RESEARCH PAPER

Comparing methods for detection of gastro-oesophageal reflux in anaesthetized dogs

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Abstract

Objective To compare the sensitivity and specificity of pH with multichannel intraluminal impedance (pH-MII), pH-metry (pH) alone and MII alone to direct observation of GOR by endoscopy in anaesthetized dogs.

Study design A prospective comparative trial in a live canine model.

Animals A group of 35 (22 females, 13 males) dogs of various breeds. The mean (range) body weight and age were 31.9 (14−40) kg and 5.6 (0.75−12) 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 used to measure oesophageal pH, MII and directly visualize reflux. Visual reflux score was (0−3) and pH was recorded on a data capture sheet. Reflux was considered to have occurred whenever oesophageal pH was < 4.0 or > 7.5, device software analysing MII data detected fluid or a visual reflux score of 2 or 3 were assigned. Receiver operator curves (ROC) analysis was used to determine sensitivity and specificity for each monitoring method to detect GOR.

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%). ROC analysis showed fair accuracy for pH-MII and pH alone, whereas MII demonstrated low accuracy.

Conclusions and clinical relevance In conclusion, 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, it lacks the ability to discern the pH of refluxate. pH alone misses reflux events with intermediate pH (4.1−7.4). Incorporation of impedance addresses some of the limitations associated with pH alone and enhances diagnostic accuracy.

Keywords dogs, endoscopy, gastro-oesophageal reflux, intraluminal impedance, pH.

Introduction

According to a recent review, gastro-oesophageal reflux (GOR) has been defined as ‘the presence of fluids, not reaching the mouth or nose, in the oesophagus’ (Fernandez Alasia et al. 2021). GOR is a common complication in dogs undergoing general anaesthesia (Lambertini et al. 2020), presenting as an undetected, transient, retrograde flow of gastric contents into the oesophagus that is not associated with vomiting (Ristic et al. 2017).

The acidity of gastric content in the oesophagus can induce erosive damage, leading to postoperative oesophagitis and discomfort in dogs (Adamama-Moraitou et al. 2002; Favarato et al. 2012). Severe instances may result in scar tissue formation and strictures, making perianaesthetic GOR responsible for up to 65% of oesophageal stricture cases in dogs (Galatos et al. 2001; Adamama-Moraitou et al. 2002). Cranial migration of gastric contents can lead to aspiration, causing pneumonitis and aspiration pneumonia that can be life-threatening (Galatos & Raptopoulos 1995). Aspiration pneumonia is one of the most common causes of death-related complications in human anaesthesia (Engelhardt & Webster 1999). Similarly, in veterinary medicine, 4% of dogs undergoing surgical intervention of brachycephalic airway syndrome developed postoperative aspiration pneumonia (Lindsay et al. 2020).

Various diagnostic modalities, including endoscopy, videofluoroscopy, nuclear scintigraphy, computed tomography and real-time magnetic resonance imaging, are used to detect GOR in human and veterinary medicine (Favarato et al. 2012; Zhang et al. 2015; Eivers et al. 2019; Benzimra et al. 2020; Grobman et al. 2020; Paran et al. 2023). In human and veterinary literature, pH and pH-multichannel intraluminal impedance (pH-MII) remain the most commonly utilized methods to detect GOR (Bredenoord 2008; Ristic et al. 2017; Lambertini et al. 2020; Fernandez Alasia et al.2021).

Oesophageal pH measurement utilizes a flexible oesophageal catheter with a pH sensor at the tip, positioned 6 cm rostral to the lower oesophageal sphincter (LOS), to detect pH fluctuations in the oesophageal lumen (Favarato et al. 2012; Zacuto et al. 2012). A reflux episode is recorded with a pH decrease below 4.0 (acidic gastric reflux) or an increase above 7.5 (alkaline biliary reflux) (Wilson et al. 2005; Johnson 2014; Lambertini et al. 2020).

