SUMMARY

of the doctoral thesis entitled:

RESEARCH OF OPTIMIZATION OF DIAGNOSIS AND THERAPEUTIC MANAGEMENT IN GALLBLADDER DISEASES IN DOGS

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The PhD thesis, entitled ***Research on optimization of diagnosis and therapeutic management of gallbladder diseases in dogs***, is structured in accordance with current provisions, and thus comprises two main parts:

-Part I corresponds to the bibliographical study associated with it and comprises 36 pages, representing 17.91% of the thesis volume.

-Part II presents the own research and comprises 159 pages, representing 82.09% of the thesis.

The research activity took place between 2016 and 2022 in the clinic Cabinet Veterinary Dr. Purice Adriana, in Rm. Valcea

Cholecystitis-associated diseases are increasing in incidence and account for a significant proportion of the caseload faced by small animal clinicians. The multitude of pathological processes that can be identified in the cholecyst, the variety of clinical signs associated with them, the complexity of the decision tree involved in making a diagnosis, the polymorphism of the paraclinical picture specific to these conditions, and the implications of the related medicinal and nutritional management on the homeostasis of the body require a detailed knowledge of this subject and oblige clinicians to a thorough study of the process.

Chapter I of the first part of the thesis focuses on the presentation of specific bibliographical data on the morphophysiological features of the gallbladder, by presenting features related to the anatomy and physiology of the gallbladder.

Chapter II of the first part of the bibliographical study of the thesis presents peculiarities of anatomical and morphofunctional disorders of the biliary system, together with the diagnostic protocols and current therapeutic options associated with it. The chapter presents the clinical and paraclinical picture related to morbid conditions, their characteristic anatomopathological changes and current

bibliographical data related to the drug, surgical and nutritional treatment associated with these pathological conditions.

Chapter III of the introductory part of this work deals with anatomopathological and pathophysiological features, together with clinical and paraclinical aspects encountered in a series of morphofunctional disorders of the cholecystis less frequently diagnosed in canids. The incidence, diagnosis and therapeutic protocols of these disorders are described.

Chapter IV of the literature review part of this thesis presents infrequently diagnosed disorders, characteristic morphofunctional aspects and a literature review on diagnostic or therapeutic peculiarities encountered in their case.

The study of gallbladder disease involves detailed knowledge of the anatomical and histological features of the organ, its functions and relationships with adjacent structures, the pathological processes encountered in the gallbladder, the diagnostic protocols available, and the appropriate therapeutic management of identified morbid conditions. The assessment of the co-morbidities encountered, the constants relating to the characteristics of the populations of individuals identified with this type of disease, together with the specific clinical and paraclinical picture and the statistical and dynamic assessment of its variation following the administration of the therapeutic protocols are of interest to clinicians involved in the treatment of small animal diseases. The detailed study of these pathological processes can provide valuable information in the early identification of gallbladder disease, in the increased efficiency of diagnostic and therapeutic protocols, in the optimisation of available resources, in the minimisation of treatment duration and associated costs, but above all, in the limitation of the intensity of the symptomatology associated with the pathology of the gallbladder and the discomfort experienced by patients and their carers.

Part II of the PhD thesis, entitled "**Personal Research**", constitutes about 80% of the total thesis and is organized in 4 chapters, general conclusions and bibliography. The results of this work are exemplified through tables, graphs and figures.

Cholecystic disorders represent a vast research topic and require a multidisciplinary and correlatively analytical approach to be addressed in their complexity. The increased prevalence of these morbid processes and the high intensity with which they sometimes manifest themselves require clinicians to study the subject in detail.

The research was carried out on a group of patients selected following a standardised clinical and paraclinical screening which allowed the identification and assessment of the alterations in clinical status and morphological and physiological constants, the evaluation of the intensity of the disease by analytically assessing the associated haematological, biochemical and ultrasonographic paraclinical picture and the staging of the related diseases. This protocol favoured the formulation of a diagnosis and the monitoring of the individually optimised therapeutic protocol.

The analysis of the results obtained by the above-mentioned paraclinical investigation methods also included their statistical processing, giving a higher degree of objectivity and confidence to the conclusions drawn from these investigations.

The first chapter of Part II, "***Research on the prevalence of cholecystitis in dogs***", outlined the clinical diagnostic process of the canid population included in the study. This involved the interpretation of anamnestic data from owners, the assessment of clinical constants of the patients presented for evaluation and their associated epidemiological data. Corroboration of these data led to the formulation of a diagnosis of suspicion and subsequently allowed the recommendation of paraclinical diagnostic methods to test it. The haematological, biochemical and ultrasound variations identified in this study allowed the formulation of a final diagnosis and the inclusion of 186 patients in the cholecystitis prelapse study.

