SUMMARY
STUDY ON OPTIMIZING THE CLINICAL-DIAGNOSTIC AND THERAPEUTIC APPROACH IN MALDIGESTION AND MALABSORPTION SYNDROMES

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The doctoral thesis, entitled Study on the optimization of the clinical-diagnostic and therapeutic approach in maldigestion and malabsorption syndromes in dogs, is structured, in accordance with the current legislative provisions, in two parts.

Part I, Bibliographic Study, presents a number of 48 pages, equivalent to 31.3% of the total volume of the thesis.

Part II, Own research, comprises 105 pages, which represents 68.6% of the volume of the thesis.

The research activity was carried out at Vet Consulting SRL and Canivet veterinary clinics in Bucharest, as well as at the Emergency University Veterinary Hospital "Prof. Univ. Dr. Alin Bîrţoiu", Bucharest, for a period of 18 months (August 2021 - February 2023).

In dogs, gastrointestinal diseases, translated, in particular, by chronic diarrhea, are a very frequent cause of presentation to the veterinarian.

Thus, in malabsorption syndrome, chronic diarrhea usually originates in the small intestine and may be accompanied by chronic vomiting, which frequently occurs on an empty stomach, which is why patients usually lose weight despite food intake grown. Chronic, intermittent episodes of abdominal pain may also occur, during which the animal adopts antalgic, uncomfortable postures.

In this context, in the syndrome of maldigestion and malabsorption, there is damage to the gastrointestinal mucosa, which leads to defects in the transport mechanisms (from the level of the intestinal membrane, to the blood or lymphatic vessels), conditions that cause a decrease in gastrointestinal motility (decreased peristalsis and appearance of stasis), disruption of the bacterial flora translated by an increase in the bacterial population in the small intestine (in particular, E. coli and Clostridium spp.) as well as intraluminal osmotic changes, which can ultimately determine , the occurrence of diarrhea.

If chronic diarrhea is accompanied by hypoalbuminemia, most often combined with hypoglobulinemia, and sometimes even hypocobalaminemia, the so-called enteropathies with protein loss are recorded, which appear following the change in the permeability of the intestinal mucosa.

In this context, knowledge of the mechanisms that lead to a defective digestion but also to poor absorption and assimilation, along with the identification of nutritional symptoms and consequences, are very important for the clinician, in order

to establish a definite diagnosis and subsequently, to ensure a therapeutic management and proper diet.

Part I of this doctoral thesis is synthetically structured in three chapters.

Chapter I, from the first part, entitled "*Bibliographic study on the interrelationship between nutrition, digestion and absorption*", includes an extensive study on the main physiological mechanisms regarding intestinal digestion and absorption. Thus, the most important data from the specialized literature are presented, regarding the particularities of digestion in dogs, but also the absorption of the main nutrients, such as proteins, carbohydrates, lipids, but also the absorption of some ions (sodium, potassium).

Chapter II, of the first part, of this doctoral thesis, entitled "*Clinical dominants in the main diseases associated with maldigestion-malabsorption syndrome*", describes a series of diseases that are based on interference between the mechanisms responsible for the enzymatic breakdown of nutrients (digestion) and absorption of degraded compounds. Thus, data are presented from the specialized literature, regarding the most important causes of maldigestion (enzyme deficiency, biliary deficiency, primary enteropathies, cholecysto-cholangiopathies), but also the main physiopathological mechanisms of the malabsorption syndrome.

In *Chapter III*, from the first part, entitled *"Bibliographic data regarding the diagnosis and therapeutic approach in maldigestion and malabsorption syndrome*", the most important data from the specialized literature regarding the main diagnostic methods used, such as the description of the different functional tests used, are presented as well as the presentation of a thorough hematological and biochemical screening, as an integrated part of the process of detecting organic and systemic pathologies. In the same chapter, general aspects regarding dietary and therapeutic management in maldigestion and malabsorption syndrome in dogs are also presented.

Part II, generically entitled "*Personal research*", is structured in three chapters, general conclusions and bibliography. The results of the studies were presented, synthetically, in 45 tables, 16 figures and 18 graphs. The present thesis also includes a total number of 239 bibliographic references, cited in the text.

The purpose of this paper is to present in detail the pathophysiological mechanisms that trigger the processes of maldigestion and/or malabsorption, as well as the clinical consequences (including both the specific symptomatology, the variations of some metabolic parameters, and other changes detected following the performance of complementary examinations), in order to establish a definite diagnosis and a correct therapeutic approach, especially due to the fact that, in maldigestion-malabsorption syndrome, diet therapy doubles enzyme therapy.

Chapter IV, an integrated part of the second part of this thesis, is entitled "*Study on the optimization of the clinical-diagnostic and therapeutic approach in enteropathies with protein loss*". The 22 canine patients with malabsorption syndrome (of different breeds and ages), included in this study, were selected from a total of 42 dogs with chronic diarrhea, following copro-parizotological examinations - to exclude parasitosis gastrointestinal, hematological and biochemical examinations (in particular, for liver and kidney parameters) - to exclude possible metabolic changes, and fecal chymotrypsin was also measured - to exclude from this study, patients with exocrine pancreatic insufficiency.

