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Short communication

PANCREATIC EVALUATION IN DOGS USING DIFFERENT ULTRASONOGRAPHIC TECHNIQUES – PRELIMINARY RESULTS

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The aim of this study is to describe the preliminary results on the accuracy of ultrasonographic techniques such as elastography, contrast enhanced ultrasound (CEUS) and Doppler in determining pancreatic changes. Twenty-five dogs, males and females, aged 1-14 years, were studied. Sixteen animals had no clinical signs of pancreatic disease (GS) and nine presented signs of pancreatic disorders (GD). All animals from GD presented sonographic changes in B-mode and qualitative elastography, with shear-wave velocity (SWV) higher (2.4±0.5m/s) in GD (p=0.014) than GS (1.9±0.3m/s) resulting in 78% sensitivity and 69% specificity in the identification of pancreatic changes. Regarding Doppler mode, no differences were observed between groups with color mapping or pulsed wave Doppler. The values obtained with CEUS did not differ between groups. Elastography is a promising technique for differential diagnosis of pancreatic changes because of its sensitivity and specificity, while the other techniques did not show diagnostic accuracy.

Key words: Ultrasonography, hemodynamics, pancreatic diseases, elastography

INTRODUCTION

Among pancreatic diseases, pancreatitis is the one that most affects the exocrine pancreas of dogs. The disease is characterized by nonspecific and intermittent gastrointestinal signs that may hinder the diagnosis [1,2], causing complications such as cell death, reduced blood supply and it might trigger systemic inflammation, leading to pancreatic dysfunction with a high mortality rate [3,4]. One report mentions a case

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of pancreatic destruction in a dog, in which it was only possible to find remnants of the parenchyma and inflammatory cells under the microscope [2].

Mild pancreatitis is a disease with low morbidity and mortality that can be reversed, if rapidly diagnosed [5].

The diagnosis of pancreatitis is still challenging, especially in mild cases, where patients present discreet clinical signs. Routinely, serum activities of amylase and lipase are used for diagnosis, because they are indicators of pancreatic inflammation, however, these assays have low specificity and sensitivity in the identification of pancreatitis in dogs and their usefulness in clinical practice is questionable [6]. Nowadays, rapid tests that measure the level of the specific pancreatic lipase in the blood are being used. Even though they have good sensitivity and specificity in cases of acute pancreatitis, they may not detect subclinical phases or they might even provide false positive results [7]

Definitive diagnosis is made by histopathological examination, however, its execution in the ante-mortem organ is rare [5]. Cytological examination by fine-needle aspiration was widely used in medicine, but nowadays its use is restricted and controversial. There are reports about secondary contamination that may occur in patients with pancreatic necrosis and the detection of the etiological agent becomes more difficult [8].

Computed tomography (CT) and Magnetic Resonance Imaging (MRI) are widely used in medicine, however, in veterinary medicine, they are not easily accessible and require general anesthesia [9].

Due to the difficulty in concluding the diagnosis of pancreatitis, the variety of differential diagnoses of acute abdomen in dogs and lack of precise assays, ultrasonography is the method of choice for the evaluation of patients with suspected pancreatitis. Despite its sensitivity of 68% [1,6], the exam provides important information about changes in the pancreatic parenchyma, already identified by other authors [5]. It is important to emphasize that a normal ultrasound examination does not rule out pancreatitis. If the disease is suspected, but there are no deviations in the first exam, ultrasound should be repeated in a few days, as the severity increases and sonographic changes tend to appear with time [6].

Although diagnostic accuracy of B mode ultrasonography is acceptable, this method cannot distinguish inflammation, necrosis and neoplasia [5], thus, the use of new non-invasive ultrasonographic techniques, such as elastography and contrast enhanced ultrasound (CEUS) could contribute in the detection of pancreatic lesions and help the diagnosis of the patients.

CEUS is a new advancement in imaging diagnosis in medicine and its application in veterinary medicine is constantly growing to assess organ perfusion, detect areas of necrosis and aid in the diagnosis of pancreatic neoplasms [4,9]. Elastography is an imaging modality that evaluates the stiffness of the organs. Studies performed with other tissues have shown a positive correlation between shear wave velocity and the

stiffness of the tissue evaluated, thus, the faster the shear wave velocity, the stiffer the tissue [10].

The aim of this preliminary study was to evaluate accuracy of different ultrasonographic techniques (B mode, Doppler, CEUS and Elastography) in assessing pancreatic changes in dogs and to describe the findings of each method in different lesions.

