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Original article

Water immersion vs. air insufflation in canine duodenal endoscopy: is the future underwater?

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Abstract

Endoscopy represents a commonly employed technique for canine enteropathies. Different trials in human intestinal endoscopy have suggested that the introduction of water for luminal distension, in place of air, improves the visualization of the mucosal texture and decreases pain.

The aim of the study was to compare water immersion (WI) vs. air insufflation (AI) during duodenoscopy in anesthetized dogs in terms of mucosal visualization and nociception.

Twenty-five dogs undergoing duodenoscopy were included. The same image of the descending duodenum was recorded applying WI and AI. Each pair of images was analyzed using morphological skeletonization, an image entropy evaluation, and a subjective blind evaluation by three experienced endoscopists. To evaluate differences in nociception related to the procedure applied, heart rate and arterial blood pressure were measured before, during and after WI/AI. To compare the two methods, a t-test for paired data was applied for the image analysis, Fleiss' Kappa evaluation for the subjective evaluation and a Friedman test for anesthetic parameters.

No differences were found between WI and AI using morphological skeletonization and entropy. The subjective evaluation identified the WI images as qualitatively better than the AI images, indicating substantial agreement between the operators. No differences in nociception were found.

The results of the study pointed out the absence of changes in pain response between WI and AI, likely due to the sufficient control of nociception by the anesthesia. Based on subjective evaluation, but not confirmed by the image analysis, WI provided better image quality than AI.

Key words: dog, duodenoscopy, water immersion, air insufflation, image analysis, nociception

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Introduction

Endoscopy of the gastrointestinal tract is a routine examination in human and veterinary medicine for the diagnosis of acute and chronic enteropathies (Jergens et al. 2016).

In order to allow the visualization of the intestinal mucosal surface, it is necessary to dilate the lumen, by using air, or better carbon dioxide, insufflation as a dilation media (Xu et al. 2016). However, as demonstrated in human medicine, insufflation of gas during colonoscopy can increase the angulation and bending of the gut, making the procedure technically more difficult and more painful for the patient (Shah et al. 2002, Xu et al. 2016). For this reason, colonoscopy is often performed under sedation. Approximately twenty years ago in order to try to reduce the side effects of the procedure by allowing the examination in non-sedated subjects, Baumann introduced the water infusion-technique into human colonoscopy as an alternative to the use of air (Baumann 1999, Leung et al. 2007, 2009, Terruzzi et al. 2012). At present, in human medicine, two different methods involving the use of water are in use: water immersion (WI) and water exchange (Leung 2013). The WI technique employs water infusion for luminal distension, with limited use of air insufflation (Lee et al. 2012, Falt et al. 2013). The water infused in the intestinal lumen is generally aspirated during the retraction of the endoscope. The water exchange technique, by means of the infusion and the almost-simultaneous suction of water, involves a continuous recirculation of the liquid in the colon, thus maintaining a layer of clear water and allowing the progression of the instrument along the cecum without employing air. This technique minimizes the distension of the organ and maximizes the cleaning of the lumen during insertion (Cadoni and Isahaq 2018). Moreover, in addition to the subjective pain reduction, the water-infusion technique is able to determine an improvement in image quality (with respect to the employment of gas) which can be quantified by measuring the increase in diagnostic capacity, namely the ability to identify a mucosal neoplasia (the so-called adenoma detection rate) (Radaelli et al. 2010, Lee et al. 2014, Wang et al. 2015). Unlike the endoscopic procedures applied in human medicine, enteric endoscopy in veterinary medicine cannot be performed without the use of general anesthesia. Although the patient is not conscious, assessment of nociception during the procedure could be verified by detection of heart rate and arterial blood pressure modification. Therefore, the aim of this study was to carry out a blind study, comparing two endoscopic techniques, WI and Air insufflation (AI) during duodenoscopy in dogs, in terms of image quality assessment and evaluation of the signs of nociception by means of the detection of cardiocirculatory alterations (heart rate and arterial blood pressure).

Materials and Methods

Inclusion and exclusion criteria

Dogs of different breeds, gender and ages requiring diagnostic duodenoscopy were included in the study. Animals were included if their body size allowed the passage of an endoscope having a diameter of 9.8 mm through the pylorus when performing a duodenoscopy. The recruitment of dogs for the study was voluntary and at no cost to the owners. Written informed consent before enrollment in the study was obtained by the owners.

