

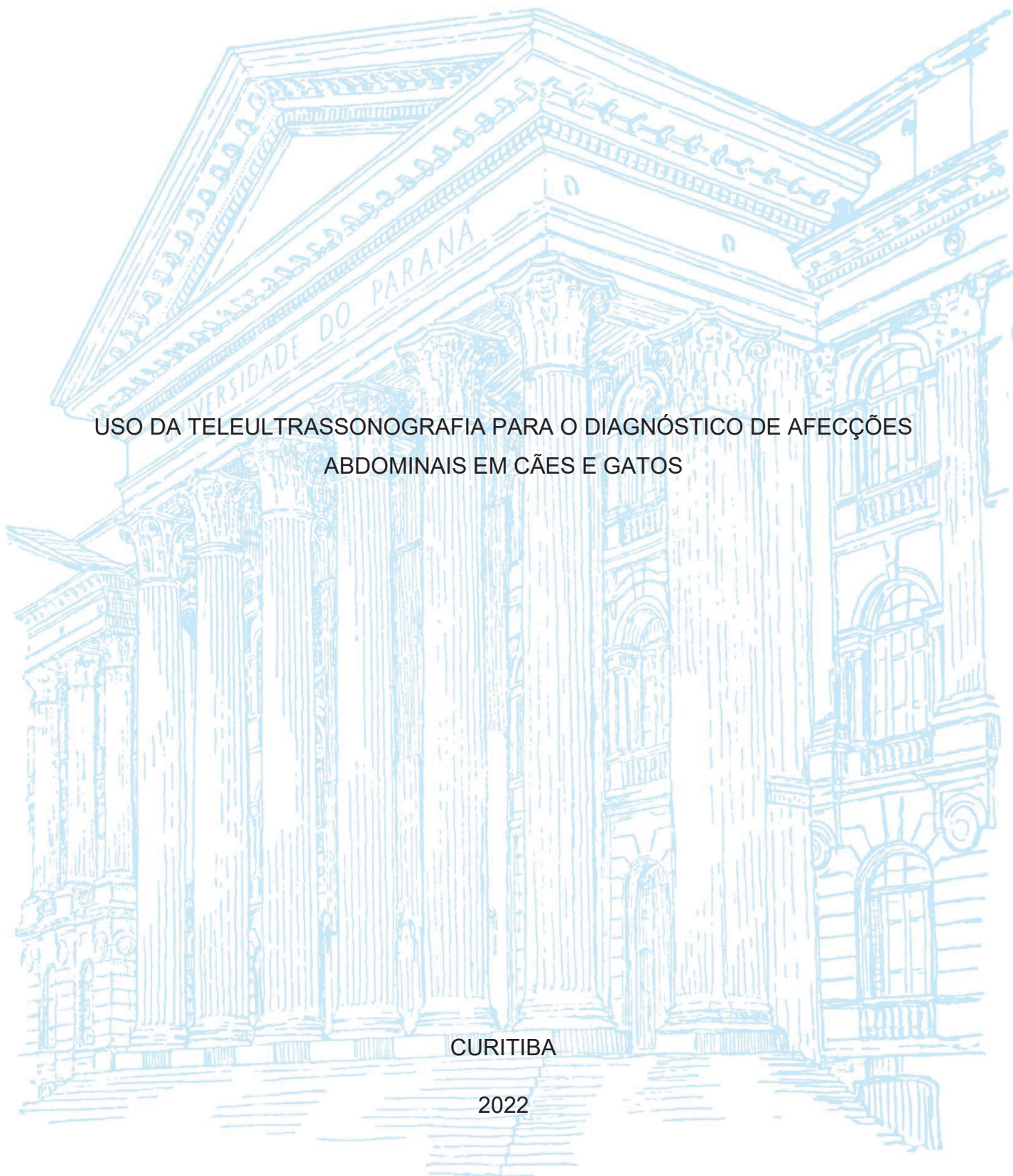
UNIVERSIDADE FEDERAL DO PARANÁ

MARINA SILVESTRE SOMBRIO

USO DA TELEULTRASSONOGRAFIA PARA O DIAGNÓSTICO DE AFECÇÕES
ABDOMINAIS EM CÃES E GATOS

CURITIBA

2022



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ABDOMINAIS EM CÃES E GATOS

Dissertação apresentada ao curso de Pós-Graduação em Ciências Veterinárias, Setor de Ciências Agrárias, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Mestre em Ciências Veterinárias.

Orientadora: Prof^a. Dr^a. Tilde Rodrigues Froes.

CURITIBA

2022

DADOS INTERNACIONAIS DE CATALOGAÇÃO NA PUBLICAÇÃO (CIP)
UNIVERSIDADE FEDERAL DO PARANÁ
SISTEMA DE BIBLIOTECAS – BIBLIOTECA

Sombrio, Marina Silvestre

Uso da teleultrassonografia para o diagnóstico de afecções abdominais em cães e gatos. / Marina Silvestre Sombrio. – Curitiba, 2022.

1 recurso online : PDF.

Dissertação (Mestrado) – Universidade Federal do Paraná, Setor de Ciências Agrárias, Programa de Pós-Graduação em Ciências Veterinárias.

Orientador: Prof. Dr Tilde Rodrigues Froes.

1. Telemedicina. 2. Diagnóstico por Imagem. 3. Ultrassonografia. I. Froes, Tilde Rodrigues. II. Universidade Federal do Paraná. III. Programa de Pós-Graduação em Ciências Veterinárias. IV. Título.

Bibliotecário: Fernando Cavalcanti Moreira CRB-9/1665



MINISTÉRIO DA EDUCAÇÃO
SETOR DE CIÊNCIAS AGRÁRIAS
UNIVERSIDADE FEDERAL DO PARANÁ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO CIÊNCIAS
VETERINÁRIAS - 40001016023P3

TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação CIÊNCIAS VETERINÁRIAS da Universidade Federal do Paraná foram convocados para realizar a arguição da dissertação de Mestrado de **MARINA SILVESTRE SOMBRIO** intitulada: **Uso da teleultrassonografia para o diagnóstico de afecções abdominais em cães e gatos, sob orientação da Profa. Dra. TILDE RODRIGUES FROES**, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua **APROVAÇÃO** no rito de defesa.

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CURITIBA, 14 de Abril de 2022.

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18/04/2022 20:08:04.0

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28/04/2022 13:39:39.0

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18/04/2022 16:59:52.0

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AGRADECIMENTOS

Agradeço sobretudo a minha família, que me apoiou em todas minhas decisões, desde quando, ainda criança, decidi me tornar veterinária, até a dar sequência com meus estudos com a pós-graduação. Obrigada por possibilitarem esse sonho de se tornar real. A minha mãe, que me ensinou a ser independente, por todo o cuidado comigo e apoio incondicional. A meu pai, pela dedicação em cuidar da família e por me proporcionar meus estudos. A Tuca e minha madrinha Nanda, por serem minhas primeiras inspirações na veterinária. A meus dindos, por todo o carinho e tudo que proporcionaram para mim.

A todos meus anjinhos da guarda, vó Lígia, vó Renata e vô Arlindo, que foram essenciais para eu chegar até aqui. Embora não estejam mais fisicamente aqui, sei que continuam torcendo por mim e me guiando a tomar as melhores decisões.

A Bill, meu companheiro de vida, que participou e esteve por perto durante mais essa etapa, comemorando comigo as vitórias e me ajudando e dando colo nos momentos de desespero. Gratidão por ser tão presente, mesmo quando distantes, por sempre me apoiar e me motivar a querer e buscar sempre mais.

A todos os animais que fizeram parte da minha vida em algum momento e a todos que tive a chance a ajudar e salvar nesses anos, é por vocês que me dedico tanto, obrigada pela oportunidade de cuidar de criaturas tão especiais. Em especial à Rupí e Bebel, meus amores de quatro patas que tornam meus dias mais alegres.

Sou grata também à minha incrível orientadora, Tilde, por tudo que me ensinou durante esses anos. Não foi fazer fácil cursar um mestrado em plena pandemia, ainda mais com todas as mudanças e adaptações que tivemos que fazer no meio do caminho, porém você conseguiu me conduzir com maestria e excelência, me ensinando muito mais que tons de cinza.

À toda a equipe do LADIV/UFPR, por me acolher e contribuir com os trabalhos fazendo os exames e disponibilizando todos os casos e imagens do arquivo. A Danielle, Daniela, Giovanna, Marília e professor Wilfried Mai, por aceitarem participar dos projetos e serem nossos avaliadores, esse trabalho não existiria sem toda a ajuda de vocês!

Agradeço também a todos meus colegas e amigos da pós, em especial Danielle, Felipe, Louise e Sabrina, por toda a ajuda com as matérias, projetos, provas e por compartilharem comigo as alegrias e os perrengues da pós-graduação. A UFPR e aos professores da pós, por me darem a oportunidade de cursar o mestrado nessa universidade de referência. Em especial aos professores Fabiano, Juan, Rafael, Rosângela e Ron, por ter o privilégio de ter tido aulas com vocês e, por ensinarem muito além de suas matérias, por me ensinarem a ter análise crítica. Ao CnPQ, pelo auxílio financeiro a um dos projetos. Ao professor Thiago Muller, por ser uma das

minhas principais inspirações na veterinária, obrigada por todas as oportunidades e conhecimento compartilhado.

A minhas amigas, Giovanna, Marília, Eloisa, Julia, Chariel, Daiane, Bia, Ketlen, Mariana e Sarah, por terem me apoiado durante esses anos e acompanhado essa jornada, mesmo que a distância, por sempre torcerem por mim e me darem forças.

E, por fim, aos professores da banca, Fabiano, Robson, Mariana e Juan, por disponibilizarem um tempo da sua rotina para contribuírem com suas experiências e sugestões com os nossos trabalhos, suas contribuições serão valiosas para nós.

"Life is like riding a bicycle.

To keep your balance, you must keep moving".

Albert Einstein

RESUMO

Os avanços da medicina veterinária e tecnologia tornaram possível a incorporação da telemedicina na rotina veterinária, em especial em diagnóstico por imagem. Apesar de já ser bem estabelecida a importância da ultrassonografia abdominal para a avaliação do trato gastrointestinal e urinário em cães e gatos, ainda há poucos estudos publicados na veterinária sobre teleultrassonografia, e nenhum que estabeleça a acurácia de teleultrassonografia para detecção de obstrução do trato gastrointestinal e de obstrução renal e ureteral em pequenos animais. Apesar de essa modalidade já estar sendo utilizada em alguns centros de imagem e hospitais, não há parâmetros e estudos que avaliem se é uma técnica confiável para ser usada na rotina e nem mesmo diretrizes publicadas para orientar os radiologistas quanto a seu uso e limitações. Dessa forma, o propósito deste trabalho foi estabelecer a acurácia e concordância interobservador da teleultrassonografia para o diagnóstico de obstrução gastrointestinal e de obstrução renal e ureteral, entre radiologistas com diferentes níveis de experiência. Para isso, este estudo consistiu em dois capítulos. Ambos foram estudos retrospectivos transversais com cães e gatos, porém o primeiro capítulo incluiu pacientes com sinais gastrointestinais e suspeita de obstrução gastrointestinal, que foram submetidos à ultrassonografia abdominal entre 2017 e 2019. Já o segundo capítulo incluiu pacientes submetidos à ultrassonografia devido a sinais do trato urinário e suspeita de obstrução do trato urinário superior, atendidos entre 2017 e 2018. Em ambos os estudos, os pacientes foram divididos em dois grupos, com e sem obstrução, e todas as imagens dos exames ultrassonográficos foram enviadas de modo aleatório para os radiologistas analisarem. Foram realizadas análises de sensibilidade, especificidade, acurácia, de valores preditivos positivos e negativos, além de concordância interobservador. A teleultrassonografia apresentou boa acurácia tanto para detecção de processos obstrutivos gastrointestinais e de obstrução renal e ureteral, porém com moderada concordância interobservador e baixo valor preditivo. Dessa forma, essa modalidade deve ser usada com cuidado nesses contextos clínicos, dado o potencial de decisão cirúrgico envolvido no diagnóstico dessas afecções.