MATERIALS AND METHODS

The study was performed and approved by the Ethics Committee in the Use of Animals (protocol n. 007976/18). Twenty-five dogs of different breeds, aged between 1-14 years and weighing between 5-29kg were selected for this study. Dogs were allocated in two groups: GS (n=16) healthy animals and GD (n=9) non-healthy animals, with suspicion of pancreatic disease. All dogs came from the clinical practice of the Veterinary Hospital of the same institution, informed and written consent were obtained from client-owned animals included in this study.

Animals were classified based on the results of physical and clinical examination, hematological results and B mode ultrasonography. GS animals did not present any alterations in these tests and GD presented pain in the epigastric region, abdominal discomfort and hematological changes, such as leukocytosis with left shift.

For ultrasound examination, all animals fasted for 8-12 hours prior to the exam, so there would not be superposition of the gastrointestinal content on the pancreas. The abdomen was clipped and acoustic gel was applied to facilitate the procedure and promote more contact between the transducer and the skin. Patients were positioned in dorsal recumbency and during the scan they were shifted to lateral (right and left) recumbency, in order to locate all portions of the organ under study (pancreas).

Scanning was performed by a single operator, with nine years of experience, using ACUSON S2000 – SIEMENS (Siemens, Munich, Germany) and linear and convex multifrequency transducers (7.5 to 9.0MHz).

B mode scanning was performed prior to elastography, aiming to evaluate the entire pancreatic parenchyma of the animals in its different anatomical portions (right and left lobes and body) and also to assess their characteristics: echogenicity (in comparison to adjacent structures), echotexture (homogenous and heterogenous), contours (regular and irregular) and dimensions (normal, increased and reduced). To locate the right pancreatic lobe, the anatomical landmarks used were right kidney, descending duodenum (coursing through the right abdominal wall) and the pancreaticoduodenal vein, located parallel to the descending duodenum.

In this study, all techniques were standardized on the right pancreatic lobe, because in GS it was the portion that could be identified in all animals. In GD, all portions were assessed, however, only the values of the right lobe were considered.

Elastography was performed with the same equipment, using the software for Acoustic Radiation Force Impulse (ARFI) qualitative characterization and quantification (Virtual Touch Tissue Quantification, 2D-SWE technique). The qualitative ARFI method resulted in colored images of the pancreatic tissue (elastogram) for the evaluation of tissue deformity (with or without deformation), in which lighter tones (bluish) represent more elastic tissues (soft) and darker tones (reddish) represent stiffer tissues (hard). Image quality was tested using the exhibition mode, in which greenish and homogenous images indicated high quality of the technique and yellowish and heterogenous images indicated low quality.

In the quantitative ARFI method, it was possible to measure shear wave velocity (SWV m/s). In this study, five measurements of the right pancreatic lobe were obtained and mean values were calculated for statistical analysis.

Using Color Doppler, vascular characteristics of the parenchyma were evaluated. In patients with suspected pancreatic disease, it was verified whether neovascularization was present. In order to obtain vascular indices of both healthy and diseased tissue, after identification of the pancreaticoduodenal vein with color mapping, the sample volume was positioned in the central portion, then, Pulsed Doppler was activated, obtaining the results of resistivity index (RI) automatically by the equipment.

For microbubble contrast enhanced ultrasound (CEUS), contrast-specific software was used (CADENCE®, Siemens, Munich, Germany), with secondary harmonic imaging, pulse inversion technique and a 9.0MHz linear transducer. The images obtained were evaluated in a specific imaging software. The contrast agent (SonoVue®, Bracco, Milan, Italy), was administered in the dosage of 0.1 mL for each animal, using a venous catheter that was maintained in the cephalic vein, followed by administration of 5.0 mL of saline solution (NaCl 0.9%).

Video clips lasting 5 minutes after injection of the contrast were obtained and registered in the internal system storage and then, they were analyzed.

This contrast exam defined parameters related to the filling (homogenous or heterogenous) of the organ by microbubbles. Additionally, the times of vascular filling were analyzed, since administration of the contrast in the bloodstream until the beginning of the organ perfusion (wash-in); peak of contrast (enhancement); and time of the contrast total output from the parenchyma (wash-out).

Statistical analysis was performed using the R software (RTM Foundation for Stastistical Computing, Vienna, Austria). Sonographic variables were compared between clinical classifications using Student's T- test. Subsequently, parameters that presented a significant difference were submitted to a discriminant analysis using ROC curves and cutoff values (CV), sensitivity, specificity and area under the curve were calculated using the logistic regression model. A significance of 5% ($p=0.05$) was established for all tests.