A pH-MII monitoring device includes a flexible catheter with seven impedance electrodes and a pH sensor at the probe tip (Ristic et al. 2017). This technique measures oesophageal pH and fluid movements, providing information of the refluxate's nature, composition, migration distance, duration and frequency (Hojsak et al. 2016). Impedance electrodes detect changes in impedance from gas or liquid in the oesophageal lumen, while the pH sensor detects pH fluctuations (Rosen et al. 2018). A GOR event is defined as a 50% increase in ohm (Ω) across two consecutive impedance channels in the distal oesophagus for more than 2 seconds (Zacuto et al. 2012). Computer software analyses pH values and impedance data, quantifying acidic, weakly-acidic and nonacidic GOR events (Hojsak et al. 2016; Rosen et al. 2018).

Although GOR has been extensively researched, the primary method of detection in veterinary medicine has been pH-metry alone (pH) (Galatos & Raptopoulos 1995; Anagnostou et al. 2015; Savvas et al. 2016; Lambertini et al. 2020), with limited utilization of pH-MII (Zacuto et al. 2012; Tarvin et al. 2016). There is a lack of studies comparing the sensitivity and specificity of different methods for detecting GOR in dogs.

This trial compared the binomial outcome (yes/no) of pH-MII, pH-metry alone and MII alone to direct endoscopic observation of GOR in anaesthetized dogs. We hypothesized that pH-MII would be more sensitive and specific for detecting GOR than pH or MII alone.

Materials and methods

Animal and husbandry

Ethics approval for the prospective comparative trial was obtained from the Research and Animal Ethics Committees of the University of Pretoria (REC204-21). This study was reported using Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines. A live canine model comprising 35 dogs was used. The sample size was calculated using a commercially available software (MedCalc Statistical Software, Version 19.5; MedCalc Software Ltd, Belgium) where the α error for the sample size calculation was set at 0.05, corresponding to a 95% confidence interval. The sample size of 35 animals was derived from the calculation.

The study population was selected from dogs scheduled for elective pelvic limb orthopaedic procedures admitted to the Onderstepoort Veterinary Academic Hospital. Before acceptance into the study, informed consent from owners was obtained. Inclusion criteria were a body mass between 10 and 40 kg, physiologic variables and blood work (creatinine, haematocrit and total serum protein) within reference intervals, and an American Society of Anesthesiologists physical classification of I or II. Dogs with recent history of respiratory or gastrointestinal disease or those on medications affecting the risk of GOR or LOS tone were excluded from the study. Dogs were admitted on the day of the procedure and housed in ward cages with comfortable bedding. Postoperatively, the dogs were recovered in the high care ward for at least 2 days for monitoring and care performed by students, nurses and veterinarians.

Study procedures

At least 1 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. 1a). After calibration, the monitoring devices used to detect GOR were assembled (assembly) in-and-around 8.5 mm internal diameter polyvinyl chloride endotracheal tube as follows: an endoscopy camera (6-LED Wifi-Endoscope Cam; Sanoxy, CA, USA) was threaded through the inside of the endotracheal tube until the distal tip was positioned at the level of the tube bevel. Electrical insulation tape (25 mm) was wrapped around the assembly to form a liquid-tight seal. Then, a disposable flexible pH-MII catheter (VersaFlexZ; Given Imaging) was affixed to the side of the assembly using narrow strips of insulation tape, positioning the tip 10 mm beyond the camera (Fig. 1b).

Measures were implemented to avoid conditions that could potentially impact data recordings and subsequent results. These included minimizing changes in body position during surgical preparation to avoid inadvertent increases in intra-abdominal pressure ensuring correct placement, thereby avoiding inadvertent placement into the stomach and preventing accidental dislodgement or removal of devices.

Food was withheld for 6–12 hours, while *ad libitum* access to water was permitted until 2 hours before premedication. Dogs were premedicated with medetomidine (Domitor, 1 mg mL^{-1}; Zoetis, RSA) at 0.01 mg kg^{-1} and morphine (morphine, 10 mg mL^{-1}; Fresenius-Kabi, RSA) at 0.3 mg kg^{-1} drawn up in separate syringes but then mixed into one syringe for a