Palavras-chave: Telemedicina. Diagnóstico por Imagem. Ultrassonografia. Cães. Gatos.

ABSTRACT

Advances in veterinary medicine and technology have made it possible to incorporate telemedicine into the veterinary routine, especially in diagnostic imaging. The importance of abdominal ultrasonography for the evaluation of the gastrointestinal and the urinary tract in dogs and cats is already well established, however few studies have been published in veterinary teleultrasonography, and none that establishes the accuracy of teleultrasonography for the detection of gastrointestinal tract and renal and ureteral obstruction in small animals. Although some imaging centers and hospitals are already using this modality, there are no parameters and studies so far that assess whether it is a reliable technique for routine use and even no published guidelines to orientate radiologists in its use and its limitations. Thus, the purpose of this study was to establish the accuracy and interobserver agreement of tele-ultrasonography for diagnosing gastrointestinal and renal and ureteral obstruction, in radiologists with different levels of experience. For this, this study consisted of two chapters. Both were retrospective cross-sectional studies with dogs and cats, but the first chapter included patients with gastrointestinal signs and suspected gastrointestinal obstruction, which underwent abdominal ultrasound between 2017 and 2019. The second chapter included patients which underwent ultrasound due to urinary tract signs and suspected upper urinary tract obstruction, between 2017 and 2018. In both studies, patients were divided into two groups, with and without obstruction, and every ultrasonographic images were sent randomly for radiologists to analyze. Analyzes of sensitivity, specificity, accuracy, positive and negative predictive values, and interobserver agreement were performed. Teleultrasonography showed good accuracy both for detecting gastrointestinal obstructive processes and renal and ureteral obstruction, but it presented moderate interobserver agreement and low predictive value. Thus, this modality should be used with caution in these clinical contexts, given the potential for a surgical decision involved in the diagnosis of these conditions.

Keywords: Telemedicine. Diagnostic Imaging. Ultrasonography. Dogs. Cats.

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LIST OF ABBREVIATIONS

Acc	- Accuracy
CEUA	- Ethics Committee on Animal Use
CI	- Confidence interval
DICOM	- Digital Imaging and Communications in Medicine
GI	- Gastrointestinal
MHz	- MegaHertz
MSc	- Master of Sciences
NPV	- Negative predictive value
Ph.D.	- Doctor
PPV	- Positive predictive value
Se	- Sensitivity
Sp	- Specificity
TUS	- Tele-ultrasonography
US	- Ultrasonographic
UT	- Urinary tract

LIST OF SYMBOLS

κ	- Cohen's Kappa
k_v	- Fleiss's kappa
n	- Absolute frequency
%	- Relative frequency
χ^2	- Chi-square test

SUMMARY

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1. CHAPTER 1 - ACCURACY AND INTERRATER RELIABILITY OF TELE-ULTRASONOGRAPHY IN DETECTING GASTROINTESTINAL OBSTRUCTION IN DOGS AND CATS

1.1 ABSTRACT

Objectives: To assess the accuracy and interobserver agreement of tele-ultrasonography for the diagnosis of gastrointestinal obstruction in small animals in radiologists with different levels of experience.

Methods: A retrospective cross-sectional study including dogs and cats admitted with gastrointestinal signs, between 2017 and 2019, that had abdominal ultrasonographic examination performed and DICOM images saved for review. Patients were classified into two groups based on final diagnosis: with gastrointestinal complete or partial obstruction (group 1) or without (group 2). Observers with four different experience levels interpreted the archived ultrasound examinations, simulating a tele-ultrasonography consultation. Analyses of accuracy, sensitivity, specificity, positive and negative predictive values were obtained for each observer for detection of gastrointestinal ultrasonographic abnormal findings and gastrointestinal obstruction. Agreement between observers for the gastrointestinal obstruction diagnosis was assessed using Fleiss's Kappa statistics.

Results: Ninety patients with gastrointestinal signs were included. Of these, 23/90 were in group 1 and 67/90 in group 2. Interpretation of the images by tele-ultrasonography showed intervals of sensitivity, specificity, accuracy, positive and negative predictive values, respectively, of 86.3-93.2%, 47.1-82.4%, 84.5-86.7%, 88.3-95.5% and 58.3-64.7% for detection of gastrointestinal ultrasonographic abnormalities and 73.9-100%, 77.6-89.6%, 78.9-87.8%, 55.9-70.8% and 90.9-100% for diagnosis of gastrointestinal obstruction. Agreement for the gastrointestinal obstruction diagnosis across all reviewers was moderate (0.63).

Clinical Significance: Tele-ultrasonography had good accuracy for detection of gastrointestinal obstructive processes, however had a rather low positive predictive value and only moderate inter-observer agreement. Therefore, this

technique should be used with caution in this clinical context, given the potential surgical decision at hand.

Keywords: veterinary teleradiology, diagnostic imaging, ultrasonography, small animals, gastrointestinal disease.

Word Count: 3815 words

1.2 INTRODUCTION

Gastric or intestinal mechanical obstruction is potentially life-threatening (Fossum, 2015) and is considered a surgical emergency (MacPhail, 2010). This cause of acute abdomen requires accurate and prompt diagnosis (Cruz-Arámulo and Wrigley, 2003). Abdominal radiography or ultrasonography can be useful diagnostic tools (Thrall and Riedesel, 2018; Neelis, *et al.*, 2021). However, abdominal ultrasound is more accurate than radiography for diagnosis of mechanical obstruction and can also identify cause and location of the obstruction (Garcia *et al.*, 2011; Sharma *et al.*, 2011; D'Anjou and Penninck, 2015).

Ultrasound is a safe procedure that requires no ionizing radiation (Britton *et al.*, 2019) and is widely available in veterinary hospitals and practices. However, it is a very dynamic, operator-dependent (Johnson, 2011; D'Anjou and Penninck, 2015) and machine-dependent technique (Blaivas, Brannam and Theodoro, 2004). For good results, high quality ultrasound images (Hoscheit *et al.*, 2018), standardized examination protocols, good interpretation skills (Papageorges, Hanson and Leveille, 2001; Xiaolong *et al.*, 2020) and additional specialized training are required (Lindquist *et al.*, 2021).

Teleradiology involves the transfer of digital images and associated data via the internet for interpretation (Thrall, 2007; Johnson, 2011). This technique has become increasingly widespread, especially in human medicine (Bashshur *et al.*, 2016), and offers modern veterinary practice the opportunity for rapid and specialized image interpretation (Johnson, 2011). Tele-ultrasonography is a submodality of teleradiology (Xiaolong *et al.*, 2020) that is clinically valuable

in human medicine and can be used to guide clinical treatment (Britton *et al.*, 2019). It is also used in veterinary medicine but there is little published information on how to perform a standard and reliable examination (Papageorges, Hanson and Leveille, 2001; Navas de Solis *et al.*, 2020).

Tele-ultrasonography can be performed both synchronously and asynchronously by store-and-forward telemedicine (Papageorges, Hanson and Leveille, 2001; Sutherland *et al.*, 2011). The rapid transfer of digital images makes teleradiology applicable to emergency scenarios, where accurate radiology reports can inform patient management decisions. Emergency veterinary hospitals are increasingly looking for this type of external support from specialists (Johnson, 2011). Tele-ultrasonography scanning for gastrointestinal obstruction in dogs and cats could also provide fast and accurate diagnostics.

The importance of ultrasonography for the diagnosis of intestinal obstruction is already well established in dogs and cats (Garcia *et al.*, 2011; Sharma *et al.*, 2011). However, there are no studies of the use of tele-ultrasonography for this purpose. The aim of this study was to establish the accuracy and interobserver agreement of tele-ultrasonography for the diagnosis of gastrointestinal (GI) obstruction in dogs and cats for observers with different levels of experience. Results were compared to clinical data available and previous ultrasound reports of a trained radiologist and in-training radiology residents.

1.3 MATERIALS AND METHODS

1.3.1 Study Design and inclusion criteria

This was a retrospective, cross-sectional study using tele-ultrasonography with asynchronous evaluation. Ethics approval was obtained from the Ethics Committee on Animal Use (CEUA/UFPR) under protocol 049/2020. Medical imaging records between January 2017 and December 2019 from a veterinary teaching hospital were retrospectively searched.

Inclusion criteria for recruitment were dogs and cats with gastrointestinal signs (such as vomiting, diarrhea, anorexia, acute abdomen), clinical suspicion of mechanical obstruction, or both. Patients that had abdominal ultrasonographic (US) examinations and DICOM images saved for review were included. Exclusion criteria were patients with less than ten gastrointestinal ultrasonographic series (images and cineloops) in their medical file, patients that had recent gastrointestinal surgery and patients with ultrasonographic signs of GI obstruction that were not confirmed surgically, endoscopically or by necropsy. A master's student (M.S.S) performed the data collection and a veterinary radiologist and professor with 20 years of experience (T.R.F) made all decisions regarding inclusion or exclusion. The data collection included information regarding patients' age, sex, species, breed, weight and final diagnosis.

All patients with confirmed gastrointestinal obstruction that met the inclusion criteria were selected for the study. Many patients without ultrasonographic signs of gastrointestinal obstruction also met the criteria, so a stratified random sampling of this group was performed.

Dogs and cats were divided in two groups depending on the medical records data: complete and partial GI obstruction (group 1), and no GI obstruction (group 2). To be included in group 1, a diagnosis of total or partial obstruction had to be confirmed surgically, by endoscopy or by necropsy. Patients in group 2 had no gastrointestinal obstruction and this was confirmed from the medical records using a combination of results of follow-up ultrasound examination, recorded clinical outcome and/or findings at surgery or necropsy.

1.3.2 Imaging procedures and techniques

Abdominal US had been performed and reported in all patients by a veterinary radiology resident with at least 6 months of training under the supervision of a veterinary radiologist with 20 years of experience (T.R.F.).

Almost all abdominal ultrasonography was performed using one of two ultrasound machines (GE Logic F6 or Philips Affiniti 50) and a variety of

transducers (linear, convex and microconvex), with different frequencies 5.0MHz – 14 MHz. The exam was performed on a different machine, a MyLab 30 Esaote, in only one dog. The patients were positioned in dorsal recumbency, hair was clipped and coupling gel applied to the skin. Standard abdominal ultrasound examination was performed as previously described (D'Anjou and Penninck, 2015). Images and cine loops of the ultrasound examinations were recorded in DICOM format and stored in the hospital database. However, not all abdominal images obtained for each case were recorded and saved appropriately, hence imaging archives in some animals were incomplete (missing some video cine-loops and static images). All cases with at least 10 images of the gastrointestinal tract on the archive were included in study to investigate the influence of incomplete image datasets on interpretation.

The observers assessed the imaging material for each patient using commonly described ultrasonographic features used to diagnose GI obstructive diseases in dogs and cats. These findings include: degree of gastric distention, type of gastrointestinal content, gastrointestinal motility, identification of foreign body or strong hyperechoic shadowing, small intestinal diameter, degrees and length of small intestinal dilatation, gastric and small intestinal wall thickness and layering and presence of intestinal plication.