RESULTS AND DISCUSSION

B mode ultrasonography and elastography were performed without difficulties and intercurrences. However, Spectral Doppler evaluation was very difficult in six GS patients (37.5%), because of the narrow nature of the organ and its small evaluation window. In the GD group, this evaluation was not possible in three patients (33.3%), due to the relentlessness and abdominal discomfort of the patients. In CEUS, it was not possible to evaluate five patients from GS (31.25%), due to the localization and dimension of the organ. These limiting factors of our study reduced the number of patients evaluated in the described techniques.

In B mode scanning of GS dogs, the mean thickness was 0.67cm and it was possible to observe the right pancreatic lobe of normal size, preserved echogenicity (isoechoic to the mesenteric fat and the adjacent hepatic lobe) homogenous echotexture and regular contours. The characteristics found in the pancreas from the GS group were compatible to a healthy tissue.

All nine sick patients (GD) showed nonspecific signs, such as anorexia, apathy, vomiting, diarrhea, "praying position", pain in the epigastric region and sonographic alterations such as changes in echogenicity, echotexture, dimensions and contours. In seven cases (77.8%) changes in the adjacent organs were observed, reinforcing the suspicion of pancreatic disease (Table 1).

The sonographic alterations found in this study (reduced echogenicity, increased or normal size of the organ, heterogenous echotexture and adjacent changes, such as reactive mesentery, free fluid in the abdomen or duodenitis) are images suggestive of acute pancreatitis, corroborating with several studies [4–7,11,12]. All alterations were noted in the right lobe, in some patients in the pancreatic body and only one patient presented alterations in all portions of the organ, which reinforces data from the literature that states that the right pancreatic lobe is more easily located in dogs [6].

Pancreatic edema and adjacent free fluid were seen in two patients. This was also observed in other studies that describe acute pancreatitis with swollen parenchyma, however, in these cases, it is necessary to consider differential diagnosis such as portal hypertension and hypoalbuminemia [12].

No sonographic signs of neoplasia were seen in any patient. This might be because of the small number of patients in this study. Although Bailey and Page [13] reported that pancreatic neoplasms (exocrine and endocrine) are rare in veterinary medicine in comparison to human medicine, pancreatitis, in contrast, is routinely detected in the canine pancreas, as observed in the present study.

In the qualitative elastography, GS group presented homogenous pancreatic parenchyma and of bluish colors (deformable), being characterized as soft. In eight GD patients, the elastogram presented deformable parenchyma, with predominantly bluish colors and some discreet stiff areas (small areas in yellowish color), suggesting

Table 1. Changes in the pancreatic parenchyma and adjacent tissues in patients in B-mode ultrasonography (Jaboticabal, 2018)

Breed	Age	Echogenicity	Echotexture	Thickness (cm)	Contours	Others	Qualitative Elastography	Quantitative Elastography
1- Mongrel 17 Kg	4	Reduced	Heterogeneous	1.78	Regular	Hypoechoic lesions	Predominantly bluish and greenish	2.26 m/s
2- Mongrel 20 Kg	7	Reduced	Homogenous	1.20	Regular	Reactive Mesentery	Predominantly bluish	1.98 m/s
3- Mongrel 5 Kg	7	Reduced	Homogenous	1.40	Regular	Reactive Mesentery	Predominantly bluish and greenish, with a small yellowish area	2.82 m/s
4- Dalmatian 18 Kg	8	Reduced	Homogenous	2.40	Irregular	Reactive Mesentery and Duodenitis	Predominantly greenish, with large reddish areas	3.48 m/s
5- Mongrel 29 Kg	10	Reduced	Homogenous	1.0	Regular	Reactive Mesentery	Predominantly bluish and greenish, with a small reddish area	2.19 m/s
6-Yorkshire 7 Kg	10	Reduced	Heterogeneous	1.0	Irregular	Reactive Mesentery	Predominantly bluish and greenish, with small yellowish and reddish areas	1.94 m/s
7- Mongrel 28 Kg	11	Reduced	Heterogeneous	1.25	Regular	Reactive Mesentery	Predominantly bluish and greenish, with small yellowish and reddish areas	2.24 m/s
8- Pitbull 26 Kg	11	Reduced	Heterogeneous	1.47	Irregular	Edema and free fluid	Predominantly bluish and greenish, with a small yellowish area	2.43 m/s
9- Cocker Spaniel 12,5 Kg	14	Reduced	Heterogeneous	1.26	Regular	Edema, reactive mesentery and free fluid	Predominantly bluish, with some greenish areas and a small yellowish area	1.70 m/s

acute pancreatic alterations. In only one case (11%), i.e. animal 4, the parenchyma showed to be heterogeneous with reddish areas, demonstrating he stiffness of the tissue, suggesting a chronic alteration (Figure 1). Many studies point that this parenchymal characteristic is related to tissue malignancy, such as in mammary neoplasms, for example [14–16], however, these changes were also seen in chronic inflammatory processes of the pancreatic parenchyma [17,18].