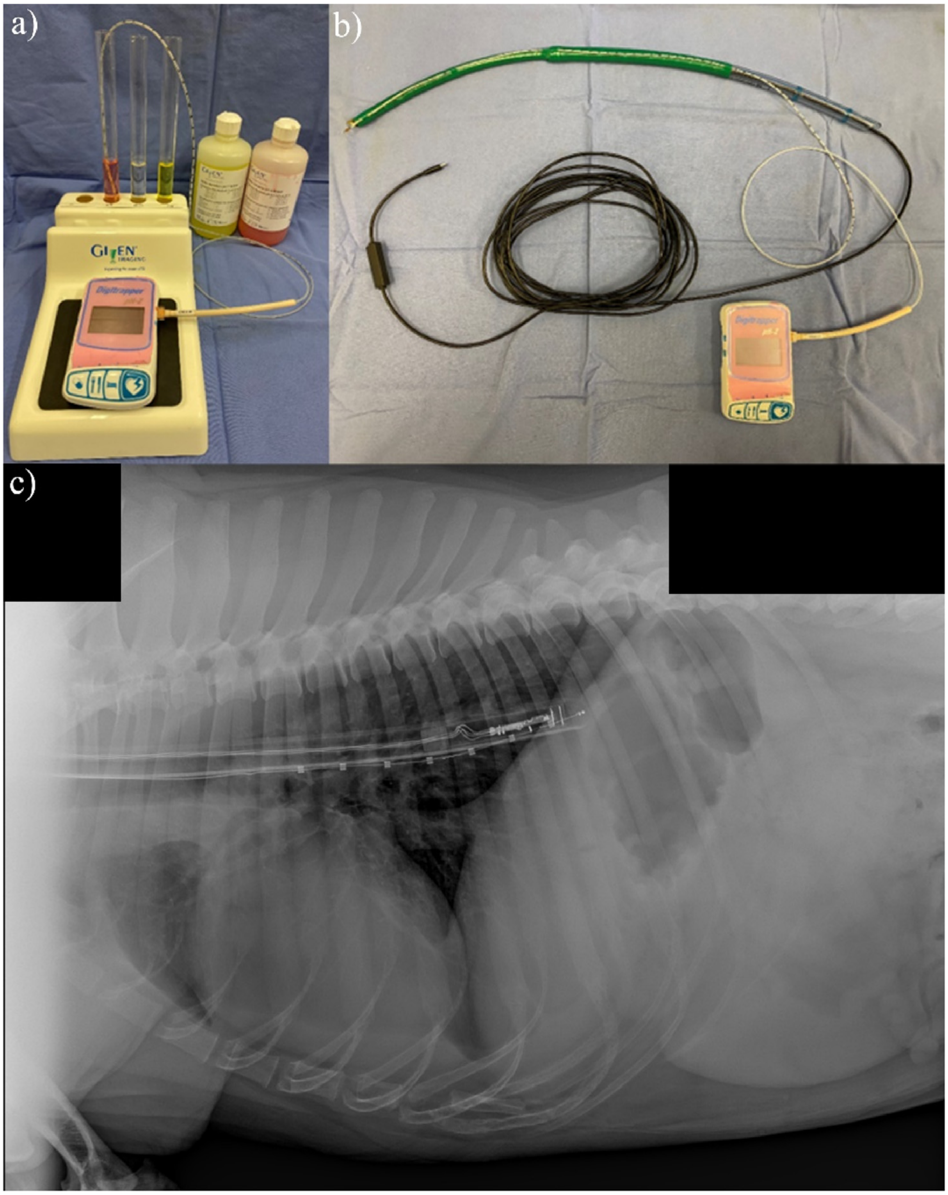

Figure 1 (a) Calibration of pH electrode in buffer solutions of pH 4.0 and 7.0. (b) Gastro-oesophageal reflux (GOR) monitoring assembly consisting of an 8.5 mm internal diameter polyvinyl chloride endotracheal tube, endoscopy camera, disposable flexible pH multichannel intraluminal impedance (pH-MII) 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 tenth rib. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

