocol Title	**Establishing a gold standard method for detection of gastro-oesophageal reflux in anaesthetised dogs**
Principal Investigator	**Dr CJ Blignaut**
Supervisors	**Prof GE Zeiler** **Dr AR Kadwa**

Dear Dr CJ Blignaut,

We are pleased to inform you that your submission conforms to the requirements of the Faculty of Veterinary Sciences Research Ethics committee.

Please note the following about your ethics approval:

1. Please use your reference number (REC204-21) on any documents or correspondence with the Research Ethics Committee regarding your research.
2. Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
3. Please note that ethical approval is granted for the duration of the research as stipulated in the original application (for Post graduate studies e.g. Honours studies: 1 year, Masters studies: two years, and PhD studies: three years) and should be extended when the approval period lapses.
4. The digital archiving of data is a requirement of the University of Pretoria. The data should be accessible in the event of an enquiry or further analysis of the data.

Ethics approval is subject to the following:

1. The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.
2. Note: All FVS animal research applications for ethical clearance will be automatically rerouted to the Animal Ethics committee (AEC) once the applications meet the requirements for FVS ethical clearance. As such, all FVS REC applications for ethical clearance related to human health research will be automatically rerouted to the Health Sciences Research Ethics Committee, and all FVS applications involving a questionnaire will be automatically rerouted to the Humanities Research Ethics Committee. Also take note that, should the study involve questionnaires aimed at UP staff or students, permission must also be obtained from the relevant Dean and the UP Survey Committee. Research may not proceed until all approvals are granted.

We wish you the best with your research.

Yours sincerely

M Oosthuizen

PROF M. OOSTHUIZEN
Chairperson: Research Ethics Committee

100 YEARS
OF VETERINARY EDUCATION

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Faculty of Veterinary Science
Fakulteit Veeartsenykunde
Lefapha la Disaense tša Bongakadiruiwa

Appendix iv

The ARRIVE guidelines 2.0: author checklist

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

Item		Recommendation	Section/line number, or reason for not reporting
Study design	1	For each experiment, provide brief details of study design including:	Y
		a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated.	
		b. The experimental unit (e.g. a single animal, litter, or cage of animals).	Y
Sample size	2	a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used.	Y
		b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done.	Y
Inclusion and exclusion criteria	3	a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established *a priori*. If no criteria were set, state this explicitly.	Y
		b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so.	Y
		c. For each analysis, report the exact value of *n* in each experimental group.	Y
Randomisation	4	a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence.	N/A
		b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	N/A
Blinding	5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	N/A
Outcome measures	6	a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes).	Y
		b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	Y
Statistical methods	7	a. Provide details of the statistical methods used for each analysis, including software used.	Y
		b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	Y
Experimental animals	8	a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight.	Y
		b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	Y
Experimental procedures	9	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including:	Y
		a. What was done, how it was done and what was used.	Y
		b. When and how often.	Y
		c. Where (including detail of any acclimatisation periods).	
		d. Why (provide rationale for procedures).	Y
Results	10	For each experiment conducted, including independent replications, report:	Y
		a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range).	
		b. If applicable, the effect size with a confidence interval.	N

Appendix v

GOR Research Data Collection Sheet

1

Name:________________

Animal ID:_____________

Age: ________________

BCS: ________________

Sex: MI ☐ MN ☐ FN ☐ FI ☐

Weight: ______________

Breed:________________

Procedure:___________________

Collector Initials: _____________

Recumb. Prep: RL ☐ LL ☐ D ☐

Recumb. Theat: RL ☐ LL ☐ D ☐

Stress Score: __________

Date:_________________

Starve Time:___________

Induc. Time: __________

Place. Time: __________

Rads. Time: ___________

Theat. Time: __________

Record of GOR Episodes																																													
Time (min)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	25	30	35	40	45	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
pH Value																																													
Cam. Reflux																																													
Oral Reflux																																													

Emesis event: Y / N Time:_________________ Laryngeal Stimulation Score:___________

Change in Position:____

Notes: ____

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Instructions:

- Animal **ID** (Number of animal enrolled in study e.g 8th animal 08, 12th animal 12)
- Record **age**, **sex**, **BCS** (score out of 9, example attached) and **stress score**. Criteria: dogs presenting for pelvic limb surgery that are > 15kg & < 40kg.
- **Exclusion** criteria are dogs weighing <15kg or >40kg, abnormal clinical exam or blood results, dogs with a history of coughing or gastrointestinal disease (oesophagitis, IBD, lymphangiectasia, food sensitivities, GERD), dogs diagnosed with hiatal hernias and dogs that have been vomiting/regurgitating for the last 24 hours prior to the surgery.
- Type of procedure should be recorded with noting the affected pelvic limb.
- Record the resting position/**recumbency** in prep and theatre.
- Record induction time (**Induc. Time**), final placement of probes (**Place. Time**), time of transport from prep to radiology (**Radio. Time**; N/A if not applicable) time of transport from prep to theatre (**Trans. Time**)
- Probe placement is **5cm** from LOS. Insert the probe to a fixed depth measuring the length from the rostral **mandibular incisors** to the cranial margin of the **10th rib**. Confirm placement with lateral **radiograph** (MAs 2.5-3.2; Kv 85-100, focal point 100cm). A strip of white zinc oxide will be used to mark the position of the probe at the level of the mandibular incisors. This is used as a reference point to see if the probe migrated during transport. The probe must be fixed in place using a small crepe bandage tied to the mandible.
- **Recording** starts 1 min after confirmed probe placement. Data captured every minute for first 20 minutes and then for every 5 minutes until patient is moved to theatre where recordings are continued every minute for 20 minutes. Recording stops 20 minutes after patient was moved to theatre. **Minimum** recording of **1 hour** duration.
- Record values for chloride (Cl), pH, and GOR confirmation during active reflux (visual oral).
- **Change in position** during study should be recorded in space provided (e.g any alteration of position during prep, excluding transport time which should be recorded in space provided (top right of sheet, see above description).
- Notes is to record times of visible/active GOR episodes with treatment administered (e.g. suction, saline rinse, omeprazole, etc). To note any pertinent information that may influence GOR episode (abnormal pressure on abdomen, prolonged starving (>12 hours), not starved (<4 hours), heat (female), vomiting after premedication, response to surgical stimulus, patient wakes up during transport, additional drugs given, etc.)