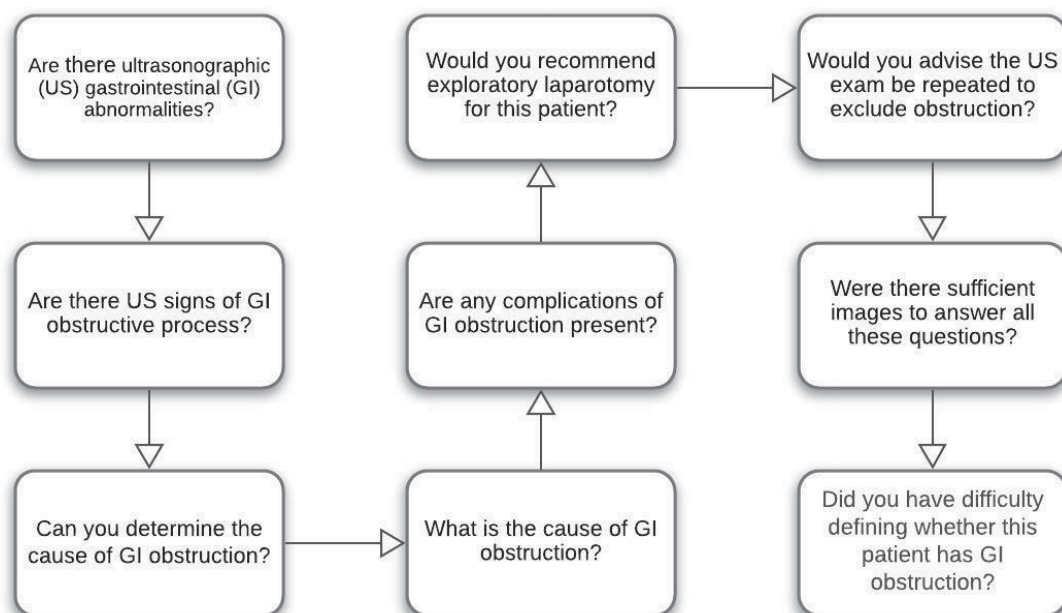
Only abnormalities in the stomach and intestines were considered gastrointestinal abnormalities for study purposes. Ultrasound findings used to diagnose a complete and partial obstruction included: severe gastric or intestinal dilatation, abnormal gastrointestinal motility, identification of a foreign body or strong hyperechoic shadowing luminal GI structure, signs of plication, changes in gastrointestinal wall and presence of masses causing obstruction. Patients were considered to have no partial or complete GI obstruction if they were lacking any of the signs described above.

A single veterinarian, master's student (M.S.S), shared the images from 16 patients per week with each observer, through a cloud server. A pilot study sharing some cases with the observers was used to identify possible bias in the questionnaire, however the observers received no standardized training. A

summary of the patient history copied from the medical records was available, but the observers were blinded to additional data and outcomes to simulate a real case scenario. All cases were randomized. Four observers interpreted all images: a board-certified diplomate of the American College of Veterinary Radiology and the European College of Veterinary Diagnostic Imaging with 22 years of experience (Observer 1), a veterinary radiologist with 12 years of experience (Observer 2), a veterinary radiologist with four years of experience (Observer 3) and a first-year veterinary radiology resident (Observer 4). The observers had not seen the images prior to this study.

The observers were instructed to interpret the ultrasound examinations using a commercially available DICOM viewer software of their choosing, in an appropriate environment (with low light and noiseless). The evaluations were carried out independently, following a script for each patient, arranged on a Google form, which was later exported to a table in Microsoft Excel. The questions to the observers are shown in figure 1.

FIGURE 1. Flowchart of the questions asked to the observers regarding each patient with gastrointestinal signs in the tele-ultrasonography study to assess gastrointestinal obstruction.



In animals that received serial ultrasound examinations, only the first examination was provided to the observers. If the observers had a suspicion of partial obstruction, they were asked if the patient should be followed up by further ultrasonographic examination. This question was not asked directly to avoid prompting the observer to consider partial obstruction or functional ileus. Even on examinations performed on site and in real time, it is sometimes necessary to repeat the ultrasound examination some hours later to exclude partial obstruction.

The observers' answers were reviewed, interpreted, and tabulated prior to statistical analysis. The master's student compared the observers' classification with the gold standard diagnosis (based on full clinical history).

1.3.3 Statistical analysis

The data was tabulated in Microsoft Excel spreadsheets and analyzed in SPSS 21.0 (IBM 2012). A descriptive analysis of the data was carried out with an estimate of absolute (n) and relative (%) frequency of the variables according to the patients and the observers, across all animals and then separately by species. Accuracy, sensitivity, specificity, positive and negative predictive values for the detection of GI ultrasound abnormalities and of GI obstructive processes were estimated. Interobserver agreement for the presence of GI obstruction was assessed using Fleiss's kappa (k_v) statistics and 95% confidence interval (CI). Agreement was classified as none ($0.0 \leq k_v \leq 0.2$), minimal ($0.21 \leq k_v \leq 0.39$), weak ($0.40 \leq k_v \leq 0.59$), moderate ($0.60 \leq k_v \leq 0.79$), strong ($0.80 \leq k_v \leq 0.90$) and almost perfect ($0.90 < k_v$) (McHugh, 2012). The answers of the observers to the question "Were there sufficient images to answer all these questions" (Fig. 1) for each case were used to consider each ultrasonography dataset sufficient or insufficient to interpretate, and it was evaluated if their answers matched the gold standard in the detection of GI ultrasonographic abnormalities and obstructive processes in each patient. The association between their classification of "sufficient or insufficient" and their ability to detect US GI ultrasonographic abnormalities and GI obstructive

processes in all patients was tested with the chi-square test (χ^2). P-values < 0.05 were considered significant.

1.4 RESULTS

1.4.1 Study Design and inclusion criteria

Three hundred forty-eight dogs and cats with gastrointestinal signs or clinical suspicion of mechanical obstruction that underwent abdominal ultrasonographic examination met the inclusion criteria. After the stratified random sampling, the sample was reduced to 132 patients, of which 42 were excluded, mainly because they had less than ten gastrointestinal ultrasonographic series (images and cine-loops) recorded in their medical file or because they did not have the surgical or by necropsy confirmation of the gastrointestinal obstruction. Thus, a total of 90 patients were left for analysis.

1.4.2 Descriptive analysis

The sample population consisted of 69 dogs (76.67%) and 21 cats (23.33%). 32/69 dogs were female (46.38%) and 37/69 dogs were male (53.62%) with a mean weight of 15.4 kg (± 12.4) and mean age of 5.7 years (± 4.2). Of the 21 cats, 13/21 were female (61.90%) and 8/21 were male (38.10%), with a mean weight of 3.4 kg (± 1.3) and mean age of 5.1 years (± 4.7).

GI obstruction (group 1) was diagnosed in 23/90 patients (25.56%) – 20 dogs and three cats. Of these, 19/23 patients (82.61%) had total obstruction and 4/23 (17.39%), partial obstruction. 67/90 animals (74.44%, 49 dogs and 18 cats) had no GI obstruction (group 2).

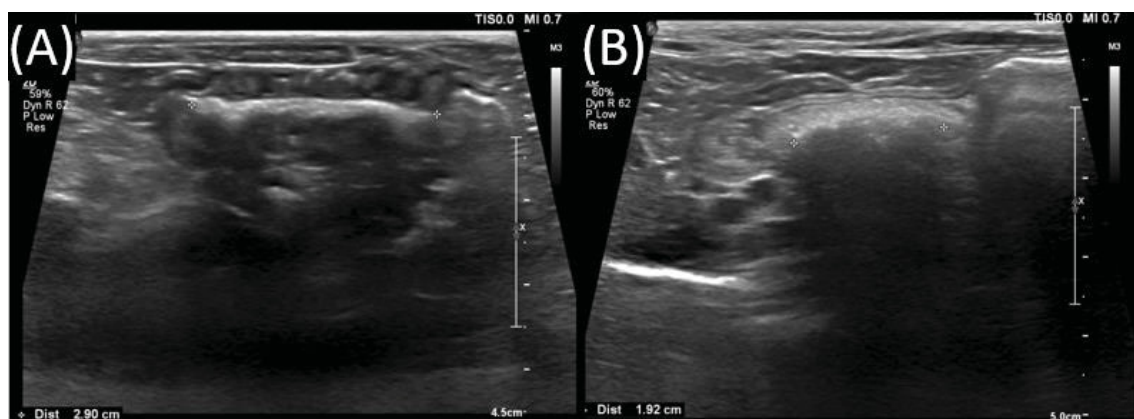
Several etiologies of GI obstruction were recorded in group 1: linear foreign bodies (7/23); gastrointestinal masses (5/23), intussusception (3/23), and nonlinear foreign bodies (4/23). Other causes included intussusception associated with foreign bodies (3/23) and combined nonlinear and linear foreign bodies (1/23). All diagnosis of total GI obstruction was confirmed surgically

(19/23). The partial obstruction cases (4/23) were confirmed surgically (2/4 patients), endoscopically (1/4) or by necropsy (1/4).

The group without GI obstruction (67/90) included patients with GI ultrasonographic abnormalities (50/67) and without (17/67). The most common GI abnormalities were functional ileus associated with gastritis, enteritis and/or colitis; followed by presumptive GI neoplastic process and incidental non-obstructive foreign bodies, or a combination thereof.

Figure 2 shows a representative example of one patient of group 1, a case of linear foreign body, correctly identified by all observers, and one patient of group 2 in which foreign bodies were identified at ultrasound examination but at exploratory laparotomy there was only cardia thickening, without signs of GI obstruction. This case was misclassified by some observers as GI obstruction because of the presence of shadowing food ingesta in the stomach.

FIGURE 2. (A) Ultrasonographic image using a high-frequency linear transducer of a young cat; this was a group 1 patient with an intestinal linear foreign body, that all observers correctly identified. Image (B) is from a 15-year-old cat of group 2, with shadowing food ingesta in the stomach that was classified as obstructed by two observers but had only cardia thickening and no obstruction at surgery.



1.4.3 Diagnostic Accuracy

Table 1 shows a summary of the statistical parameters for detection of GI ultrasonographic abnormalities and detection of GI obstructive processes for each observer. For this assessment, the gold-standard was defined by the

surgical, endoscopy or necropsy data in group 1 (partial or complete obstruction) and by available clinical and imaging follow-up data in group 2 (no obstruction). The sensitivity of each observer for detection of GI ultrasonographic abnormalities with tele-ultrasonography ranged between 86.3% and 93.2%, the specificity was 47.1-82.4%, the positive predictive value was 88.3-95.5%, the negative predictive value ranged between 58.3% and 64.7% and accuracy was 84.5-86.7%. Tele-ultrasonography had a sensitivity of 73.9-100%, a specificity of 77.6-89.6%, a positive predictive value of 55.9-70.8%, a negative predictive value of 90.9-100% and accuracy of 78.9-87.8% for diagnosis of GI obstructive process.

Table 1. Sensitivity, specificity, accuracy, positive and negative predictive values for each observer for detection of gastrointestinal (GI) ultrasonographic abnormalities and ultrasonographic GI obstructive processes in all animals, dogs and cats.

Observer s	All animals (n = 90)					Dogs (n = 69)			Cats (n = 21)		
	Se	Sp	Acc	PPV	NPV	Se	Sp	Acc	Se	Sp	Acc
Ultrasonographic GI abnormalities											
Observer 1	86.3 %	82.4 %	85.6 %	95.5 %	58.3 %	87.9 %	81.8 %	87%	80%	83.3 %	81%
Observer 2	89%	70.6 %	85.6 %	92.9 %	60%	89.6 %	63.6 %	85.5 %	86.7 %	83.3 %	85.7 %
Observer 3	93.2 %	47.1 %	84.5 %	88.3 %	61.5 %	94.8 %	54.6 %	88.4 %	86.7 %	33.3 %	71.4 %
Observer 4	91.8 %	64.7 %	86.7 %	91.8 %	64.7 %	94.8 %	63.6 %	89.9 %	80%	66.7 %	76.2 %
Ultrasonographic detection of GI obstruction											
Observer 1	73.9 %	89.6 %	85.6 %	70.8 %	90.9 %	70%	91.8 %	85.5 %	100%	83.3 %	85.7 %
Observer 2	100%	83.6 %	87.8 %	67.7 %	100%	100%	85.7 %	89.9 %	100%	77.8 %	81%
Observer 3	95.7 %	82.1 %	85.6 %	64.7 %	98.2 %	95.0 %	85.7 %	88.4 %	100%	72.2 %	76.2 %
Observer 4	82.6 %	77.6 %	78.9 %	55.9 %	92.9 %	80.0 %	81.6 %	81.2 %	100%	66.7 %	71.4 %

Abbreviations: Se = sensitivity; Sp = specificity; Acc = accuracy; PPV = positive predictive value; NPV = negative predictive value.