single intramuscular administration into the quadriceps muscle group. After 30 minutes, a cephalic vein was aseptically cannulated using a 20 gauge, over-the-needle intravenous (IV) catheter (Jelco; Smiths Medical, UK). Anaesthetic induction ensued with propofol (Propofol 1% Fresenius Injection, 10 mg mL^{-1}; Intramed, RSA) administered IV, titrated to effect to achieve tracheal intubation. Endotracheal intubation was facilitated with the help of an illuminated laryngoscope using a cuffed polyvinyl chloride endotracheal tube (KRUUSE PVC Endotracheal tube with cuff; KRUUSE, Denmark). A cuff inflation leak test was performed by administering a single positive airway pressure of 20 cmH_20, followed by inflating the endotracheal tube cuff using an injectable syringe until the audible air leak noise ceased. All dogs were administered the same perioperative drug therapy, which included preoperative meloxicam [0.2 mg kg^{-1}, subcutaneously (SC), Metacam, 5 mg mL^{-1}; Boehringer Ingelheim, RSA], cefazolin [20 mg kg^{-1}, IV, Zefkol; Acino Pharma (Pty) Ltd, Namibia] and various locoregional blocks of the pelvic limb using bupivacaine [0.1 mL kg^{-1} per perineural injection, Macaine, 5 mg mL^{-1}; Adcock Ingram Critical Care (Pty) Ltd, RSA]. All dogs were administered postoperative analgesia in the form of morphine (0.3 mg kg^{-1} every 4 hours, IV, 10 mg mL^{-1}; Fresenius-Kabi) and meloxicam (0.1 mg kg^{-1} daily, SC).

Subsequently, the dogs were connected to a semi-closed, rebreathing system (22 mm Flextube breathing system; Intersurgical, RSA) equipped with a precision vaporizer (Ohmeda Isotec 5; BOC Health Care, UK). The vaporizer dial was adjusted between 2.0% and 2.5% and an initial fresh gas flow rate set to 100 mL kg^{-1} minute^{-1} to maintain anaesthesia using isoflurane (Isofor; Safeline Pharmaceuticals, RSA) in oxygen. After 10 minutes, the fresh gas flow rate was adjusted to 50 mL kg^{-1} minute^{-1}. The dogs were placed in lateral recumbency, with the nonaffected pelvic limb positioned on the dependent side, and thorax and abdomen positioned atop a digital radiography detector plate (VIVIX-S; VIEWORKS Co. Ltd., Republic of Korea). Lactated Ringer's solution (Ringers Lactate Solution; Fresenius-Kabi) was administered IV at a rate of 5 mL kg^{-1} hour^{-1} using an electronic infusion pump (MedCaptain HP60; MedCaptain Medical Technology Co. Ltd., Guangdong, China).

Following induction of anaesthesia, the endoscope was linked to a laptop computer (Lenovo E50; Lenovo, Hebei, China) to provide real-time video analysis. To ensure correct placement of the assembly, a measurement was taken from the maxillary incisors to the level of the tenth rib, as previously described by Waterman & Hashim (1991). The measured length was demarcated on the assembly with tape to guide the depth of advancement into the oesophagus. During advancing, endoscopy video was monitored to detect visual reflux and prevent accidental advancement into the stomach. The primary investigator performed the placement and positioning to ensure consistent placement. A lateral thoracic radiograph, using a portable x-ray generator (ULTRA 9020BT Diagnostic X-ray unit; Ecoray Co. Ltd., Republic of Korea), was performed to verify correct positioning of the assembly (Fig. 1c).

Following confirmation of correct positioning, the assembly was secured firmly to the dog's maxilla using 25 mm ribbon gauze (Cutisoft Gauze; BSN Medical, Germany), to mitigate inadvertent displacement during data collection. The pH-MII catheter was connected to a portable data recording and monitoring device (Digitrapper; Medtronic, RSA), and the endoscope provided real-time visual analysis of the distal oesophagus. Continuous monitoring of pH and MII values was 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). Throughout the initial 20 minutes of the surgical preparation, pH values and visual reflux score (Table 1) were recorded every minute on a data capture sheet. 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.

During surgical preparation and data collection, a veterinary anaesthetist not involved in the study monitored physiological variables and adjusted the depth of anaesthesia, if required. These variables were systematically recorded on a monitoring sheet at 5 minute intervals. After 45 minutes, the dogs were moved to the surgical theatre.