Anesthetic protocol

The standard anesthetic protocol consisted of intramuscular premedication with dexmedetomidine (Dextroquillan®, Fatro S.p.A. Italy) (0.001 - 0.005 mg/kg) and butorphanol (Nargesic®, ACME S.r.l. Italy) (0.2 mg/Kg), followed by intravenous induction with propofol (Proposure®, Boehringer Ingelheim Animal Health Italia S.p.A. Italy) titrated to effect (1-4 mg/Kg). During the procedure, the patient was kept under anesthesia with isofluorane (Isoflo®, Esteve S.p.A. Italy) in oxygen.

Endoscopy

The endoscopic procedure consisted of a duodenoscopic examination carried out for diagnostic purposes. The animals were positioned in left lateral recumbency in order to allow easier trans-pyloric passage of the endoscope.

Endoscopic examination of duodenum was performed before the full examination of the stomach, to avoid paradoxic motion of the instrument and difficulty in crossing the pyloric sphincter, due to the stomach's distension.

The pattern of the mucosal surface, the shag carpet appearance created by the villi, the major (and occasionally the minor) duodenal papilla and the Peyer's patches were examined.

All the procedures were performed by the same operator (MP), employing the same endoscope (Pentax EG-2931 K, diameter: 9.8 mm).

Water immersion and air insufflation

The duodenal dilation was obtained with the use of both water (WI) and air (AI) in every dog, starting

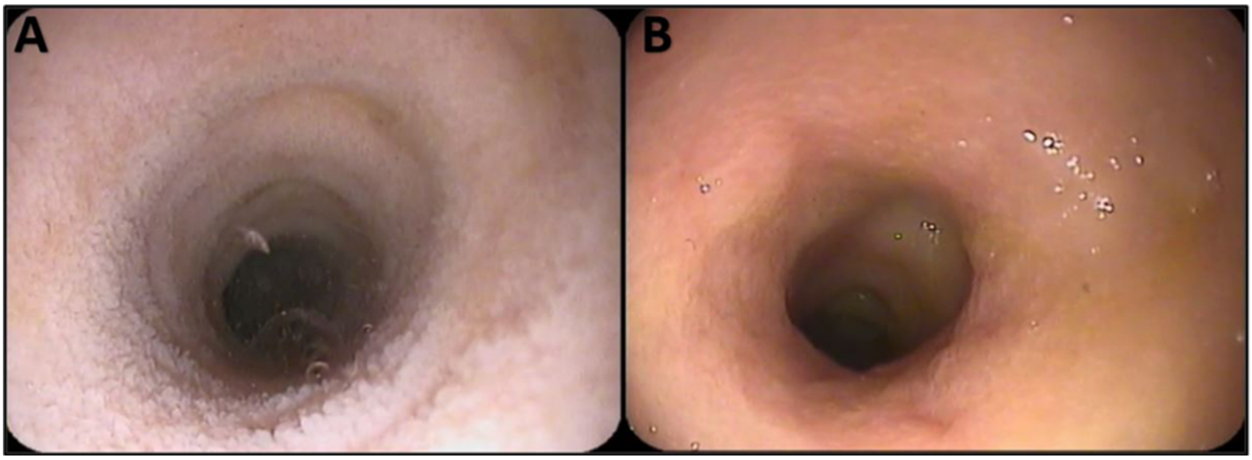

Fig. 1. Example of the same images of the canine descending duodenum recorded with the two endoscopic methods described in the study (A: water immersion, B: air insufflation).