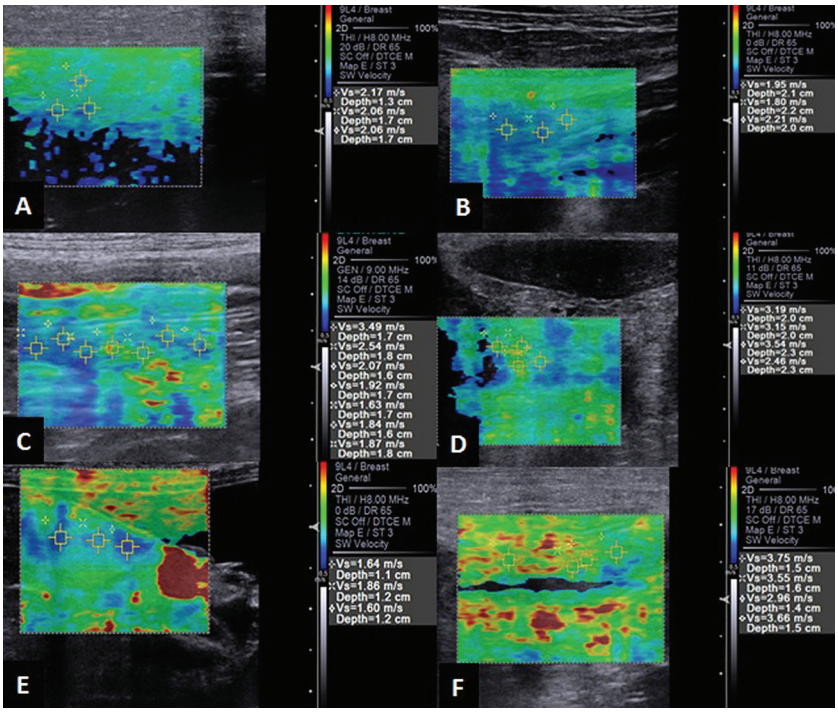

Figure 1. Qualitative and quantitative ARFI elastography images of the pancreatic parenchyma of the dog. **A.** and **B.** Healthy pancreas, with a homogenous pattern in bluish colors. **C.**, **D.**, and **E.** Images of the parenchyma with acute alterations, deformable with predominantly bluish colors and small areas of stiffness **F.** Images of the parenchyma with chronic alterations, heterogenous pattern, with reddish areas, characterizing the tissue's stiffness.

Quantitative elastography presented mean shear wave velocity of 1.91±0.32m/s in GS and 2.35 ±0.53m/s in the GD group. This difference was considered significant (P = 0.014), thus, when pancreatic SWV is higher than 1.98m/s it might indicate pancreatic disease, with a 78% sensitivity, 69% specificity and 78% AUC (Figure 2). Among GD, the changes caused by acute inflammatory processes apparently resulted in lower SWV than in chronic alterations.

Goertz et al. [18] cited that, in human patients, chronic inflammation leads to fibrotic changes of the pancreatic tissue and, in some cases, calcifications. These characteristics decrease elasticity of the diseased pancreas, promoting elastographic findings of greater stiffness [19], as observed in the present study. When tissues with sonographic

aspects of acute (inflammatory) cases were observed, they presented discreet areas in yellowish and reddish colors in eight cases. It suggests an increase in parenchyma stiffness in comparison to the healthy group, which presented bluish and greenish on the elastogram (not hard). In one patient with findings compatible with chronicity, a higher stiffness was observed, with bigger and more frequent reddish areas. Although in the present study there was no suspicion of neoplastic lesions, it is suspected that stiffness of these tissues may be higher than in chronic lesions, which might aid in the differential diagnosis of chronic pancreatitis and pancreatic neoplasms, which is still challenging [20]. Further studies are necessary in order to contribute for diagnosis of these alterations.

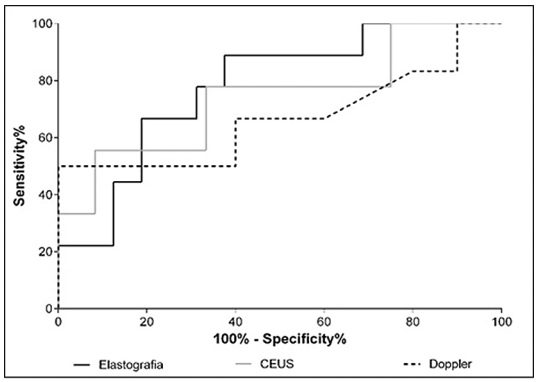

Figure 2. ROC curves representing sensitivity and specificity (%) of each ultrasonographic method in determining pancreatic lesions.