Dogs that manifested GOR events were administered omeprazole [1 mg kg^{-1}, IV; Nexipraz, 8 mg mL^{-1}; Ranbaxy Pharmaceuticals (Pty) Ltd, RSA] every 12 hours and sucralfate (0.5 g per dog in dogs weighing < 20 kg and 1 g per dog in dogs weighing > 20 kg orally; Ulsanic, 200 mg mL^{-1}; Aspen Pharmacare, RSA) once daily for 5 days. In dogs that exhibited overt oropharyngeal refluxate, their oral cavity was rinsed, swabbed dry and oesophagus lavaged with saline before termination of general anaesthesia and tracheal extubation. These interventions aimed to mitigate the incidence of aspiration and minimize oesophageal stricture formation.

Data and statistical analysis

A dichotomous outcome (yes/no) was assigned for each method used to monitor GOR events at each time point. A 'yes' was assigned for pH method when the distal oesophageal pH was < 4.0 (indicative of acidic reflux) or > 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 analyse the MII data to assign a 'yes' for fluid 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). For each GOR monitoring method, receiver operator curve (ROC) analysis (DeLong et al. 1988) 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 were plotted against the true outcome detected by the pH method. Area under the curve (AUC) was used to discern an accurate method from a nonaccurate 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

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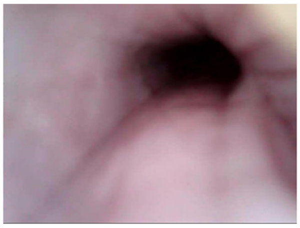
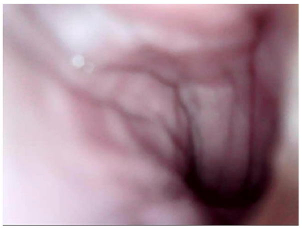
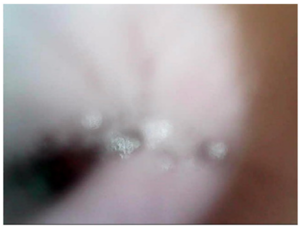
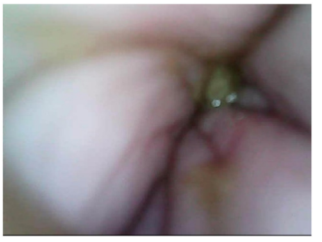
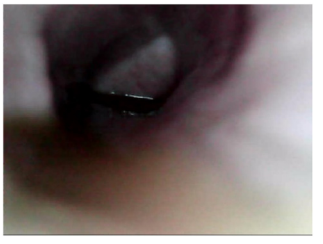
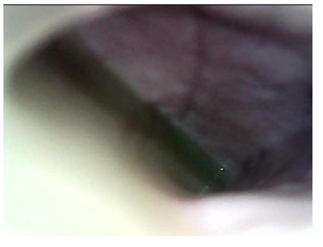
Table 1 Visual reflux score used to grade refluxate within the distal oesophagus in 35 anaesthetized dogs with an endoscope placed at the level of the tenth rib.

Visual Reflux Score			
Score	**Classification**	**Description**	**Picture**
0	None	No reflux visible on camera	 
1	Mild	A 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.	 

(*continued on next page*)

Table 1 (*continued*)

Visual Reflux Score			
Score	**Classification**	**Description**	**Picture**
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 gastro-oesophageal reflux content.	 
3	Severe	Near to complete obliteration of camera view with reflux content in the oesophageal lumen.	 

0.8−0.9) and excellent accuracy (AUC ≥ 0.9) (Nahm 2022; Swets 1988).

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 determining method reliability. Inter-rater agreement between pH and pH-MII and between pH and MII was also analysed. For all tests, where applicable, a significance was interpreted as a p-value < 0.05.

Results

The mean (range) mass and age of the dogs (22 female; 13 male) of various breeds were 31.9 (14−40) kg and 5.6 (0.75−2) years, respectively. Endoscopy identified GOR events in 20/35 dogs, constituting 57% of the study population, while pH-MII monitoring detected GOR events in 19/35 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 (Table 2). Among the 13 dogs identified by the pH method, 12 exhibited acidic reflux (pH < 4), whereas one experienced alkaline reflux (pH > 7.5). During the study, endoscopy view was obstructed, whether temporarily or permanent, in 10 of the 35 dogs.