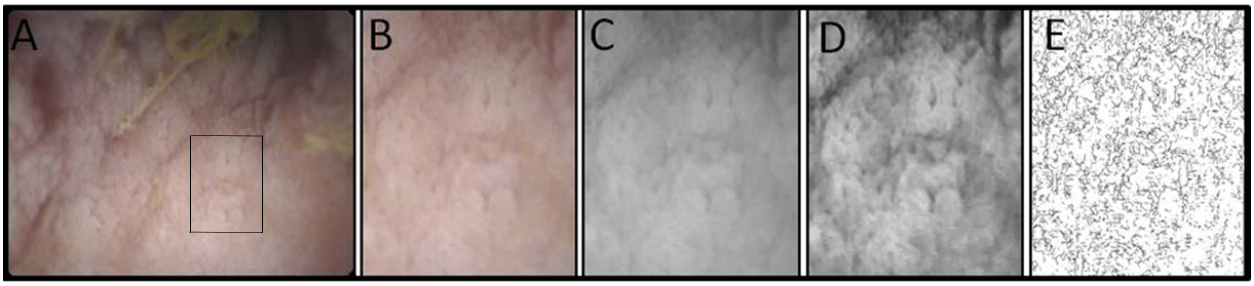

Fig. 2. Example of the application of the texture analysis method (morphological skeletonization). The method employed used the ImageJ software using the Fiji plugin. The same image of the descending duodenum was recorded with AI and WI. From the original image (A), a homogenous and not blurred rectangular portion of the image was selected (B) and the area of the rectangle was calculated in pixels. The image was then converted to a gray scale with the function "RGB (Red, Green, Blue) to luminance" (C) and then contrast enhanced with the "CLAHE" function (D). The algorithm called "Find edges" was subsequently applied to highlight the sharp intensity changes of the image; the binary option was then used to obtain a binary image. Finally, the Skeletonize option (E) was applied, and the length of the image obtained was measured in pixels. The ratio between the rectangle and the skeletonized area was considered; of the two images (obtained with WI or AI); the image having a lower ratio was considered to be of the best quality.

randomly with either the first or the second method. For WI, an amount of warm to the touch water (from 40 to 100 ml until the duodenal lumen was completely filled, depending on the size of the dog) was instilled in the descending duodenum with a syringe through the biopsy channel. If the procedure started with AI, WI was always preceded by air aspiration and, conversely, by water aspiration if the procedure started with WI. All the procedures were recorded using a software package (Pinnacle Studio 22 Plus, Corel Corp., Ottawa ON, Canada). When the lumen was completely dilated with water and the duodenal lumen became transparent, an image of the duodenum was captured and saved by means of dedicated computer software (Adobe Premiere Elements, Adobe Inc, San Jose, CA, US) (Fig. 1A). The water was then removed from the aspiration channel, the lumen dilated with air, and an endoscopic image of the same bowel portion was acquired (Fig. 1B). If the procedure started randomly with water, the steps were reversed.

Image analysis

All pairs of images acquired from each dog, recorded under WI and AI, were analyzed both by applying two different computer analysis procedures (morphological skeletonization and image entropy evaluation), and by a subjective blind evaluation. The first method, derived from a human medicine study in which the comparison, carried out on images of the duodenum of healthy patients vs. patients affected by celiac disease, was used to evaluate the degree of villous atrophy (Ciaccio et al. 2011). In brief, the images were analyzed by employing ImageJ software with the Fiji plugin (National Institutes of Health, Bethesda, Maryland, US) (Figs. 2A-E). A homogenous and unblurred rectangular portion of each image was selected and its area was calculated in pixels. The RGB (Red, Green, Blue) image was then converted to a gray scale, and the contrast was enhanced. A high-pass "edge finding" filtering algorithm was subsequently applied in order