Appendix vi

agriculture, land reform & rural development

Department:
Agriculture, Land Reform and Rural Development
REPUBLIC OF SOUTH AFRICA

Directorate Animal Health, Department of Agriculture, Land Reform and Rural Development
Private Bag X138, Pretoria 0001

Enquiries: Ms Marna Laing • Tel: +27 12 319 7442 • Fax: +27 12 319 7470 • E-mail: MarnaL@dalrrd.gov.za
Reference: 12/11/1/1/8 (2265JD)

Responsible person: Dr Christiaan Johannes Blignaut
Institution: Onderstepoort Veterinary Academic Hospital, Old Soutpansberg Road, Onderstepoort, 0110
Email: christiaan.blignaut@up.ac.za

Dear Dr Blignaut,

PERMISSION TO DO RESEARCH IN TERMS OF SECTION 20 OF THE ANIMAL DISEASES ACT, 1984 (ACT NO 35 OF 1984)

Title of research project / study: "Establishing a gold standard method for detecting gastro-oesophageal reflux in anaesthetised dogs"

Your application dated 21 November 2021, requesting permission under Section 20 of the Animal Diseases Act, 1984 (Act No 35 of 1984) to perform the research project or study stipulated above, refers.

Based on the information provided in your application, your study does not fall under the scope of Section 20 of the Animal Diseases Act, 1984 (Act no 35 of 1984) provided that statements 1 to 7 hereunder (as applicable) are, and remain, accurate in relation to your research project.

Should the accuracy of any of the statements 1 to 7 hereunder change in any way in relation to your project, you are required to inform the Section 20 Secretariat. You may not proceed with any activities until written permission to do so have been granted by the National Director of Animal Health.

1. No work will be done with any controlled and/or notifiable animal diseases (list of diseases can be obtained from this office), which also includes any animal diseases which do not occur in South Africa;

– 1 –

2. No work will be done with any pathogen, disease, vector, micro-organism, parasite or animal material (including vaccine, serum, test kit, toxin, anti-toxin, antigen, biological product which consists or originates from a micro-organism, animal or parasite);
3. No imported material of animal origin or imported animal pathogens will be utilized in the study;
4. No samples that originate from a biobank will be used in the study;
5. No clinical studies will be performed in the target species, either in a laboratory or in the field;
6. The areas where the samples are to be collected are not under restriction for controlled or notifiable animal diseases to which the species of animal, from which the samples are obtained, is susceptible;
7. No samples or products will be obtained from an abattoir.

Written permission from the Director of Animal Health must be obtained prior to any deviation from the conditions. Application must be sent in writing to MarnaL@dalrrd.gov.za Failure to obtain written permission as above may be considered a contravention of the Animal Diseases Act, 1984 (Act no 35 of 1984).

Kind regards,

Dr Mpho Maja
DIRECTOR: ANIMAL HEALTH
Date: 2022 -01- 11

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SUBJECT: Permission to do research in terms of Section 20 of the Animal Diseases Act, 1984 (Act No 35 of 1984)

Appendix vii

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YUNIBESITHI YA PRETORIA

Faculty of Humanities
Fakulteit Geesteswetenskappe
Lefapha la Bomotho

Humanities 100.
Since 1919

25 June 2024

Dear Dr CJ Blignaut

Project Title:	Establishing a gold standard method for detection of gastro-oesophageal reflux in anaesthetised dogs
Researcher:	Dr CJ Blignaut
Supervisor(s):	Prof GE Zeiler Dr AR Kadwa
Department:	Companion Animal Clinical Studies
Reference number:	29003352 (HUM013/0622)
Degree:	Masters

Thank you for the application that was submitted for ethical consideration.

The Research Ethics Committee notes that this is a literature-based study and no human subjects are involved. The application has been **approved** on 25 June 2024 with the assumption that the document(s) are in the public domain. Data collection may therefore commence, along these guidelines.

Please note that this approval is based on the assumption that the research will be carried out along the lines laid out in the proposal. However, should the actual research depart significantly from the proposed research, a new research proposal and application for ethical clearance will have to be submitted for approval.

We wish you success with the project.

Sincerely,

Prof Karen Harris
Chair: Research Ethics Committee
Faculty of Humanities
UNIVERSITY OF PRETORIA
e-mail: tracey.andrew@up.ac.za

Room 7-27, Humanities Building, University of Pretoria, Private Bag X20, Hatfield 0028, South Africa
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Declarations

The authors declare no conflict of interest.

The authors declare that artificial intelligence was not used in this study or during the preparation of the manuscript.

The data set is available upon reasonable request.

CJ Blignaut