The AUC for the ROC of endoscopy was 1.0 and demonstrated 100% sensitivity and 100% specificity. By using endoscopy as the true diagnostic outcome, pH and pH-MII both showed a fair accuracy in discerning GOR events (Fig. 2b and

Table 2 Outcome of gastro-oesophageal reflux events detected over a 45 minute period using four different methods of detection in 35 anaesthetized dogs positioned in lateral recumbency directly after induction of anaesthesia.

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

ID, identification number; NGD, no gastro-oesophageal reflux detected; pH-MII, pH with multichannel intraluminal impedance.
Acidic reflux is classified as gastro-oesophageal pH < 4, and alkaline reflux is classified as gastro-oesophageal pH > 7.5. Endoscopy view temporarily* or permanently† obstructed during study.

c). Notably, MII demonstrated a low accuracy in discerning GOR events (Table 3). 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%).

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 were 94% and 99%, respectively, in dogs with a pH < 4 and 94% and 12%, respectively, in dogs with a pH > 7. Similarly, comparing pH as the true diagnostic outcome with pH-MII showed excellent test accuracy in discerning GOR events (Table 3). 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.

Inter-rater kappa agreement analysis revealed fair agreement between endoscopy and pH, as well as endoscopy and pH-MII (Table 4). 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.

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 1995; Wilson et al. 2005; Lambertini et al. 2020; Paran et al. 2023). Our study, focused on dogs undergoing 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. However, pH alone and MII alone had a lower detection rate of GOR events.

The application of pH-MII in veterinary medicine is rare. 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 anaesthetized dogs that we could find. The substantial variability in reported GOR prevalence in previous investigations is broad and prompts scrutiny of the accuracy of the current methods of detection with the adoption of a standardized technique across studies. There are a large number of predisposing risk factors for the occurrence of GOR in anaesthetized dogs, which include administration of certain anaesthetic drugs, body position, type of food and preoperative fasting times, deep-chested breeds, body weight, pregnancy, increased intra-abdominal pressure, abdominal surgery and older dogs (Galatos & Raptopoulos 1995; Raptopoulos and Galatos, 1997; Wilson et al. 2005; Savvas et al. 2009; Anagnostou et al. 2015, 2017). In a comprehensive review, Savvas et al. (2022) summarized several factors influencing GOR development in dogs during general anaesthesia, potentially contributing to the observed variations between previous studies. However, the review did not investigate the potential role of detection methods or techniques utilized as probable contributing factor to the variability in reported prevalence. Our study is the first to compare the accuracy of endoscopy, pH alone and pH-MII to detect GOR in anaesthetized dogs. It is evident that there is a need to establish a well-defined effective technique to detect the occurrence of GOR in anaesthetized dogs. Further investigations are required to determine the 'gold