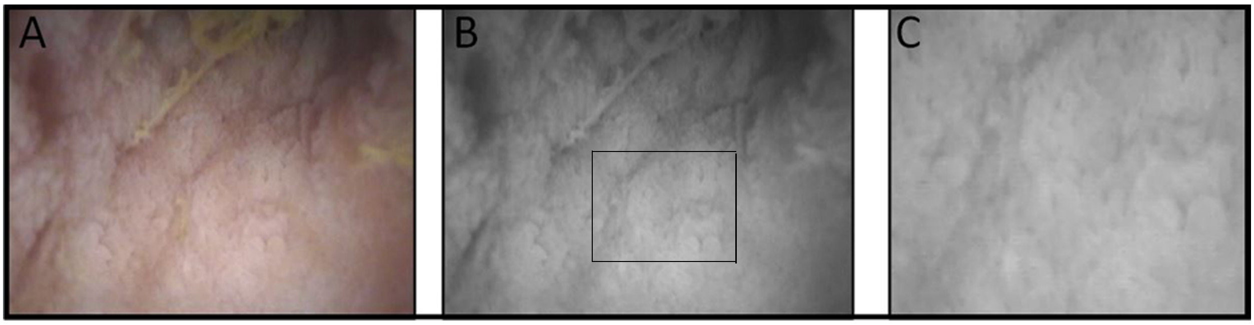

Fig. 3. Example of the application of the texture analysis method (entropy). The method employed used ImageJ software with the GLCM (Gray-Level Co-occurrence Matrix) plugin for texture analysis. The same image of the canine descending duodenum was recorded using AI and WI (A). All the images were converted to a gray scale (function "RGB [Red, Green, Blue] to luminance") (B), and a homogenous and not blurred rectangular portion was selected (C). The application of the GLCM algorithm allowed obtaining a numerical value which quantified the entropy. Of the two images (obtained with WI or AI), the image characterized by lower entropy was considered to be of the best quality.

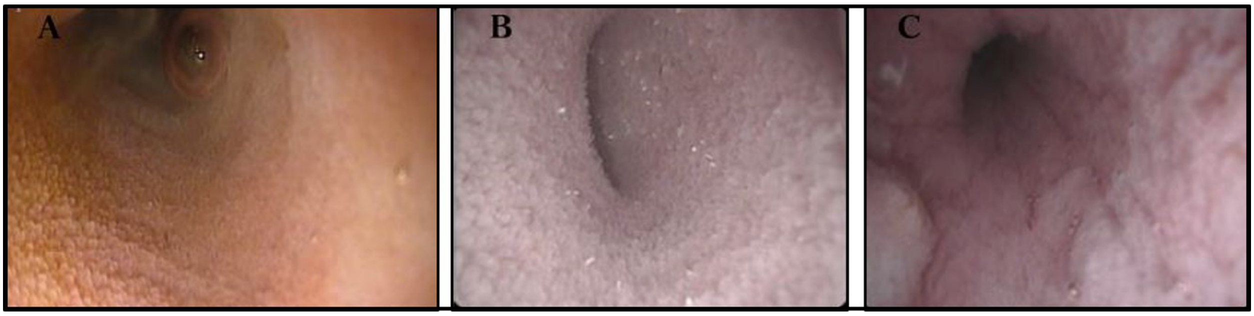

Fig. 4. Example of the parameters considered for subjective evaluation; the first image (A) shows a portion of the canine duodenal lumen in which the villi profile can easily be defined on the mucosal surface, corresponding to a high definition of the mucosal texture; in the second image (B), there are several white spots distributed in the mucosa which correspond to severe cystic lacteal dilation. The third image (C) explains the granulation, defined as the presence of longitudinal grooves in the mucosa. In this image, they are evident in the right-ventral portion of the picture.

to highlight the sharp intensity changes of the image; a binary image was then obtained. Finally, a skeletonization procedure was applied and the lengths of the lines were measured in pixels. The ratio between the rectangle and the skeletonized area was calculated and compared between the two images (obtained with WI or AI); the image having a lower ratio was qualitatively higher in definition. The second method employed (Figs 3A-C) evaluated the entropy, defined as the amount of information given by an image. This method used ImageJ software with the GLCM (Gray-Level Co-occurrence Matrix) plugin for texture analysis (National Institutes of Health, Bethesda, Maryland, US). All the RGB images were converted to a gray scale, and a homogenous and unblurred rectangular portion was selected. The application of the GLCM algorithm allowed obtaining a numeric value, quantifying the entropy. Between the two images (obtained with WI or AI), the image characterized by lower entropy was considered to be of higher quality.