Table 2 shows the association between the accurate or inaccurate answers regarding detection of GI abnormalities and GI obstruction and the subjective assessment of completeness of the set of tele-ultrasonography images in each examination by the observers. There was no statistically

significant association between the number of correct and incorrect detections of GI abnormalities and obstructive disease and the subjective amount of tele-ultrasonography images available, except for observer 4 and diagnosis of GI obstruction. This observer (1st year resident) had a slightly higher rate of correct diagnosis of obstruction when they deemed the imaging material provided as sufficient versus insufficient.

Table 2. Association of the subjective assessment of sufficient or insufficient dataset of ultrasonographic (US) images according to each observer and their ability to detect US gastrointestinal (GI) US abnormalities and GI obstructive processes in all patients.

Subjective amount of available US images according to the observers (n = 90)						
Observers		Insufficient		Sufficient		P-value
		N	%	N	%	
Ultrasonographic detection of GI abnormalities						
Observer 1	Accurate answer	28	31.11%	49	54.44%	0.374*
	Inaccurate answer	7	7.78%	6	6.67%	
Observer 2	Accurate answer	34	37.78%	43	47.78%	0.169*
	Inaccurate answer	9	10%	4	4.44%	
Observer 3	Accurate answer	30	33.33%	46	51.11%	0.66*
	Inaccurate answer	7	7.78%	7	7.78%	
Observer 4	Accurate answer	25	27.78%	53	58.89%	0.457*
	Inaccurate answer	2	2.22%	10	11.11%	
Ultrasonographic detection of GI obstruction						
Observer 1	Accurate answer	28	31.11%	48	53.33%	0.529*
	Inaccurate answer	7	7.78%	7	7.78%	
Observer 2	Accurate answer	38	42.22%	41	45.56%	1.0*
	Inaccurate answer	5	5.55%	6	6.67%	
Observer 3	Accurate answer	33	36.67%	44	48.89%	0.607*
	Inaccurate answer	4	4.44%	9	10%	
Observer 4	Accurate answer	17	18.89%	54	60%	0.032
	Inaccurate answer	10	11.11%	9	10%	

* Statistically not significant ($P > .05$).

1.4.4 Interobserver analysis

Fleiss's kappa agreement for the GI obstruction diagnosis across all four observers was moderate for all animals, dogs and cats, respectively of 0.63, 0.64 and 0.62. Interobserver agreement for detection of GI ultrasonographic

abnormalities was weak: 0.48 for all animals, 0.49 for dogs and 0.44 for cats (Table 3).

Table 3. Fleiss's Kappa interobserver agreement (across all four observers) for detection of gastrointestinal (GI) ultrasonographic abnormalities and GI obstructive processes in all animals, dogs and cats through tele-ultrasonography.

	All animals (n = 90)			Dogs (n = 69)			Cats (n = 21)		
	k_v	95%CI	P-value	k_v	95%CI	P-value	k_v	95%CI	P-value
GI US abnormalities	0.48	0.42-0.55	<0.001	0.49	0.41-0.56	<0.001	0.44	0.30-0.57	<0.001
GI obstruction	0.63	0.57-0.70	<0.001	0.64	0.56-0.71	<0.001	0.62	0.49-0.76	<0.001

Abbreviations: k_v = Fleiss's Kappa; CI = confidence interval; GI = gastrointestinal; US = ultrasonographic.

1.5 DISCUSSION

This study examined the use of tele-ultrasonography in the diagnosis of GI mechanical obstruction, with interpretation of store-and-forward ultrasound images. Trained readers had a good accuracy in detecting GI obstruction ranging between 78.9% and 87.8% (Table 1), showing us that it is possible to routinely use tele-ultrasonography to help clinicians identify GI mechanical obstruction in dogs and cats. In comparison to other study (Sharma *et al.*, 2011), our values of accuracy were a little lower than the overall accuracy of real time, on-site abdominal ultrasound for diagnosing small-intestinal mechanical obstruction (95%). The three more experienced observers had similar accuracies to detect GI obstruction (85.6-87.8%) and it was higher than the accuracy of the less experienced observer (78.9%), in agreement with a previous teleradiology study that showed that more experienced radiologists had a greater overall accuracy compared to less experienced ones (Noel *et al.*, 2016). However, for the overall detection of ultrasonographic GI abnormalities, all observers showed similar good accuracies (84.5-86.7%).

Few studies evaluated real time on-site ultrasonography to identify GI obstruction in dogs and cats, Garcia *et al.* (2011) and Winter *et al.* (2017) found an excellent sensitivity (100%). We observed a larger range in sensitivities of

tele-ultrasonography to diagnose obstruction in our study (73.9-100%, Table 1). This could be explained by differences in observer training and quality of stored images. It is known that less experienced radiologists have a higher sensitivity and lower specificity (Noel *et al.*, 2016) and the large differences in observers' experience in our study may have contributed to this. Another factor explaining some differences in sensitivities is that Garcia *et al.* (2011) only considered intestinal obstruction while in our study patients with gastric foreign bodies were included (2/23) and interpretation of the significance of gastric foreign material as obstructive can be challenging and lead to more variation in interpretation. One of our observers commented that the absence of clear images of the pyloroduodenal junction challenged the interpretation of cases in which gastric foreign material was present.

The board-certified radiologist had the highest specificity to rule out GI abnormalities (82.4%) and GI obstruction (89.6%). This observer also had the highest positive predictive value (PPV) to detect GI abnormalities (95.5%) and GI obstruction (70.8%). While his negative predictive value (NPV) for obstruction was the lowest, it remained excellent (90.9%). Specificity tends to increase with experience (Britton *et al.*, 2019), and it was very evident in our study, with an increase in specificity for GI obstruction (77.6-89.6%) as the observers' experience increased (Table 1).

The PPV was lower than previously reported with real-time ultrasound for the diagnosis of mechanical GI obstruction in dogs (93%) (Winter *et al.*, 2017). The NPV was high (90.9-100%) (Table 1), similar to the ones found in previous studies (100%) (Winter *et al.*, 2017; Garcia *et al.*, 2011). However, comparisons to the study by Winter *et al.* (2017) need to consider the much higher prevalence of obstruction in their study group (81.25% vs. 25.56%), since PPV increases with a higher prevalence (Scrivani, 2002). In the context of telemedicine, the PPV and NPV of the test have some substantial importance, as the practitioner will likely make a surgical decision based on the outcome of the exam. A higher PPV may be desired to avoid un-necessary invasive and costly surgery; it is hence questionable whether tele-ultrasonography is an appropriate test to offer practitioners given the relatively low PPV (55.9-70.8%).

There was moderate interobserver agreement (McHugh, 2012) for correct identification of GI obstruction for all animals ($n = 90$, $k_v = 0.63$), dogs ($n = 69$, $k_v = 0.64$) and cats ($n = 21$, $k_v = 0.62$) (Table 3), despite the differences in observers' experience. However, the level of agreement is not as high as one would desire when there is a potential surgical decision to be made; in such clinical scenarios, a strong to near-perfect interobserver agreement (i.e., >0.80) would be more desirable. This finding raises up further questions as to the usefulness of tele-ultrasonography for detection of GI obstruction in dogs and cats. The interobserver agreement was higher than has been reported for serial abdominal radiographs ($\kappa = 0.36-0.53$) (Elser *et al.*, 2020) in diagnosing GI obstruction, this difference could be explained because they only considered obstruction due to non-radiopaque foreign bodies. Another explanation is that ultrasound is superior to radiography to diagnose intestinal obstruction (Sharma *et al.*, 2011) and GI foreign bodies (Tyrrel and Beck, 2006), and it appears that so is tele-ultrasonography.

Some of the cases in which there were disagreements in the detection of GI obstruction were the cases that had an intestinal mass or granuloma concurrent with GI partial or complete obstruction ($n=5$) and they could be due to different interpretation approaches and training. Only in one of these five cases all observers agreed regarding the presence of GI obstruction; this was a case of complete obstruction. There were also three cases in which all observers achieved false positive diagnosis for obstruction. One patient had intestinal foreign material within an oral dilated intestinal loop with hypermotility, but in the follow-up exam the foreign material was seen in the descending colon. Another case was a cat in which the stomach and small intestine were distended by amorphous shadowing intestinal material, but at exploratory laparotomy a thickened intestinal loop was noted, with no GI obstruction. These two cases highlight the subjectivity of US diagnosis of GI obstruction in some cases, because the features on initial exam may suggest an obstruction but this can resolve sometimes with medical management such as fluid therapy. The last case that all observers misclassified as false positive had presumed gastric wall edema associated with hypoalbuminemia (Murakami *et al.*, 2020) with

incidental intestinal foreign material. A possible contributory factor in this case was that there were no cine-loops following the oral and aboral extent of the intestinal foreign material.

Other possible reasons for the low agreements in diagnosis made by the observers in this study include the presence of subtle abnormalities which were not easily identified in stored images, and the quality and number of tele-ultrasonography images available for interpretation. Except for the least experienced observer, we found overall no association between accurate and inaccurate sonographic detection of GI obstruction and the perception of the observers of the set of stored images as “sufficient” or “insufficient” (Table 2). However, the quantity of sonographic stored images was considered only in a subjective way for this analysis and this approach may have led to a false negative result. On the other hand, a previous study suggested the quality and quantity of the ultrasound imaging data is essential to tele-ultrasonography (Xiaolong *et al.*, 2020). These findings might help establishing a protocol for how the US images should be acquired in a standard manner to maximize the radiologist performance in store-and-forward telemedicine, with minimum database required and detailed cine-loops of the affected portion and the adjacent segments (oral and aboral), besides the identification of localization marked in each image.

This study has some limitations. First, the retrospective nature of the data and lack of standardization of the images sent may have affected interpretation. Secondly, the large difference between the number of cases in different species could have influenced some results, such as sensitivity for feline GI obstruction that may have been overestimated. Thirdly, field vs. laboratory bias is inherent to studies with this design because all the observers were aware that their response to each case had no clinical consequence. Fourth, there was a significant difference in training between the observers and between the veterinarians acquiring the images.

It was said that it is complicated to render a meaningful interpretation from another sonographer’s static images or videoclips (Mattoon and Berry,

2021), but a recent pilot study evaluating the feasibility of telehealth in equine ultrasound (Navas de Solis *et al.*, 2020), showed that tele-ultrasonography is feasible in veterinary medicine. Even though some veterinary radiologists are not pleased with remote reporting associated with this imaging modality (Johnson, 2011) and the potential loss of image quality and change in grey scale (Burgul, Gilbert and Undrill, 2000), a great number of applications of tele-ultrasonography have been reported in human medicine (Hussain *et al.*, 2004; Robertson *et al.*, 2017; Britton *et al.*, 2019; Goldsmith *et al.*, 2020).

1.6 CONCLUSION

In conclusion, this study suggests that tele-ultrasonography is a feasible modality in dogs and cats with a clinical suspicion of GI obstruction. However, although the overall accuracy was good, the PPV and inter-observer agreement were rather low, questioning the usefulness of the technique in this clinical context, particularly given the potential surgical decision at hand. The lack of standardization and insufficient amount of imaging material provided may be reasons for these observations. Further prospective studies are necessary to evaluate the influence of standardized techniques for ultrasonographic image acquisition and the impact of real time (synchronous) teleradiology on the diagnostic accuracy for different diseases through veterinary tele-ultrasonography.

1.7 ACKNOWLEDGEMENTS

The primary author, Marina Silvestre Sombrio, conducted the research as part of a project funded by CNPq-Br. The authors thank Camila Marinelli Martins for statistical consultation.

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2. CHAPTER 2: ACCURACY AND INTERRATER RELIABILITY OF TELE-ULTRASONOGRAPHY IN DETECTING RENAL AND URETERAL OBSTRUCTION IN SMALL ANIMALS

2.1 ABSTRACT

Objectives: To assess the accuracy and interobserver agreement of tele-ultrasonography for the diagnosis of renal and ureteral obstruction in dogs and cats in radiologists with different levels of experience.