Characterizing the population of canids diagnosed with cholecystitis and analyzing the particularities that establish the coordinates of the groups of sex, race, age and hormonal status of the evaluated individuals was one of the main objectives of this study and of this PhD thesis.

The research carried out during the doctoral internship included a total of 612 canine patients with specific symptoms of the digestive system, among which 186 (30.14%) individuals were identified with cholecystitis.

Integrative analysis of the results obtained allowed the identification of the increased prevalence of this condition among females, in the proportion of 62.90% (n=117). Cholecystitis was predominantly found in the case of the half-breed patients, 22.58% (n=42), then among the Bichon Maltese breed patients 18.27% (n=34) and Yorkshire Terrier 14.51% (n=27).

In the survey, the age category identified as most affected was patients aged 6-10 years, in a proportion of 43.01% (n=80). From the point of view of the characterisation of the population in terms of its hormonal status, the condition could be reported predominantly in unsterilised females, in 67.52% (n=79) of the individuals included in this research.

The data obtained from the anamnestic analysis indicate an acute type of symptomatology, with high intensity, reported by the owners in the majority of 58.06% (n=108) of the patients, the remaining 41.93% (n=79) of them showing symptoms of chronic type, with a lower intensity and frequency than the first group.

In the research on the prevention of cholecystitis in canidae, the frequency of vomiting episodes was notable, this being considered the main symptom manifested by the individuals included in the research. 76.34% (n=142) of them had one or more vomiting episodes. The majority of patients presented following two or more episodes of emesis within 3 days, 50.70% (n=72) of them, 32.39% (n=46) of them presented following 3 or more episodes of vomiting within a week, and 16.90% (n=24) of patients presented following a single episode.

Interpretation of the data on the symptoms of those included in the research allowed the identification of the second most prevalent symptom as apathy, manifested by 128 patients (68.81%), followed by capricious appetite, among 73 of them (39.24%). 43.54% (n=81) of the canids included in this research did not tolerate being palpated in the abdominal area or frequently changed their position as a result of abdominal discomfort. Last but not least, an important symptom manifested by patients diagnosed with cholecystitis was febrile episodes or subfebrility encountered in 51 (n=41%) of the canids, marking an important systemic response of the body regarding general clinical impairment.

The diagnostic algorithm applied among 64 patients included in this research revealed that the biochemically and haematologically results of 32.81% (n=21) of the patients showed changes of high severity, highlighting the systemic impact on the canine organism. The main variations were inflammatory leukogram in 22% (n=14) of the patients and hyperbilirubinemia and elevated liver transaminases in 9.35% (n=6) and 15.62% (n=10) of the cases analyzed, respectively.

Abdominal ultrasound is a minimally invasive diagnostic technique with high accuracy and sensitivity that can be performed easily, without major inconvenience to the patient, owner and clinician. It is highly relevant in the diagnosis of gastrointestinal disorders and is beneficial in identifying mucosal variations, intraluminal content, position and shape of the cholecyst, having a high significance in establishing the diagnosis and the evolution of the therapeutic protocol indicated for patients.

In this study, a standardized ultrasonographic evaluation of 22 patients was performed, and the processing and centralization of the results obtained by this type of paraclinical imaging investigation allowed the evaluation of the integrity and changes reported at the levels of the gallbladder mucosa. The characterization of its appearance alternated from mucosa with variable hyperrepresentation, ranging in size from 1-4.6mm in 65.51% (n=18), to biliary mucosa with uniform appearance in 48.27% (n=14) and with non-uniform appearance in 13.79% (n=4). The presence of intravesical sediment, with variable size between 0.2-0.87cm, was reported in 37.93% (n=11) of cases.

The analysis of intraluminal collections identified following ultrasonographic examination allowed to highlight the sediments identified as being able to unblock the gallbladder, as having variable structure and as being hyperechogenic in relation to the anechoic, transsonic content specific to the cholecyst. Also, 10.34% (n=3) of the patients presented intravesical masses of variable size of 1-5mm generating acoustic shadow phenomenon and inducing additional gallbladder distention, giving it a hypotonic character. In 10.34% (n=3) of cases, the presence of biliary sediment was also reported.

Following the centralization of all the data obtained from the anamnestic examination, clinical examination and paraclinical investigations, the presence of acute cholecystitis could be assessed in 58.06% (n=108) of the cases, accompanied by

cholestasis in 43.51% (n=47) of the cases, microlititis in 29.35% (n=32), comorbidities in 20.37% (n=22) or caused by trauma 6. 48% (n=7). 41.93% (n=78) of cases investigated had a chronic presentation of the condition, accompanied by cholestasis in 29.48% (n=23) and bladder lithiasis in 12.82% (n=10). A number of comorbidities evolving concomitantly with chronic cholecystitis were identified in 27 patients (34.61%), microlithiasis in 9 of them (11.53%) and biliary mucoceles in 6.41% (n=5) among investigated patients.