Lastly, a subjective blind evaluation was carried out. Three endoscopists, each with a minimum of 5 years expertise in the field, participated in the study. All the endoscopists involved were external to the project. Each pair of images (WI vs. AI) underwent a blind evaluation carried out by the three experts (A; B; C) who independently selected the best image by analyzing three parameters: 1) mucosal texture, considered as villi definition; 2) sharpness of the eventual lymphangiectasia and 3) eventual granulation, defined as the presence of longitudinal grooves in the mucosa (Figs 4A-C). When comparing the two images, the operators had to give a score of 0 (worst image) or 1 (best image) for each parameter. After adding up the scores for each image, the image receiving the highest score was considered to be the best one. Therefore, for each pair of images, three distinct results were indicated, corresponding to the choice of each of the three experts; only at the end of the evaluation were the 75 (25 X 3) results thus obtained decoded and attributed to each of the two groups (WI and AI). No single evaluator was aware of the method applied in the image recording nor of the endoscopic diagnosis. It should be clarified that an expert endoscopist, based on his/her own experience, could differentiate between the two methods, but the evaluation was made considering the quality of each individual parameter in the images compared to try to transform the subjective judgement into an objective evaluation.

Cardiocirculatory parameters

For the purpose of the nociception evaluation and with the aim of achieving a stable anesthetic plane before each examination, the anesthesia and the endoscopic procedure were divided into four phases. Baseline (phase 1) began soon after the induction of the general anesthesia and ended with the beginning of the water/air phases. The water/air or air/water phases (phase II and III) consisted of the application of air or water, as dilation media, applied in a random order. Outcome (phase IV) was considered to be the end of the evaluation, beginning with water/air reabsorption. Each step was recorded for not less than one minute. During each phase, the heart rate was monitored using a Datex-Ohmeda S5 monitor (GE Healthcare Italia, Milano, Italy) and the blood pressure was measured from the brachial artery using a Pettrust sphygmomanometer (BioCare Corp., Taiwan, China). At baseline, the isoflurane vaporizer was adjusted in order to obtain a stable plane of anesthesia characterized by relaxed jaw tone, an absent palpebral reflex and a ventral position of the eyeball. The vaporizer setting was maintained constant throughout the four phases of evaluation. In case of an insufficient anesthetic plane, a bolus of propofol was administered IV and its eventual administration was recorded. At the end of the outcome phase, the vaporizer was adjusted by the anesthetist in charge based on the patient's requirement in order to complete the endoscopic examination.

Statistical analysis

The statistical analyses were carried out using software MedCalc® 16.8.4 (MedCalc Software bvba, Ostend Belgium). Randomization of the procedure (WI vs AI) was carried out by applying a Fisher-Yates algorithm. Assessment of the data for normality was carried out applying the D'Agostino and Pearson Omnibus normality test. For the image analysis, a t-test for paired data was applied for morphological skeletonization and image entropy evaluation, while a Fleiss' Kappa evaluation was applied for the subjective evaluation. For the anesthesiological parameters, the median measured values during each of the four phases were compared using the Friedman test.

Significance was set at $p<0.05$ for all the analyses.

Institutional animal care or other approval declaration

The study was approved by the University Scientific Ethics Committee for Experimentation on Animals (Approval No. 1087/2019).

Results

A total of 25 dogs were enrolled in the study, 5 mixed breed and 20 pure breeds (3 Boxers, 2 German Shepherd dogs, 2 Miniature Dachshunds, 2 Miniature Poodles, 2 Pugs, 1 Bernese Mountain Dog, 1 Bull Mastiff, 1 Golden Retriever, 1 Irish Setter, 1 Maltese, 1 Miniature Pinscher, 1 Standard Schnauzer, 1 Samoyed and 1 Yorkshire Terrier). Thirteen were male [9 intact males (36%) and 4 neutered males (16%)], and 12 were female [3 intact females (12%) and 9 spayed females (36%)]. The age of the dogs ranged from 8 months to 13.5 years (mean 5.11 years) and body weight ranged from 3 to 42 Kg (mean weight: 19.4 Kg). The definitive diagnosis was lymphocytic-plasmacytic enteritis in 23 dogs, a gastric adenocarcinoma in 1 dog while no alterations on endoscopy and histologic examination were identified in one case. The time required to complete the WI procedure ranged from 60 to 318 seconds (mean 102±62.7 seconds). Air Insufflation is commonly performed during duodenoscopy; therefore, it was not considered as an additional procedure in terms of time. Even considering the additional time, the total time of the anesthesia was influenced very little. No side effects related to the duodenal water instillation were evidenced during the trial and up to one hour after the examination.