In both GS and GD, Color Doppler ultrasound did not show neovascularizations and the vascular pattern was homogenous. In Spectral Doppler, mean value of resistive index was 0.74±0.09 in GS and 0.79±0.13 in GD, with no significant difference between these groups (P=0.386). Changes in these parameters were observed in cases of lesions suggestive of malignance in other tissues, such as presence of neovascularization, vessels with a tortuous aspect, elevated systolic and diastolic velocities and changes in the resistive index [21–23].

Microbubble contrast-enhanced ultrasonography presented homogenous filling of the pancreas. Both groups had no side effects due to the use of the contrast agent. Mean values obtained in the contrast filling times did not have significant differences between groups; wash in (P=0.128), peak enhancement (P=0.181) and wash-out (P=0.169) (Table 2). Although there was no difference between groups, animal 4 (Dalmatian) from the GD presented a markedly prolonged time in the three moments: 12s for the entrance, 32s peak enhancement and 110s the contrast agent was still present in the parenchyma. Studies performed by Rademacher et al. [4] and Lim et al. [9] also presented an increase in the time of entrance and peak enhancement in dogs with pancreatitis.

Table 2. Mean values obtained in wash-in, peak and wash-out in GS and GD (Jaboticabal, 2018)

Group	Wash-in	Peak enhancement	Wash-out
GS = 11	3.08±1.51 s[a]	7.50±2.39 s[a]	89.00±28.61 s[a]
GD = 9	4.67±3.00 s[a]	10.89±8.05 s[a]	108.56±34.07 s[a a]

Same letters without significant difference

Results of CEUS technique were not effective in differentiation of altered tissues in most cases, however, it was possible to detect microvascularization, contrary to the study performed by Cai et al. [24] which demonstrated that in cases of acute pancreatitis, this method allows successful identification of changes in comparison to B mode ultrasonography, with an accuracy of 78.0% (103/132) and 47.7% (63/132), respectively. Vanderperren et al. [25] also verified a differentiation of exocrine and endocrine pancreatic tumors using CEUS: adenocarcinomas were presented hypovascular and insulinomas were hypervascular.

CONCLUSION

Elastography is presented as a promising technique for differential diagnosis of pancreatic alterations due to its sensitivity and specificity, while the other techniques did not show diagnostic accuracy. Further studies with a larger number of patients are required in order to test the efficiency of Doppler and CEUS.

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Authors' contributions

LMA participated in the design of the study, carried out the ultrasonographic studies and drafted the manuscript. FMAR, URAR and JCC conceived of the study, and participated In its design and coordination and helped to draft the manuscript. MMC, SPA, SAPR, SP, GB and PL participated the ultrasonographic studies and helped to draft the manuscript. PR and ALPN helped to draft the manuscript. All authors read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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PROCENA PANKREASA KOD PASA UPOTREBOM RAZLIČITIH ULTRAZVUČNIH TEHNIKA- PRELIMINARNI REZULTATI

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Cilj ove studije je da se opišu preliminarni rezultati tačnosti ultrazvučnih tehnika kao što su elastografija, kontrastno pojačani ultrazvuk (CEUS) i Doppler prilikom procenjivanja promena na pankreasu. Ispitano je ukupno dvadeset i pet pasa, mužjaka i ženki, starosti 1-14 godina. Šesnaest pasa nije imalo kliničke znake oboljenja pankreasa (GS), dok je devet imalo znakove poremećaja pankreasa (GD). Sve životinje GD grupe su imale sonografske promene u B-modusu i kvalitativnoj elastografiji, sa brzinom talasa

(SWV) većom (2,4±0,5m/s) u GD grupi (p=0,014) u odnosu na GS (1,9±0,3m/s) grupu, što je rezultiralo osetljivošću od 78% i specifičnošću određivanja promena na pankreasu od 69%. Što se tiče rezultata dobijenih putem Dopplera, nisu uočene razlike među grupama primenom kolor ili pulsnog Doppler-a. Vrednosti dobijene pomoću CEUS-a se nisu razlikovale između grupa. Obzirom na njenu osetljivost i specifičnost, elastografija je tehnika koja obećava sa aspekta diferencijalne dijagnoze promena na pankreasu, dok ostale tehnike nisu pokazale dijagnostičku tačnost.