Methods: A retrospective cross-sectional study including dogs and cats admitted with urinary tract signs and clinical suspicion of obstruction, between January 2017 and December 2018, that had abdominal ultrasonographic examination performed and DICOM images saved for review. Patients were classified into two groups based on final diagnosis: Group 1 – with renal or ureteral obstruction; group 2 - without renal or ureteral obstruction. Observers with three different experience levels interpreted the archived ultrasound examinations, simulating a tele-ultrasound consultation. Analyses of sensitivity, specificity, positive and negative predictive values and accuracy were obtained for each observer for detection of urinary tract ultrasonographic abnormal findings, of renal and ureteral obstruction and of hydronephrosis. Agreement between observers was assessed using Fleiss's Kappa statistics.

Results: Eighty-four patients were included. Of these, 11/84 (13.1%) were in group 1 and 73/84 (86.9%) in group 2. Interpretation of the images by tele-ultrasonography showed intervals of sensitivity, specificity, accuracy and positive and negative predictive values, respectively, of 94.9-98.7%, 20-100%, 94.1-96.4%, 95.1-100% and 50-62.5% for detection of urinary tract US abnormalities, 81.8-100.0%, 91.8-97.3%, 88.7-97.6%, 62.5-84.6% and 97.3-100% for renal and ureteral obstruction and 100%, 93.3-98.7%, 94-98.8%, 64.3-90.0% and 100% for detection of hydronephrosis. Interobserver agreement across all three reviewers was weak for detection of urinary tract US abnormalities (0.58), was moderate for renal and ureteral obstruction diagnosis (0.73) and strong for the detection of hydronephrosis for all animals (0.83).

Clinical Significance: Tele-ultrasonography had good accuracy for detection of renal and ureteral obstructive processes, however had a rather moderate inter-

observer agreement and low positive predictive value. So, this technique should be used with caution in this clinical context, given the potential surgical decision at hand.

Keywords: diagnostic imaging, telemedicine, veterinary teleradiology, ultrasound, urinary tract disease.

Word Count: 4374 words

2.2 INTRODUCTION

Canine and feline nephroureteral obstruction is a complex disease process (Palm and Culp, 2016), whose incidence is increasing in veterinary practice (Berent, 2011) and it requires surgical or interventional treatment (Palm and Westropp, 2011). To effectively diagnose and treat patients with upper urinary tract (UT) obstructions, it is important to identify the underlying causes for these obstructions, such as nephroliths and ureteroliths, ureteritis, scarring from previous inflammation, and/or cellular or crystalline debris (Palm and Culp, 2016).

The clinical signs are variable and are often related to the rate at which obstruction develops; patients with acute obstruction are often more painful as compared with patients with more insidious obstructions (Brenner, 2008). Non-specific signs can also appear and include reduced appetite, weight loss, lethargy and hiding (Palm and Westropp, 2011).

Ureteral obstructions can be difficult to diagnose and treat in veterinary medicine (Berent, 2011). However, some imaging modalities such as radiograph and ultrasonography can perform a straightforward diagnosis in most cases (Palm and Culp, 2016). For some authors, the sensitivity for abdominal ultrasound for canine ureteral calculi was 100% versus 88% for radiographs (Snyder *et al.*, 2004), whereas for feline ureteral calculi, it was very similar between modalities (77% vs. 81%).

Abdominal ultrasonography can also provide early detection of abnormalities of the renal pelvis, renal pelvic diverticula, and ureters (Widmer, Biller and Adams, 2004). It can help delineate the severity of hydronephrosis, determine if hydroureter are present (Palm and Westropp, 2011) and can show the exact location of the obstructive lesion (Berent, 2011). Ultrasonography is a safe (non-ionizing) and portable tool, capable of generating images that can be analyzed in real-time by a veterinarian in locum or at a distance through tele-ultrasonography (Britton *et al.*, 2019).

Tele-ultrasonography (TUS) is a modality of telemedicine (Xiaolong *et al.*, 2020) that involves performing ultrasound examination at a particular location and interpreting it in another location (Britton *et al.*, 2019), through the transfer of digital images and associated data via the internet (Johnson, 2011). This tool has become commercially available and viable due to the portability of ultrasound devices, the design of new technologies to support data transmission with greater global connectivity, and cost reduction (Britton *et al.*, 2019). And it offers veterinary practice the opportunity for specialized and fast image interpretation (Johnson, 2011).

However, ultrasonography exam is a very dynamic, operator-dependent technique (Mattoon and Berry, 2021). So, for good results, additional specialized training (Lindquist *et al.*, 2021), standardized examination protocols, good interpretation skills (Papageorges, Hanson and Leveille, 2001; Xiaolong *et al.*, 2020), and high-quality ultrasound images (Hoscheit *et al.*, 2018) are required. Tele-ultrasonography has also been used in veterinary medicine however there is little published information (Papageorges, Hanson and Leveille, 2001; Navas de Solis *et al.*, 2020).

The importance of ultrasonography for diagnosing upper urinary obstruction is already established in small animals (D'Anjou, Bédard and Dunn, 2011; Monnet and Kyles, 2013; Palm and Culp, 2016). However, there are no studies using tele-ultrasonography to scan for renal and ureteral obstruction. The aim of this study was to establish the accuracy and interobserver agreement of tele-ultrasonography for the diagnosis of renal and

ureteral obstruction in dogs and cats for observers with different levels of experience.

2.3 MATERIALS AND METHODS

2.3.1 Study Design and inclusion criteria

This was a retrospective, cross-sectional study using tele-ultrasonography with asynchronous evaluation. Ethics approval was obtained from the Ethics Committee on Animal Use (CEUA/UFPR) under protocol 010/2021. Medical imaging records between January 2017 and December 2018 from a veterinary teaching hospital were retrospectively searched.

Inclusion criteria for recruitment were dogs and cats that underwent abdominal ultrasonographic (US) examination with a Philips Affiniti 50 ultrasound machine because of UT and non-specific signs (such as hematuria, polyuria, stranguria, pollakiuria, abdominal pain, lethargy, vomiting, hyporexia/anorexia, weight loss), clinical suspicion of upper UT obstruction, or both. Exclusion criteria were patients with less than twelve DICOM ultrasonographic images and cineloops saved for review, patients without ultrasonographic images of the UT recorded and patients without confirmation of the urinary obstruction surgically or by necropsy. When patients had undergone more than one abdominal ultrasonographic exam, only one exam was included in the study.

A master's student (M.S.S.) performed the data collection and a veterinary radiologist and professor with 20 years of experience (T.R.F.) helped with the decisions regarding inclusion or exclusion. The data collection included information regarding patients' age, sex, species, breed, weight, on-site ultrasonographic findings and final diagnosis. All patients with confirmed renal or ureteral obstruction that met the inclusion criteria were selected for the study. Many patients without obstruction also met the criteria, and a stratified random sampling of this group was performed.

Dogs and cats were divided in two groups depending on the medical records data: Renal or ureteral obstruction (group 1) and without renal or ureteral obstruction (group 2). To be included in group 1, a diagnosis of obstruction had to be confirmed surgically, or by necropsy. Patients in group 2 had no obstructive lesion involving the upper urinary outflow tract and this was confirmed from the medical records using a combination of results of follow-up ultrasound examination, recorded clinical outcome and/or findings at surgery or necropsy.

2.3.2 Imaging procedures and techniques

Abdominal ultrasound had been performed and reported in all patients by a veterinarian resident with at least 6 months of training under the supervision of a veterinarian radiologist with 20 years of experience (T.R.F.).

All abdominal ultrasonography was performed using a Philips Affiniti 50 ultrasound machine and a variety of transducers (linear, convex and microconvex), with different frequencies 5.0MHz – 14 MHz. The patients were positioned in dorsal recumbency, hair was clipped and coupling gel applied to the skin. Standard abdominal ultrasonography examination was performed as previously described (D'Anjou and Penninck, 2015). Images and cine-loops of the ultrasound examinations were recorded in DICOM format and stored in the hospital database. However, not all abdominal images obtained for each case were recorded and saved appropriately, hence imaging archives in some animals were incomplete (missing some video cine-loops and static images). All cases with at least twelve ultrasonographic images on the archive were included in study because we wanted to simulate a real environment of teleradiology where unfortunately not always the cases come with all necessary images. In animals that received serial ultrasonographic examinations, only one aleatory examination was provided to the observers.

2.3.3 Tele-ultrasonography asynchronous interpretation scheme

The observers assessed the imaging material for each patient using commonly described ultrasonographic features used to diagnose renal and

ureteral obstructive diseases in dogs and cats (Widmer, Mattoon and Vaden, 2021).

Only abnormalities in the kidney, ureters, bladder, and urethra were considered UT abnormalities for study purposes. The observers should classify the renal alterations into one of three classes: incidental findings (R1), non-incidental findings with mild or moderate clinical relevance (R2), or non-incidental findings with important clinical relevance (R3). Incidental findings should be considered when there were only mild abnormalities such as small cysts and dystrophic renal mineralization and variations due to exam or transducer settings. Non-incidental findings with mild or moderate clinical relevance should be taken into consideration when the kidneys' findings were reduced corticomedullary distinction, small renal calculus, and pyelectasia without signs of megaureter associated. And last, findings with important clinical relevance, such as nephromegaly, obstructive lithiasis (total or partial), presence of mass, abscess, pseudocyst, renal parasitism, signs of chronic kidney disease.

Ultrasonographic findings used to diagnose upper UT obstruction (group 1) included mostly hydronephrosis and ureteral distension. The ureter dilation could abruptly been blunted caudally at the level of the obstruction (D'Anjou and Penninck, 2015). A renal pelvic dilation more than 8 mm in dogs and 5 mm in cats should have raised concern for urinary obstruction (Having and Byron, 2015) to the observers. A pelvic height ≥ 1.3 cm measured on a transverse image was considered as hydronephrosis and predictive of outflow obstruction (D'Anjou, Bédard and Dunn, 2011). The observers should have searched for causes of pelvic or ureteral obstruction, that include calculus (hyperechoic structures usually associated with acoustic shadowing), stricture, cellular debris, blood clot, retroperitoneal mass (neoplasia, abscess, granuloma) or infiltrative mass at ureterovesical junction, ureter or renal pelvis (D'Anjou and Penninck, 2015).