Chronic cholecystitis was also identified as a consequence of extrahepatic obstruction during pathological episodes in patients with bile duct dysfunction in 5.12% (n=4) of cases.

In Chapter VI, "***Research on the therapeutic effects of krill oil in hyperlipidemia in dogs***" a study group of 87 patients, previously diagnosed with hypertriglyceridaemia and hypercholesterolaemia, whose owners were informed in advance about the pathological implications of the condition, the available therapeutic means, the duration of treatment and the recommended doses, is presented. Correction of dyslipidaemic status is an important issue for clinicians who are faced with its consequences for their patients. The increasing frequency of this type of condition, coupled with the current context of pet life, requires a proper assessment of all therapeutic options used to combat or limit the negative effects associated with it. The curative approach to them by taking Krill oil, as a sustainable source of Omega 3 polyunsaturated fatty acids rich in eicosapentaenoic and docosahexaenoic acids or alternatively, Salmon oil as an alternative natural source of polyunsaturated fatty acids, is one of these recommended healthy options for improving dyslipidaemic status. Assessing the efficacy of these therapeutic protocols is the main objective of this chapter.

Following the centralization of the results obtained in this research and their subsequent statistical processing using the two-tailed Student's t-test, an objective and highly reliable method of assessment, it was concluded that the administration of Krill oil in therapeutically recommended doses has a significant influence on circulating triglyceride and cholesterol levels and is an effective adjuvant nutraceutical in the treatment of diabetic conditions, cholestasis, Cushing's, hypothyroidism, obesity and pancreatitis for patients with a body score (WSAVA scale) less than 5 and greater than 5 who have been given the medication for 80 days at the minimum recommended doses. Comparing the mean levels of the parameters analysed, the efficacy of salmon oil administered over the same period of time is lower in terms of lowering the mean triglyceride levels of dyslipidaemic patients with body score greater than 5, but still considered statistically significant in lowering cholesterol for both categories of patients.

Another partial conclusion that can be drawn from this research is that the use of the maximum doses recommended by the manufacturers in the adjuvant treatment of dyslipidemia has no significant limiting capacity, and in fact resulted in a 1.61%

smaller change in cholesterol lowering compared to the first study group and a 4.72% decrease in triglyceride lowering for patients with a body score greater than 5.

The results obtained from the concentrated and integrated analysis of the cases included in this research recommend the increased efficacy of Krill oil in the management of conditions such as diabetes, cholestasis, hypothyroidism, Cushing's, pancreatitis or hepatopathies.

The third chapter of the second part of the PhD thesis, entitled "***Research on the efficacy of urseodeoxycholic acid administration in the treatment of cholestasis associated with hyperlipidaemic diseases in canines***", addresses the efficacy of therapeutic protocols that constitute the treatment of cholestasis reported in canine patients with hyperlipidaemic conditions. The increasing frequency of this type of pathological condition, correlated with the increasing sedentarism of canine individuals as their lifestyle harmonizes with that of their owners, with the personification of pets and the consequent modification of their diet, requires the development of therapeutic protocols that can limit the physiological repercussions that this type of condition entails. The use of effective curative means, involving the shortest possible duration of treatment and the easiest possible route of administration, is an extremely important objective for patients, their carers and clinicians. The use of ursodeoxycholic acid may be salutary because of its specific mechanism of action stimulating biliary secretion, its protective effects on biliary mucosal epithelium, its inhibition of hepatocyte apoptosis and its characteristic detoxification, and its efficacy can be proven using highly reliable statistical means.

In this study, monitoring the clinical and paraclinical evolution of biochemical parameters of 70 patients allowed an objective analysis of the effects of this type of standardised treatment.

The characterisation of the population included in this research in terms of breed-related coordinates reveals that Bichon Maltese individuals are over-represented among the dyslipidaemic patients included in the study, in the proportion of 47.14% (n=33).

Analysis of the age categories of the individuals present in this research indicates that patients aged 6-10 years constituted 60% (n=42) of the group included in this study and represented the majority population among those identified with this pathology.

The increased prevalence of dyslipidemic status was identified, especially among females, in a proportion of 52.85% (n=37) and in a higher proportion of 67.14% (n=47), of unsterilized patients.

The drug management administered to these individuals was applied in accordance with the symptoms manifested by the patients included in this research, dominated by episodes of emesis, expressed acutely in 91.48% (n=43) and chronically in 60.86% (n=14), followed then by the presence of stools with altered consistency in 31.91% (n=15) and forms of inappetence in 87.23% (n=41) of the cases with acute

presentation, respectively inappetence in 82.60% (n=19) and weight loss in 73.91% (n=17) of the patients with chronic presentation.