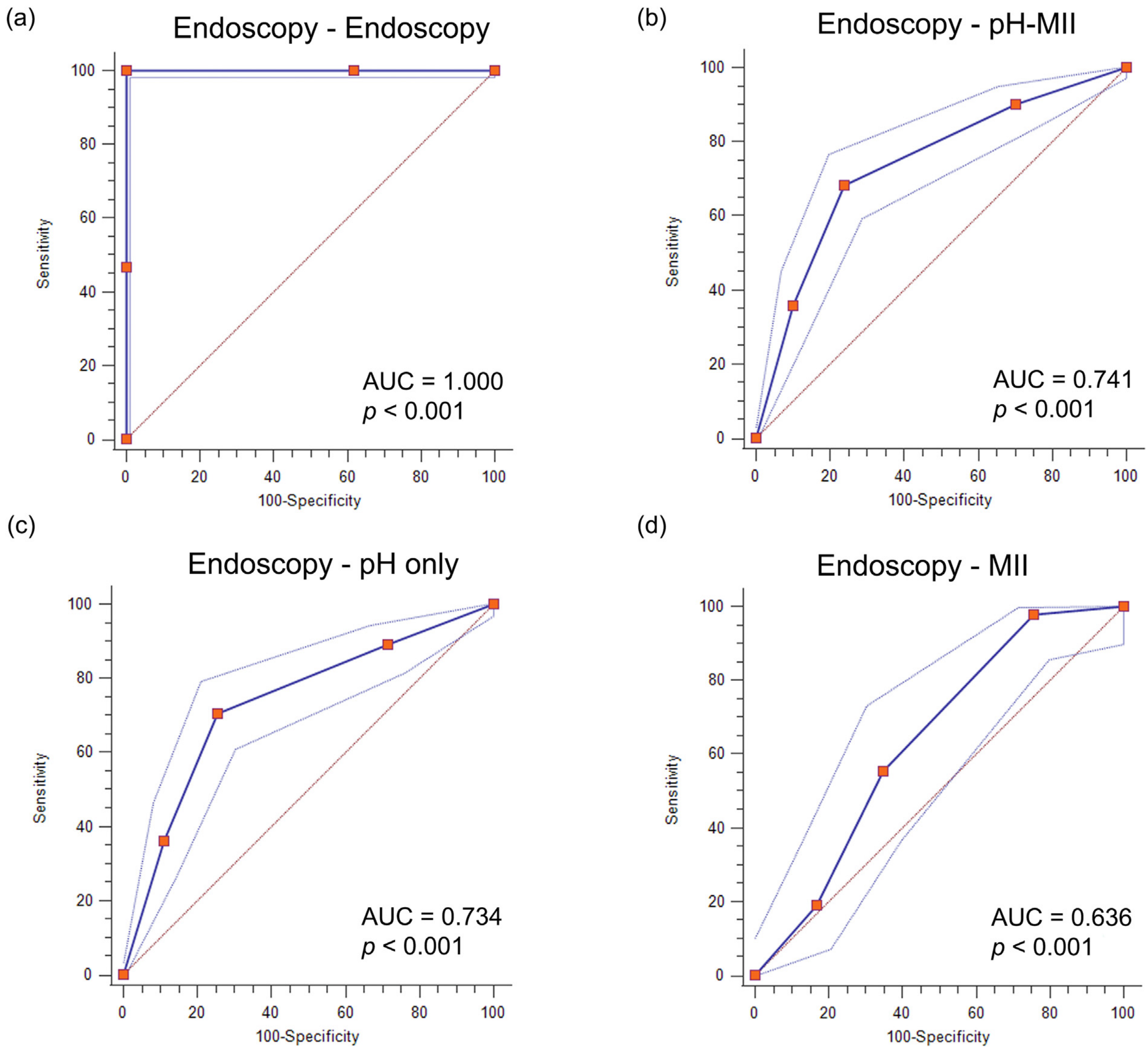


Figure 2 Receiver operator curve (ROC) graphs used to determine sensitivity and specificity between the different monitoring methods for detecting gastro-oesophageal reflux (GOR) in 35 anaesthetized dogs. The true outcome detected by the endoscopy method (a) was used and plotted against each data point for pH multichannel intraluminal impedance (pH-MII) (b), pH alone (c) and impedance (MII) alone (d). Included in ROC curve graphs b, c and d are 95% confidence interval (CI) lines for sensitivity and specificity were . Area under the curve (AUC) was used to detect an accurate method and a nonaccurate 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).

standard' method for use in veterinary medicine and further validate the efficacy of combined pH-metry with MII across a larger and more diverse cohort of anaesthetized dogs.

We noted that out of the 19 dogs detected by pH-MII, approximately a third were exclusively detected by pH alone 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 with 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 failed to detect reflux events with intermediate pH values (i.e. pH between 4 and 7.5), resulted in a potential underreporting of the frequency of GOR events and provide a possible explanation for these findings. Based on this summary, endoscopy and pH-MII are superior modalities for identifying GOR events during anaesthesia in dogs. Incorporating impedance addresses some of the limitations associated with pH

Table 3 Sensitivity and specificity for detecting gastro-oesophageal reflux (GOR) in 35 dogs anaesthetized 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 were plotted against the true outcome detected by the pH alone method.

Variable	Prevalence (%)	ROC AUC	ROC 95% CI	*p*	Sensitivity (%)	Specificity (%)
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

AUC, area under the curve; CI, confidence interval; MII, multichannel intraluminal impedance; pH-MII, pH with intraluminal impedance, ROC, Receiver operator curve.

Table 4 Statistical analysis using inter-rater kappa (κ) 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 anaesthetized 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 κ	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

CI, confidence interval; MII, multichannel intraluminal impedance; pH-MII, pH with intraluminal impedance.

alone and significantly improves 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 utility in veterinary anaesthesia.