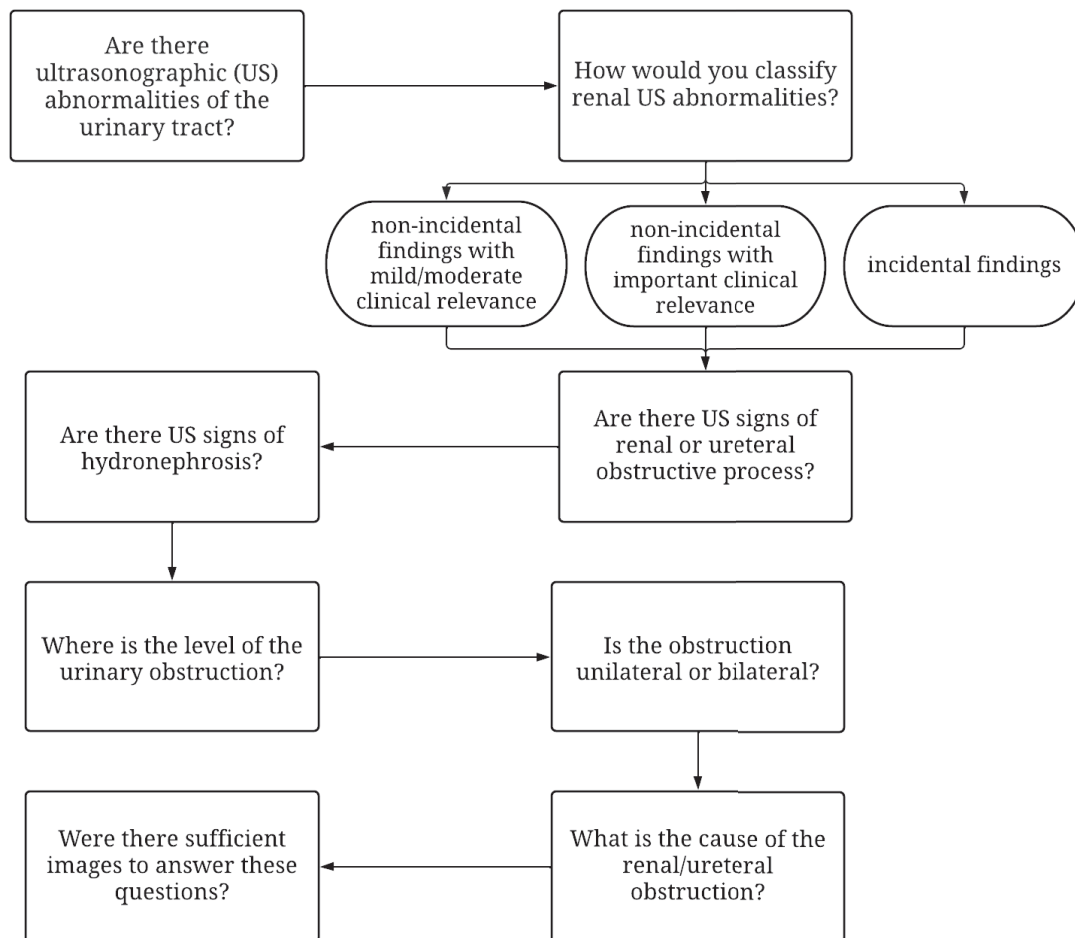
2.3.4 Data collection and validation

A single veterinarian, master's student (M.S.S.), shared the images from 11 patients per week for each observer through a cloud server. A pilot study

sharing some cases with the observers was used to identify possible bias in the questioning; however, the observers received no standardized training. A summary of the patient history copied from the medical records was available, but the observers were blinded to additional data and outcomes to simulate a real case scenario. All cases were randomized. Three observers interpreted all images: a Ph.D. veterinary radiologist with more than ten years of experience (Observer 1), an MSc veterinary radiologist with more than ten years of experience (Observer 2) and an MSc veterinary radiologist with four years of experience (Observer 3). The observers had not seen the images prior to this study.

The observers were instructed to interpret the ultrasonographic examinations using a commercially available DICOM viewer software of their choosing in an appropriate environment (with low light and noiseless). The evaluations were carried out independently, following a script for each patient, arranged on a Google form, which was later exported to a table in Microsoft Excel. The questions to the observers are shown in figure 3. The classification of renal abnormalities should be done based on the most abnormal renal silhouette, if they had different aspects.

FIGURE 3. Flowchart of the questions asked to the observers regarding the urinary tract of each patient in the tele-ultrasonography study.



The observers' answers were tabulated prior to statistical analysis. Results were compared to the clinicopathologic data, previous live ultrasonographic findings, and surgical data, when available. The master's student classified the renal abnormalities according to the previous live ultrasonographic findings obtained from the report of each patient to compare to the observers' classification. The observers' answers about the presence of renal or ureteral obstructive process were compared with the golden standard diagnosis (surgical confirmation or by necropsy).

2.3.5 Statistical analysis

The data was tabulated in Microsoft Excel spreadsheets and analyzed in SPSS 21.0 (IBM 2012). A descriptive analysis of the data was carried out with

an estimate of absolute (n) and relative (%) frequency of the variables according to the patients and the observers, across all animals and then separately by species. Sensitivity, specificity, accuracy, positive and negative predictive values for the detection of UT ultrasound abnormalities, of renal and ureteral obstructive processes and of hydronephrosis were estimated. The observer's renal classification for each patient was compared to the classification obtained through the on-site previous ultrasonographic findings. Interobserver agreement for the presence of UT US abnormal findings; renal and ureteral obstruction and detection of hydronephrosis was assessed using Fleiss's kappa (k_v) statistics and 95% confidence interval (CI). Agreement was classified as none ($0.0 \leq k_v \leq 0.2$), minimal ($0.21 \leq k_v \leq 0.39$), weak ($0.40 \leq k_v \leq 0.59$), moderate ($0.60 \leq k_v \leq 0.79$), strong ($0.80 \leq k_v \leq 0.90$) and almost perfect ($0.90 < k_v$) (McHugh, 2012). P-values < 0.05 were considered significant.

2.4 RESULTS

2.4.1 Study Design and inclusion criteria

Three hundred twenty-eight dogs and cats met the inclusion criteria. Of them, 216 were eliminated due to the exclusion criteria, which reduced the study sample to 112 patients. After the stratified random sampling, there were a total of 84 patients left for analysis.

2.4.2 Descriptive analysis

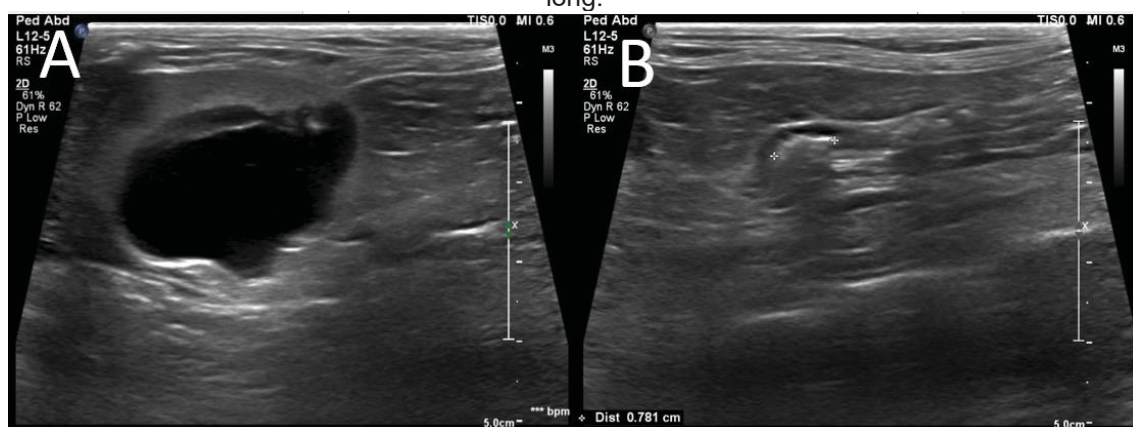
The sample population consisted of 64 dogs (76.19%) and 20 cats (23.81%). 28/64 dogs were female (43.75%) and 36/64 were male (56.25%), with a mean weight of 9.62 kg (± 5.66) and mean age of 9.5 years (± 4.4). Of 20 cats, 9/20 were female (45.0%) and 11/20 were males (55.0%), with a mean weight of 3.94 kg (± 1.43) and a mean age of 9.0 years (± 4.8).

Renal and ureteral obstruction (group 1) was diagnosed in 11/84 (13.10%) – nine dogs and two cats. 73/84 animals (86.90%, 55 dogs and 18 cats) had no upper urinary obstruction (group 2).

Several etiologies of renal and ureteral obstruction were recorded in group 1: lithiasis (4/11), extrinsic masses (4/11), and a renal mass involving the pelvic outflow (1/11) and the cause could not be clearly determined by the surgeon in 2/11. There was only ureteral lithiasis in 3 of 4 cases of lithiasis, and in one, it was located both within the renal pelvis and ureter. Of the extrinsic masses' cases, 2 of 4 cases were at the ureterovesical junction and the others were surrounding a ureter. In 2 of 4 of the extrinsic masses, it was performed a histopathological exam, one resulted in leiomyoma at ureterovesical junction and the other one was a histiocytic sarcoma adjacent to a ureter. The case of the renal mass also underwent histopathological exam and resulted in renal papillary adenoma. The diagnosis of obstruction was confirmed surgically (10/11), or by necropsy (1/11). Figure 4 shows a representative example of a 12-years old female cat classified as group 1, a patient with obstructive right side hydronephrosis correlated to a ureteral obstruction by lithiasis confirmed by exploratory laparotomy and correctly identified by all observers.

Regarding the presence of hydronephrosis, it was detected in 9/84 patients (10.71%) – seven dogs and two cats. 75/84 patients (89.29%) had no ultrasonographic signs of hydronephrosis.

FIGURE 4. Sequence of ultrasonographic images of a 12-years old female cat, using a high-frequency linear transducer, with ureteral obstruction by lithiasis with concurrent hydronephrosis confirmed by exploratory laparotomy. This case was correctly identified by all observers in the tele-ultrasound study. A) Longitudinal image of the right kidney with hydronephrosis. B) Longitudinal image of the right proximal ureter showing a distended ureter with two hyperechoic foci with acoustic shadowing, the bigger one is 0.78 cm long.



The group with no renal and ureteral obstruction (73/84) included patients with UT ultrasonographic abnormalities (65/73) and without (5/73). A variety of medical conditions were identified in group 2 including chronic kidney disease, renal parasitism (*Dioctophyma renale*), urethral obstruction, pyelonephritis, urolithiasis, cystitis, hepatopathy and pancreatitis.

2.4.3 Diagnostic Accuracy

Ultrasonographic renal abnormalities were compared to the clinical data available and on-site ultrasonographic findings and the cases were classified into three classes: incidental findings (R1), non-incidental findings with mild to moderate clinical relevance (R2) or non-incidental findings with important clinical relevance (R3). According to the clinical data available and on-site ultrasonographic findings (gold-standard classification), 14/84 patients were classified as R1 (16.66%), 35/84 as R2 (41.67%) and 35/84 as R3 (41.67%). Table 4 shows the comparison of the gold-standard classification with the classifications according to the observers' tele-ultrasonography interpretations. Overall, observer 1 correctly classified 77/84 (91.7%) animals, observer 2 correctly classified 56/84 (66.7%) animals, and observer 3 correctly classified 60/84 animals (71.4%). The class that all observers correctly classified most of patients was R3.

Table 4. Comparison of the observers' classification profile for each patient through tele-ultrasonography interpretations in three classes: R1 (incidental findings), R2 (non-incidental findings with mild to moderate clinical relevance) or R3 (non-incidental findings with important clinical relevance) versus the gold-standard classification, specifically for urinary tract findings in dogs and cats of the study.

Observers' classification	CLASSIFICATION								P-value	
	CLASS 1 (R1)		CLASS 2 (R2)		CLASS 3 (R3)		Total			
	N	%	N	%	N	%	N	% total		
Observer 1	R1	14	100.0%	4	11.4%	0	0.0%	18	21.4%	<0.001
	R2	0	0.0%	30	85.7%	2	5.7%	32	38.1%	
	R3	0	0.0%	1	2.9%	33	94.3%	34	40.5%	
	Total	14	100.0%	35	100.0%	35	100.0%	84	100.0%	
Observer 2	R1	12	85.7%	4	11.4%	1	2.9%	17	20.2%	<0.001
	R2	2	14.3%	23	65.7%	13	37.1%	38	45.3%	
	R3	0	0.0%	8	22.9%	21	60.0%	29	34.5%	
	Total	14	100.0%	35	100.0%	35	100.0%	84	100.0%	

Observer 3	R1	7	50.0%	1	2.8%	0	0.0%	8	9.5%	<0.001
	R2	7	50.0%	24	68.6%	5	14.3%	36	42.9%	
	R3	0	0.0%	10	28.6%	30	85.7%	40	47.6%	
	Total	14	100.0%	35	100.0%	35	100.0%	84	100.0%	

Bolded : patients in which their answers matched the gold standard classification.

Table 5 shows a summary of the statistical parameters for detection of UT ultrasonographic abnormalities, of renal and ureteral obstructive processes and of hydronephrosis for each observer. For this assessment, the gold-standard was defined by the surgical, or necropsy data in group 1 (with obstruction) and by available clinical and imaging follow-up data in group 2 (no obstruction).

Table 5. Sensitivity, specificity, accuracy, positive and negative predictive values for each observer for detecting urinary ultrasonographic abnormalities, renal and ureteral obstructive processes and hydronephrosis in all animals, dogs and cats through tele-ultrasonography.