Correlative and integrated analysis of the paraclinical results of the biochemical and ultrasonographic tests recommended at the beginning of the treatment period revealed that the most dramatic variation was found among serum transaminases in 75% (n=9) of the cases. Following standardized ultrasonographic assessment, distention by content of the cholecyst was identified in 91.66% (n=11) of the 12 patients investigated sonographically, followed by hyperrepresentation of the parietal mucosa of the cholecyst, with dimensions varying between 2-4.76mm in 75% of cases (n=9). In 16.66% (n=2) of situations, focal mucosal reaction was identified. The biliary sediment spotted had dense, hyperechogenic echostructure in relation to the gallbladder lumen and its anechoic , transonic physiological content. The dimensions of this type of sediment varied between 0.4-3.42 cm, without the posterior shadowing phenomenon typical of mineral or gaseous collections.

The assessment of the effects of administering the therapeutic protocol over a fixed period of time with variable doses was assessed using the Student's t-test, an objective analytical method with a high confidence level. Centralisation of the results obtained from this research allowed the assessment of the efficacy of the therapeutic protocol indicated for the patients included in this research by identifying statistical variations in the biochemical paraclinical values associated with the patients investigated. Following the application of the 2-tailed Student's t-test for the biochemical parameters aspartate aminotransferase, alanine aminotransferase, total bilirubin and alkaline phosphatase, it is possible to reject the initially formulated null hypothesis and accept the alternative hypothesis that the administration of ursodeoxycholic acid leads to a significant improvement of their values in patients with cholestasis. In the case of gammaglutamyltransferase, this hypothesis cannot be rejected.

Centralizing all the results obtained in this research, it can be concluded that the administration of the treatment scheme had salutary effects on the patients investigated, leading to statistically significant improvement in the biochemical parameters analyzed and associated clinical signs, thus representing a valuable therapeutic option for the adjuvant treatment of dyslipidemias.

In Chapter VII, "***Therapeutic non-surgical management of gallbladder mucocele in canides*** ", 7 canines, diagnosed with mucoceles following clinical and paraclinical investigations, were managed medically for its dissolution, according to the limitations imposed by the current clinical condition or the wishes of their caregivers.

Integrative and comparative analysis of the data obtained in this study reveals that higher incidence of the condition was identified in females in 57, 14% (n=4) and in sterilized animals in 71.42% (n=5). A positive correlation of 42.85% (n=3) between the presence of mucoceles and hypothyroidism was also noted.

Participants' gastrointestinal distress was amended by exclusively administering Hill's Id Low Fat and Royal Canin Gastrointestinal Low Fat, and the switch to these diets was well tolerated by all study participants.

Therapeutic management involved the amendment of various degrees of emesis, episodes of inappetence and episodes of diarrhoea manifested by the patients by using central antivomiting medication such as maropitant (Cerenia) in a dose of 1mg/kg/24hr and drotaverine (No-spa) in a dose of 2mg/kg/12/24hr, respectively, proton pump inhibitors such as omeprazole (Omeprazole-Richter) at a dose of 10mg/kg/12hr/5days, mucosal protectants such as sucralfate (Venter) at a dose of 1mg/kg/day and H2-receptor antagonists such as famotidine (Famotidine) at a dose of 1g/kg/24 hr.

Optimisation of therapeutic management also involved the inclusion in the therapeutic protocol of levothyroxine (Thyroxsanil) at a dose of 0. 02mg/kg/12hr for 4 weeks to correct endocrine imbalance, ursodeoxycholic acid (Ursofalk) at a dose of 20mg/kg/24hr for min 4 weeks to solubilise bile and Krill Oil at a dose of 1000mg/10kg/24hr for a minimum of 4 weeks as an adjuvant to regulate their dyslipidaemic status.

Febrile episodes required the use of amoxicillin with clavulanic acid at a dose of 12.5mg/kg/12h for 15 days, depending on the therapeutic response, in cases where patients manifested this symptom.

Centralization of the data obtained by processing the results of this study showed that the response to the recommended therapeutic package was favourable, with the digestive forms improving and the parameters evaluated normalizing. Liver transaminases decreased in 85.71% (n=6) of patients, cholesterol and triglycerides in 71.42% (n=5). Improvement of haematological parameters was found in all investigated cases. Also, dynamic ultrasound evaluation of gallbladder contents showed dissolution and considerable reduction of its echogenicity.

The synthetic study of the data collected in this research following the association of the results obtained from clinical and paraclinical examinations allowed the outline of a genuine therapeutic protocol of some pathological processes specific to the gallbladder, such as cholestasis or mucoceles, in order to optimize the therapeutic management associated with these conditions

The sum of the research included in the studies completed completes the knowledge and analysis of the gallbladder diseases in canines, by studying the incidence and prevalence of the affected individuals, by deepening their clinical and paraclinical consequences and by the statistical assessment of the therapeutic protocols usually associated with them.

Chapter VII includes 149 bibliographical sources cited in the text.