Image analysis

All 25 pairs of images obtained during the duodenal dilation with water and air were compared. No significant difference was observed between the pairs of images with regards to morphological skeletonization (WI 0.12±0.03; AI 0.13±0.05; p=0.46), and image entropy evaluation (WI 5.65±0.41; AI 5.62±0.60; p=0.85). The subjective blind evaluation of each pair of images carried out by three experienced endoscopists (25 X 3 = 75 pairs of images), evaluating mucosal texture, and sharpness of the eventual lymphangiectasia and granulation, showed a significant predilection for the WI technique (71/75) as compared to the AI technique (4/75).

The Fleiss' K evaluation had a K value of 0.74 (range: 0.61 - 0.80), showing substantial agreement between the results provided by the endoscopy experts, supporting the WI method.

Cardiocirculatory parameters

The cardiocirculatory parameters analyzed referred to 24 dogs since one dog was excluded due to the lack of data at the fourth step (outcome). None of the dogs required an additional bolus of propofol throughout the four phases of the examination period. The mean

Table 1. Cardiocirculatory parameters (HR: heart rate; SAP: systolic arterial pressure; DAP: diastolic arterial pressure; MAP: mean arterial pressure; Min: Minimum; Max: Maximum) divided into four phases. There were no significant differences among the four phases in any of the parameters.

PARAMETER	BASELINE			WATER			AIR			OUTCOME			*p*
	Median	Min	Max	Median	Min	Max	Median	Min	Max	Median	Min	Max	
HR (bpm)	90	49	164	79	47	180	82	42	174	82	49	155	0.829
SAP (mmHg)	106	80	157	110	75	189	105	71	141	109	78	135	0.511
DAP (mmHg)	65	49	130	64	45	137	61	44	113	63	44	108	0.299
MAP (mmHg)	79	60	141	78	57	153	77	56	156	80	55	120	0.358

fraction of expired isoflurane was 1.5 +/- 0.4 %. Heart rate and blood pressure values (systolic, diastolic, and mean) of the samples are reported in Table 1. With regard to the heart rate and blood pressure values, no significant differences were observed among the four different steps of the procedure (baseline, water, air, outcome).

Discussion

The study, which aimed at applying human endoscopy procedures to dogs, differed from studies on humans, primarily due to the part of the intestinal tract involved: the colon in humans vs. the duodenum in dogs. The reason for the different sites depends on the particular diagnostic needs characterizing the different species. In human medicine, endoscopy of the colon is a procedure frequently applied to the diagnosis of polyposis or colon cancer while, in veterinary medicine, endoscopic examination of the small bowel (duodenum and ileum) is more frequently requested for diagnosing chronic enteropathy (Jergens and Simpson 2012, Lee et al. 2014). When analyzing the results of the present study, it can first be noted that computer analysis of the endoscopic images obtained using water did not show any difference from those using air as a spacer. In the medical field, the use of texture analysis is a valid method of overcoming the subjectivity provided by the operator's judgment (Holli et al. 2010). In particular, the choice of using image analysis (morphological skeletonization and image entropy evaluation) derives from studies carried out in human medicine, even if they involved other intestinal areas than the one analyzed in this study (Holli et al. 2010, Ciaccio et al. 2011, Cannellas et al. 2018). However, it is necessary to emphasize that no previous studies in veterinary medicine have compared these methods of image processing to collate two different procedures of enteric endoscopy. Moreover, unlike what had been reported in a previous study carried out in human medicine (Ciaccio et al. 2011), in the present study, the comparison was not carried out in order to differentiate between a pathological versus a healthy condition, but to compare two different endoscopic techniques applied to the same intestinal area in the same subject. Therefore, it is plausible that the differences between the two images were not as evident as they would be when comparing the enteric surfaces of healthy and sick patients.

In addition, the lack of statistical differences between the WI and the AI images, applying the skeleton measurement, could be linked to the quality of the images themselves which were derived from the freeze frames acquired from digital videos and, therefore, were often not perfectly in focus. On the other hand, the application of image entropy evaluation as an index of comparison between two images has already been applied in human medicine in order to differentiate between benign and malignant intestinal neoplasms (Cannellas et al. 2018). However, in the present study, the use of this analysis technique as a comparison approach did not provide any significant difference in relation to the media used to dilate the bowel, water vs. air. As previously discussed regarding morphological skeletonization, the reason for this result could be attributable to the quality of the images. The images obtained during an endoscopic examination are not well defined when compared to those obtained by other imaging procedures, such as computed tomography, which have already been successfully evaluated by entropy (Cannellas et al. 2018).