Observers	All animals (n = 84)					Dogs (n = 64)			Cats (n = 20)		
	Se	Sp	Acc	PPV	NPV	Se	Sp	Acc	Se	Sp	Acc
Ultrasonographic detection of urinary abnormalities											
Observer 1	94.9%	100%	95.2%	100%	55.6%	96.7%	100%	96.9%	89.5%	100%	90%
Observer 2	96.2%	100%	96.4%	100%	62.5%	98.3%	100%	98.4%	89.5%	100%	90%
Observer 3	98.7%	20%	94.1%	95.1%	50%	98.3%	0%	92.2%	100%	100%	100%
Ultrasonographic detection of renal and ureteral obstruction											
Observer 1	81.8%	97.3%	95.2%	81.8%	97.3%	77.8%	100%	96.9%	100%	88.9%	90%
Observer 2	90.9%	91.8%	88.7%	62.5%	98.5%	88.9%	89.1%	89.1%	100%	100%	100%
Observer 3	100%	97.3%	97.6%	84.6%	100%	100%	100%	100%	100%	88.9%	90%
Ultrasonographic detection of hydronephrosis											
Observer 1	100%	98.7%	98.8%	90%	100%	100%	100%	100%	100%	94.4%	95%
Observer 2	100%	97.3%	97.6%	81.8%	100%	100%	98.3%	98.4%	100%	94.4%	95%
Observer 3	100%	93.3%	94%	64.3%	100%	100%	94.7%	95.3%	100%	88.9%	90%

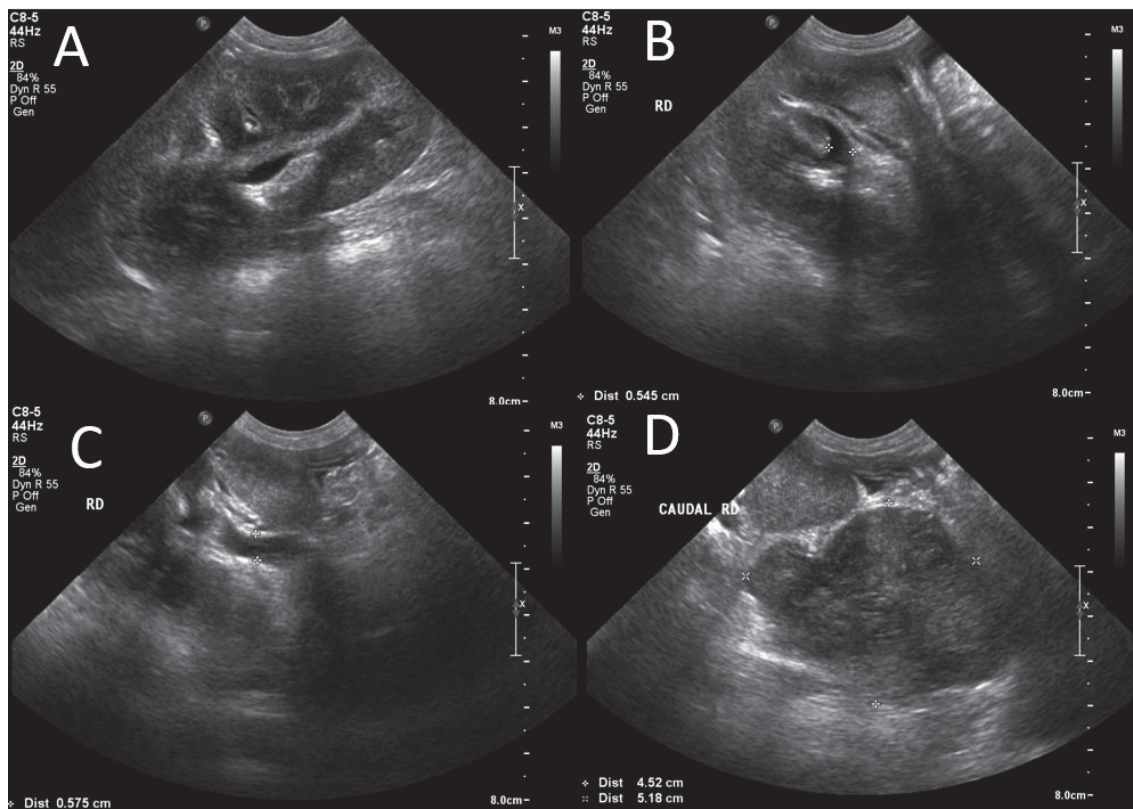
Abbreviations: Se = sensitivity; Sp = specificity; Acc = accuracy; PPV = positive predictive value; NPV = negative predictive value.

The sensitivity of each observer for detection of UT US abnormalities with tele-ultrasonography ranged between 94.9% and 98.7%, the specificity was 20-100%, accuracy ranged between 94.1% and 96.4%, the positive predictive value was 95.1-100% and the negative predictive value ranged between 50.0% and 62.5%. Tele-ultrasonography had the sensitivity of 81.8-100%, the specificity of 91.8-97.3%, accuracy of 88.7-97.6%, the positive predictive value of 62.5-84.6% and the negative predictive value of 97.3-100% for diagnosis of renal and ureteral obstructive process. The sensitivity of each observer for detection of hydronephrosis was 100%, the specificity ranged

between 93.3 and 98.7%, accuracy ranged between 94% and 98.8%, the positive predictive value was 64.3-90% and the negative predictive was 100%.

Figure 5 shows a representative example of a 14-years old female non-spayed dog classified as group 1, a patient with ureteral obstruction by extrinsic mass confirmed by exploratory laparotomy that were the only case two observers misclassified as group 2.

FIGURE 5. Ultrasonographic images of a 14-years old female non-spayed dog, using a low-frequency micro-convex transducer, with ureteral obstruction caused by multiple extrinsic masses confirmed by exploratory laparotomy. This group 1 patient were the only case two observers misclassified as group 2. A) Longitudinal image and B) transverse image of the right kidney, evidencing the kidney pelvis moderately distended. C) Distended proximal ureter is apparent, communicating with the pelvis. D) One of the abdominal masses nearby the right kidney and ureter, causing the ureteral obstruction.



2.4.3 Interobserver analysis

Fleiss's kappa agreement for the renal and ureteral obstruction diagnosis across all observers was moderate for all animals, dogs and cats, respectively of 0.73, 0.75 and 0.67. Interobserver agreement for detection of urinary ultrasonographic abnormalities was weak for all animals (0.58) and cats (0.54) and moderate for dogs (0.60). Regarding US detection of hydronephrosis,

interobserver agreement was strong for all animals (0.83) and dogs (0.88) and moderate for cats (0.67) (Table 6).

Table 6. Fleiss's Kappa interobserver agreement (across all three observers) for detection of urinary tract ultrasonographic abnormalities, renal and ureteral obstruction, and hydronephrosis in all animals, dogs and cats through tele-ultrasonography.

	All animals (n = 84)			Dogs (n = 64)			Cats (n = 20)		
	k_v	95%CI	P-value	k_v	95%CI	P-value	k_v	95%CI	p-value
Urinary tract US abnormalities	0.58	0.49-0.67	<0.001	0.60	0.50-0.70	<0.001	0.54	0.36-0.72	<0.001
Renal and ureteral obstruction	0.73	0.64-0.82	<0.001	0.75	0.65-0.85	<0.001	0.67	0.49-0.85	<0.001
Hydronephrosis	0.83	0.74-0.91	<0.001	0.88	0.78-0.98	<0.001	0.67	0.49-0.85	<0.001

Abbreviations: k_v = Fleiss's Kappa; CI = confidence interval; US = ultrasonographic.

2.5 DISCUSSION

We examined the use of tele-ultrasonography in diagnosing renal and ureteral obstruction, with an interpretation of store-and-forward ultrasonographic images. Trained readers had a good accuracy in detecting renal and ureteral obstruction ranging between 88.7% and 97.6% (Table 5), showing us that it is possible to use tele-ultrasonography to identify upper urinary obstruction in small animals in veterinary practice. Ultrasonography is dependent on the experience and skill of the sonographer with clinical decisions based on real-time imaging (Sharma *et al.*, 2011). However as all the observers had at least four years of ultrasound training, their accuracies to detect UT US abnormalities (94.1-96.4%) and of hydronephrosis (94-98.8%) were similar and excellent (Table 5).

The accuracy and sensitivity of ultrasonography for the detection of renal and ureteral obstruction in small animals has not been investigated extensively, however in comparison to other study in medicine (Ellenbogen *et al.*, 1978), our values of sensitivity (Table 5) were similar to or lower than the overall sensitivity of real time, on-site abdominal ultrasound for diagnosing UT obstruction (98%)

however their specificity was lower (74%). We observed a larger range in sensitivities of tele-ultrasonography to diagnosis renal and ureteral obstruction in our study. This could be explained by differences in observers' experience and quality of stored images, due to the potential loss of image quality and change in grey scale (Burgul, Gilbert and Undrill, 2000). The large differences in observers training and experience in our study may also have contributed to our findings, as it was observed an increase in sensitivity with the decrease of observers' experience in our study, in concordance with Noel *et al.* (2016) that described that less experienced radiologists may have lower specificity and higher sensitivity.

There were only two cases in which there were false negatives assessments for renal and ureteral obstruction. One patient was the only case two observers misclassified as group 2: a 14-years old female non-spayed dog with ureteral obstruction caused by multiple extrinsic masses confirmed by exploratory laparotomy, represented in Figure 5. These disagreements may be because many times it was not provided longitudinal and transverse US images and cine-loops of both kidneys and following all the ureter length. And the other case with a low agreement was a 12-years old male dog with distended pelvis and obstruction by a kidney mass confirmed surgically and with histopathological exam (renal papillary adenoma). This disagreement may be due to different interpretation approaches and training and because it was a case with a partial obstruction only, making the US interpretation harder.

Hence, it should be established a protocol for how the ultrasonographic images should be acquired, with minimum database required, in agreement with a previous study that suggested the quality and quantity of the ultrasound imaging data is essential to tele-ultrasound (Xiaolong *et al.*, 2020). It should include images and cine-loops of longitudinal and transverse clear views of renal pelvis and the entire ureter length, besides the identification of localization marked in each image.

In dogs, TUS had a sensitivity of 77.8-100% and specificity of 89.1-100% (Table 5) for detection of renal and ureteral obstruction. Lower sensitivity (73%)

and specificity (77%) have been reported for the diagnosis with doppler ultrasonography of induced UT obstruction for dogs (Nyland *et al.*, 1993), but their small number of patients may have contributed to this difference. A higher sensitivity was achieved in cats, and all observers correctly confirmed renal and ureteral obstruction when it was present (100% sensitivity) (Table 5). In a previous study (Kyles *et al.*, 2005) in cats with confirmed ureterolithiasis, sensitivity of ultrasonography alone was 77%, and sensitivity of a combination of survey radiography and ultrasonography reached 90%. It is possible that this excellent sensitivity for TUS was in part due to the high resolution of the images achieved with the high frequency transducer; also, in smaller size patients such as cats, higher quality examinations are typically achieved as compared to large breed dogs in which ultrasound depth of exploration, and gas interposition can challenge imaging quality. Nevertheless, there was a lower number of cats with obstruction in comparison to dogs in our study and in comparison to the previous study (Kyles *et al.*, 2005), which may also have contributed to an overestimated value.

The most experienced radiologist had the highest specificity to rule out UT US abnormalities (100%) and renal and ureteral obstruction (97.3%). This observer also had the highest positive predictive value (PPV) to detect UT US abnormalities (100%). Although her negative predictive value (NPV) in detecting obstruction was the lowest, it remained excellent (97.3%). Regarding US detection of UT abnormalities, there was a large amplitude between the two most experienced observers (100%) and the least experienced observer (20%). It is described that specificity tends to increase with experience (Britton *et al.*, 2019), however the presence of only a few cases without UT US abnormalities (5/84, 5.96%) is possibly the main reason for this large difference.