Examining the data point detection rates, we noted that endoscopy outperformed pH alone, pH-MII and MII alone. This disparity suggests that endoscopy possesses heightened accuracy in detecting intermittent GOR events. However, the diagnostic capability of pH and MII alone may have been influenced by the assembly's construction affecting the performance of the catheter if it was situated on the nondependent 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 probe. Although pH-MII demonstrated marginal superiority in discerning GOR events compared with pH alone, the disparity in our results were not as significant as those reported by Hojsak et al. (2016). In the aforementioned study, pH alone did not recognize gastro-oesophageal reflux disease (GORD) in 52.3% children compared with 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 with that in dogs (Youngberg et al. 1985; Sagawa et al. 2009). Notably, observed postprandial gastric pH in humans is higher than that of dogs, which demonstrated a decrease in gastric pH. GORD studies in humans were performed in conscious children and infants over a 24 hour period in which meals were consumed. This discrepancy in postprandial events potentially explains why pH alone detected more GOR events in our dogs than in human studies.

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 suggests that combining these methods may enhance diagnostic accuracy and reliability. MII alone showed none to slight agreement with pH true outcome, indicating this to be an unreliable standalone method for GOR detection. The observed patterns of agreement emphasize the benefits of combining pH and impedance monitoring techniques, thereby improving diagnostic accuracy and reliability in detecting GOR events.

Endoscopy presented inherent challenges and limitations, being labour-intensive, time-consuming and requiring constant direct supervision, detracting the investigator from other tasks. Additionally, endoscopy equipment is cumbersome, fragile and expensive. pH-MII equipment is equally, if not more, costly compared with pH alone and this may limit its use in veterinary studies. Despite allowing quantification of the volume of reflux, endoscopy lacks the ability to determine the pH of the refluxate. Evidence has shown that a mixed alkaline refluxate, with both gastric and duodenal enzymes, causes a more profound inflammatory injury of the oesophageal mucosa compared with an acidic gastric or alkaline bile reflux alone (Nehra et al. 1999; Galatos et al. 2001; Oh et al. 2006; Favarato et al. 2012). By providing a pH value, pH alone and pH-MII have the benefit of identifying the type of refluxate present.

Additionally, if endoscopy is used as the reference standard, it introduces potential biases considering human error and overinterpretation challenges. Inconsistencies may result from the overinterpretation of GOR events in instances where large volumes of mucus or foamy saliva may be mistaken for a GOR event. pH-MII has a distinct advantage as it possesses software that excludes gas reflux from analysis. Additionally, endoscopy only evaluates the oesophagus at a fixed point within its length. If the fluid bolus is cranial to the endoscope, the GOR event will probably 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. The use of air or fluid bolus to clean the lens cannot be used as this can result in false interpretation of a fluid bolus or affect the lower oesophageal tone by introducing air into the oesophagus. Furthermore, while the endoscopist was blinded to the MII values, they were not blinded to the pH values, potentially introducing bias into their score assessment. Nonetheless, efforts were made to minimize this bias by following a definitive scoring table.

A review of GOR in anaesthetized dogs noted several inconsistencies between studies that could influence results and methodological 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 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 tenth 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 was used. 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 their effect. 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.

Despite its limitations, our findings suggest that pH remains an acceptable method for detecting GOR in anaesthetized dogs. However, in human studies, pH monitoring has been reported to miss up to 40.052.3% of GORD episodes in human children and infants (Hojsak et al. 2016; Ristic et al. 2017). Furthermore, a study utilizing endoscopy and pH monitoring in anaesthetized dogs found that pH failed to identify 50% of reflux episodes identified by endoscopy (Favarato et al. 2012). In our study, endoscopy and pH-MII detected 35% and 32% more cases than pH, respectively. Although our detection rates were not as pronounced as those in the aforementioned studies, with a larger number of dogs, the number of detected GOR episodes would probably be significant. 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.

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

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Authors' contributions

CJB, GEZ: study design, data collection, data interpretation, statistical analysis and manuscript drafting. ARK: study design, data collection. All authors contributed with the manuscript editing.

Conflict of interest statement

Authors declare no conflict of interests.

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