With regard to the images evaluated subjectively, those obtained by dilation of the lumen with water, when compared to those obtained using air, showed improved visualization of the duodenal surface with a higher degree of definition of the lymphangiectasia and of the granulation, if present.

The preference of the WI method, with respect to the AI method, was in agreement with numerous studies carried out in human medicine, which have shown how the use of the water technique could determine a greater cleaning of the intestinal lumen, leading to better visualization of the mucosal surface and greater distension of the villi (Fuccio et al. 2018), with an increase in polyp and adenoma detection (Cadoni et al. 2017, Cadoni and Leung 2017). In the present study applied to the duodenum, the cleaning of the mucosa was not as important as it is in the colon since the first intestinal tract is always cleaner than the large bowel. However, the better visualization under WI could depend on the fact that the villi did not collapse which is a common feature observed during AI endoscopy. An additional aim of the study was to evaluate nociception in relation to the application of water or air to dilate the intestinal lumen during endoscopy. In human medicine, unlike what happens in veterinary medicine, endoscopy is a procedure which can be performed without sedation, or under a low degree of sedation, allowing patients to be conscious and reporting their own feelings of pain and discomfort (Bushnell et al. 2013, Al-Zubaidi et al. 2016). Therefore, based on the results obtained directly from non-anesthesized human patients, water-assisted endoscopy minimizes patient discomfort and reduces the need for sedation (Leung et al. 2007, 2009, Terruzzi et al. 2012, Xu et al. 2016). In canine patients, endoscopic evaluation is carried out only in anesthetized patients (McFadzean et al. 2017), and this obviously prevents the use of pain scales for objective pain evaluation which are applicable only in non-anesthesized animals (Reid et al. 2007). Even if, to the Authors' knowledge, nociception has never been evaluated in anesthetized dogs undergoing endoscopic examination, changes in heart rate and blood pressure have already been used for the evaluation of nociception in dogs during surgical procedures (Höglund et al. 2011, Caniglia et al. 2012). In the present study, neither the heart rate nor the blood pressure changed significantly with air insufflation vs water immersion. Considering this, in the population in the present study, the water technique did not have any advantage over the air-assisted technique in terms of nociception. However, it should be considered that all the dogs were premedicated with dexmedetomidine and butorphanol which produce analgesia and deep sedation in dogs (Nishimura et al. 2018). Therefore, it is believed that these drugs, along with propofol and isoflurane, provided a stable anesthetic plane and prevented changes in the physiologic parameters in spite of the stretching of the walls of the gastrointestinal tract which is considered to be the most painful stimulation induced by endoscopic examination. In fact, dexmedetomidine has already been described to provide hemodynamic stability, effective sedation and comfort for human patient undergoing colonscopy (Dere et al. 2010); therefore, it cannot be excluded that it also has the same effect on dogs. In addition, when heart rate and blood pressure were evaluated in human patients during colonscopy, various authors observed that propofol sedation prevented significant changes in these parameters which were, instead, observed in non-anesthesized patients (Gasparović et al. 2003).

It should be emphasized that this study has some limitations, the first being related to the quality of the images collected which could have influenced the results of the image analysis, and the second to the short time between the experimental phases. The latter may have limited the response of the animal to the mechanical insult of air and water. For ethical reasons, since the animals in the study were patients and not experimental animals, it was impossible to have a longer time between the phases because it would have extended the anesthesia for longer than what was required for the classic procedure.

The results of this study indicated that the introduction of water instead of air during duodenoscopy in dogs provided an increase in the quality of the endoscopic images, documented by subjective evaluation, although not by the texture analyses.

In conclusion, WI was a cheap, non-time consuming, easy to apply, alternative technique to AI endoscopy. Therefore, although no clinical post-WI evidence was found and no observable side effects were recorded during and one hour after the trial, additional studies are needed to investigate any possible general influence of the WI method.

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