The PPV for detecting UT US abnormalities was excellent (95.1-100%), but it was lower for renal and ureteral obstruction (62.5-84.6%) and for hydronephrosis (64.3-90%) (Table 5). Besides the ureter being a frustrating area to gain access to for both diagnostic and therapeutic purposes (Berent, 2011), in a previous study (Lamb, Cortellini and Halfacree, 2018) no significant differences in ultrasound signs were found between cats with obstructed and

non-obstructed ureters confirmed by pyelography, in other words, there is not exclusive US findings and measures to diagnosis and exclude ureteral obstruction reliably by ultrasound exam.

The predictive values of the exams (PPV and NPV) will help the clinician establishes a decision (in this case, surgical) based on the outcome of the TUS exam, so it has substantial clinical importance. A low PPV, as observed for renal and ureteral obstruction and hydronephrosis, implies that the probability of the test being a false-positive is high (Scrivani, 2002). Therefore, it is questionable whether TUS is a suitable exam to offer practitioners to scan for these diseases given its relatively low PPV. Other imaging tests, such as excretory urography and computed tomography, can be required in patients in which a clear diagnosis could not be made by using ultrasonography alone (D'Anjou and Penninck, 2015).

Renal US abnormalities (Table 4) were classified mainly in non-incidental findings with mild to moderate clinical relevance (35/84) or with important clinical relevance (35/84), and this last class (R3) was the one the observers classified more accurately, probably because it is easier to detect more evident US abnormalities. It was not assessed separated by species in our study, but it has been reported that kidney US imaging in cats with unilateral ureteral calculi suggested that preexisting renal parenchymal disease was common in cats with ureterolithiasis (Kyles *et al.*, 2005) and with ureteral obstruction (Lamb, Cortellini and Halfacree, 2018). In one study, 39% of patients with ureteral obstruction had US signs compatible with pre-existing chronic nephropathy, including irregular kidney shape, cortical scars and reduced kidney size (Lamb, Cortellini and Halfacree, 2018).

In our study the mainly causes of renal and ureteral obstruction were lithiasis (4/11) and extrinsic masses (4/11), and all cases of lithiasis causing obstruction were associated with hydronephrosis, as represented in Figure 4. Sensitivity of detecting hydronephrosis (Table 5) was excellent and higher than described to men in medicine (98%) (Ellenbogen *et al.*, 1978). In agreement with our study, lithiasis is also a common cause of renal and ureteral

obstructions in both dogs (Widmer, Mattoon and Vaden, 2021) and cats (Kyles *et al.*, 2005), generally resulting in unilateral hydronephrosis (Widmer, Mattoon and Vaden, 2021), as it happened, all four patients with lithiasis had concomitant hydronephrosis. Less common causes of hydronephrosis include blood clots, fibroepithelial polyps, or extrinsic masses (Widmer, Mattoon and Vaden, 2021), however extrinsic masses, represented in Figure 5, also were responsible for 4/9 cases of hydronephrosis (44.44%) in our study.

Moderate interobserver agreement (McHugh, 2012) was observed for identification of renal and ureteral obstruction for all animals (0.73), dogs (0.75) and cats (0.67) (Table 6), in spite of the differences in observers' experience. Nevertheless, a higher interobserver agreement with excellent reproducibility ($k_v > 0.75$) (Regan *et al.*, 2005) would be more desirable with the potential surgical decision in hand. There was a rather strong interobserver agreement for detecting hydronephrosis in small animals in general (0.83) and dogs (0.88), but it was only moderate for cats (0.67).

There were a number of limitations to the present study, mainly the use of retrospective data and lack of standardization of the US images sent might have influenced the interpretation. The difference between the number of patients in the groups and of different species could also have affected the results, such as sensitivity for feline renal and ureteral obstruction and hydronephrosis. All observers were aware that their response to each case had no clinical consequence to the patients, so the laboratory vs. field bias is inherent this study due to its design. Lastly, there was an important difference in training between the observers and between the radiologists that acquired the images.

2.6 CONCLUSION

In summary, this study suggests that tele-ultrasonography has a good accuracy in small animals with non-specific signs and clinical suspicion of renal and ureteral obstruction. However, its interobserver agreement were moderate and its positive predictive values were low, it is hence questionable its utility in

this clinical context, especially given the potential surgical decision to be made. The reasons for these observations might be the insufficient amount of imaging material provided in some cases and lack of standardization of ultrasonographic images due to the retrospective nature of the data. Prospective studies are recommended to assess the impact of a standardized protocol for ultrasonographic image acquisition and the influence of synchronous tele-ultrasonography on the accuracy for abdominal diseases.

2.7 ACKNOWLEDGEMENTS

The authors thank Camila Marinelli Martins for statistical consultation.

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3. FINAL CONSIDERATIONS

Abdominal ultrasonographic examinations are commonly used for evaluation of gastrointestinal and urinary tract of small animals. With the advances in veterinary medicine and technology, tele-ultrasonography has become commercially available and viable, and it has a number of advantages, however, few papers have been published yet that approaches its use in veterinary practice.

So, we presented two studies simulating a tele-ultrasonography consultation for evaluation of gastrointestinal, and renal and ureteral obstruction in dogs and cats in radiologists with different experience levels and we assessed the accuracy and interobserver agreement of tele-ultrasonography. These studies may be useful for both small animal clinicians and diagnostic imaging veterinaries when considering the implementation of this tool in clinical veterinary practice.

Overall, we suggest that TUS is a feasible modality in small animals due to its good accuracies for both detection of renal and ureteral obstructive processes and for gastrointestinal obstruction in dogs and cats, however it presented a low positive predictive value and only moderate inter-observer agreement. Despite its good accuracies, given the potential surgical decision on hand, this technique should be used with caution in these clinical contexts, knowing its benefits and possible limitations. Further prospective studies are recommended to assess the influence of standardized techniques for ultrasonographic image acquisition and the impact of synchronous TUS on the diagnostic accuracy for diagnosing small animal diseases.

APPENDIX



UNIVERSIDADE FEDERAL DO PARANÁ
SETOR DE CIÊNCIAS AGRÁRIAS
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o protocolo número 049/2020, referente ao projeto de pesquisa “Confiabilidade da teleultrassonografia para o diagnóstico de obstrução gastrointestinal em cães e gatos”, sob a responsabilidade de Tilde Rodrigues Froes – que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de Outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) DO SETOR DE CIÊNCIAS AGRÁRIAS DA UNIVERSIDADE FEDERAL DO PARANÁ - BRASIL, com grau 2 de invasividade, em 06/11/2020.

Finalidade	Pesquisa
Vigência da autorização	Novembro/2020 até Fevereiro/2022
Espécie/Linhagem	<i>Canis lupus familiaris</i> e <i>Felis catus</i> (canino e felino)
Número de animais	60 cães e 40 gatos
Peso/Idade	Variável/Variável
Sexo	Macho e fêmea
Origem	Sob tutela.

*A autorização para início da pesquisa se torna válida a partir da data de emissão deste certificado.

CERTIFICATE

We certify that the protocol number 049/2020, regarding the research project “Reliability of teleultrasonography for diagnosis of gastrointestinal obstruction in dogs and cats” under Tilde Rodrigues Froes – which includes the production, maintenance and/or utilization of animals from Chordata phylum, Vertebrata subphylum (except Humans), for scientific or teaching purposes – is in accordance with the precepts of Law nº 11.794, of 8 October 2008, of Decree nº 6.899, of 15 July 2009, and with the edited rules from Conselho Nacional de Controle da Experimentação Animal (CONCEA), and it was approved by the ANIMAL USE ETHICS COMMITTEE OF THE AGRICULTURAL SCIENCES CAMPUS OF THE UNIVERSIDADE FEDERAL DO PARANÁ (Federal University of Paraná, Brazil), with degree 2 of invasiveness, in session of 11/06/2020.

Purpose	Research
Validity	November/2020 until February/2022
Species/Line	<i>Canis lupus familiaris</i> e <i>Felis catus</i> (canine e feline)
Number of animals	60 dogs and 40 cats
Weight/Age	Variable/Variable
Sex	Male and female
Origin	Under guardianship.

*The authorization to start the research becomes valid from the date of issue of this certificate.

Curitiba, 11 de novembro de 2020

Simone Tostes de Oliveira Stedile
Coordenadora CEUA-SCA



UNIVERSIDADE FEDERAL DO PARANÁ
SETOR DE CIÊNCIAS AGRÁRIAS
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

OFÍCIO Nº 001/2021

Para: Tilde Rodrigues Froes

Assunto: Protocolo 049/2020

Prezado(a) pesquisador(a),

Após avaliação sobre o pedido de aumento do número de animais a serem utilizados no projeto/aula sob sua responsabilidade, de protocolo número 049/2020 intitulado “**Confiabilidade da teleultrassonografia para o diagnóstico de obstrução gastrointestinal em cães e gatos**”, a Comissão de Ética no Uso de Animais do Setor de Ciências Agrárias – UFPR entendeu que:

1. Tendo em vista que a origem dos laudos a serem acrescidos foi devidamente esclarecida, aprova-se a alteração de 60 cães (30 machos e 30 fêmeas) para 75 cães (41 machos e 34 fêmeas).

Curitiba, 26 de janeiro de 2021.

Atenciosamente,

Simone Tostes de Oliveira Stedile
Coordenadora da Comissão de Ética
no Uso de Animais
SCA - UFPR



UNIVERSIDADE FEDERAL DO PARANÁ
SETOR DE CIÊNCIAS AGRÁRIAS
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o protocolo número 010/2021, referente ao projeto de pesquisa “Confiabilidade da teleultrassonografia par ao diagnóstico de obstrução renal e ureteral em cães e gatos”, sob a responsabilidade de Tilde Rodrigues Froes – que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de Outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) DO SETOR DE CIÊNCIAS AGRÁRIAS DA UNIVERSIDADE FEDERAL DO PARANÁ - BRASIL, com grau 1 de invasividade, em 06/04/2021.

Finalidade	Pesquisa
Vigência da autorização	Abril/2021 até Fevereiro/2022
Espécie/Linhagem	<i>Canis lupus familiaris</i> (canino)/ <i>Felis catus</i> (felino)
Número de animais	180
Peso/Idade	Variável
Sexo	Machos e fêmeas
Origem	Sob tutela

*A autorização para início da pesquisa se torna válida a partir da data de emissão deste certificado.

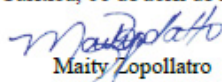
CERTIFICATE

We certify that the protocol number 010/2021, regarding the research project “Reliability of teleultrasonography for diagnosis of renal and ureteral obstruction in dogs and cats” under Tilde Rodrigues Froes – which includes the production, maintenance and/or utilization of animals from Chordata phylum, Vertebrata subphylum (except Humans), for scientific or teaching purposes – is in accordance with the precepts of Law nº 11.794, of 8 October 2008, of Decree nº 6.899, of 15 July 2009, and with the edited rules from Conselho Nacional de Controle da Experimentação Animal (CONCEA), and it was approved by the ANIMAL USE ETHICS COMMITTEE OF THE AGRICULTURAL SCIENCES CAMPUS OF THE UNIVERSIDADE FEDERAL DO PARANÁ (Federal University of Paraná, Brazil), with degree 1 of invasiveness, on 2021, April 6th.

Purpose	Research
Validity	April/2021 until February/2022
Specie/Line	<i>Canis lupus familiaris</i> (canine)/ <i>Felis catus</i> (feline)
Number of animals	180
Weight/Age	Various
Sex	Male/Female
Origin	Under guardianship

*The authorization to start the research becomes valid from the date of issue of this certificate.

Curitiba, 06 de abril de 2021


Maity Zopollatro

Coordenadora pro-tempore
CEUA/AG/UFPR

Submission Confirmation



Thank you for your submission

Submitted to

Journal of Small Animal Practice

Manuscript ID

JSAP-2022-0089

Title

Accuracy and reliability of tele-ultrasonography in detecting gastrointestinal obstruction in dogs and cats

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