After performing the biochemical examination, we found that some of the patients with malabsorption syndrome had hypoproteinemia, while others did not. Thus, the 22 clinical cases with malabsorption syndrome selected for this study were divided into two groups based on the presence or absence of hypoproteinemia (serum protein level, below 4.5 g/dL) and the recommended immunosuppressive treatment.

By synthetically analyzing the clinical manifestations of the selected patients, it can be found that, regardless of whether or not they had a normal level of proteinemia, they showed, in general, all the symptoms specific to this syndrome (chronic diarrhea, weight loss, vomiting, increased appetite, amount large amount of faeces, light in color, the presence of undigested food in the faeces), but with varying intensities, from patient to patient.

Patients with malabsorption syndrome and who presented hypoproteinemia (3.8±0.3 g/dL) and hypo-albuminemia, (1.8±0.2 g/dl), recorded statistically significant differences ($p < 0.05$), compared to the mean values of patients in the dog group who had a normal protein level (6.1±0.3 g/dL and 2.9±0.07 g/dL, respectively). In patients with malabsorption syndrome, protein loss is usually the result of both albumin and globulin loss, and because of this, hypoalbuminemia, and more so hypoproteinemia, results in a decrease in oncotic pressure that can lead to their extravasation fluid in the body's cavities, which may subsequently cause ascites and/or peripheral edema.

Also, in the patients selected for this study, we also dosed cobalamin because in patients with digestive disorders, the absorption of this vitamin in the small intestine is reduced, which can ultimately lead to the appearance of cobalamin deficiency. Following the ultrasound examination in these patients, an obvious and uniform thickening of the intestinal mucosa with moderately increased echogenicity was observed (n=16/22). In dogs with hypoproteinemia, respectively, hypoalbuminemia (n=7/12), pleural fluid and ascites are observed, hypoechoic striations are present, perpendicular to the mucous layer, parietal edema between 4.4-11.3 mm.

The therapeutic management of canine patients with malabsorption syndrome, but who had a normal protein level, consisted of the administration of *prednisone*-based immunosuppressive treatment, and the patients who presented with hypoproteinemia received a combined immunosuppressive treatment of *prednisone, azathioprine and metronidazole*. It is worth noting that all the dogs selected for this study followed a dietary treatment composed of easily digestible carbohydrates and proteins, with a low fat and fiber content, oligosaccharides and a ratio of omega-3:omega-6 fatty acids between 1:5 and 1:10 (formulas found in the diets of certain specialized companies, which are found on the commercial market).

10 days after the start of the treatment, the patients were called for control, and it was observed that the majority (with some exceptions of some more serious forms), presented normal stools, of a darker color, and during the immunosuppressive treatment (subsequent control periods occurred 30 and 90 days after starting treatment), no further cases of diarrhea were reported.

Following the immunosuppressive and dietary treatment, the clinical signs improved a lot, due to the reduction of the osmotic pressure in the intestinal lumen, and the recovery of serum protein levels was also observed, following not only the restoration of intestinal absorption, but also the normalization mucosal permeability, affected, as a result of this chronic inflammatory condition.

In *chapter V,* entitled "*Research on the clinical and therapeutic approach in exocrine pancreatic insufficiency in dogs*", the study group was formed after measuring the pancreatic secretion capacity of the patients, using pancreatic functional tests (canine Trypsin-like immunoreactivity - cTLI, and pancreatic lipase canine - cPL), correlated with clinical signs. Canine patients presenting cTLI values <5, and >200 µg/L were included in this group. In these patients, maldigestion and malabsorption of nutrients, followed by diarrhea, are the main functional insults responsible for severe progressive emaciation (ie, decreased weight gain in youth), along with the development of consecutive vitamin-mineral deficiencies.

The main clinical sign in patients with IPE was diarrhea, which had a frequency that varied from case to case. Just over half of the patients with exocrine pancreatic insufficiency (51%) who presented defecated between 3-8 times/day.

In the canine patients included in the study, we performed hematological and biochemical examinations, focusing in particular on the monitoring of liver biochemical parameters, in order to detect the effects of this disease (which causes maldigestion and, secondarily, malabsorption of nutrients), on liver integrity. Thus, the activity of the three liver biochemical parameters, ALT (alanine aminotransferase), ALP (alkaline phosphatase) and AST (aspartate aminotransferase), was significantly higher, compared to the group of healthy patients. Also, in patients with exocrine pancreatic insufficiency, the mean value of cobalamin was distinctly significantly ($p<0.01$) lower (244.5±19 ng/l) compared to that of clinically healthy patients, which means that absorption of this vitamin is deficient in patients with digestive disorders (followed by malabsorption), necessitating, for this reason, oral or parenteral supplementation of cobalamin.

Ultrasound examination of the intestine and pancreas in the case of these patients is very important, because through this non-invasive technique, it is possible to observe the parietal reactions of the small intestine, but also the decrease of the pancreatic parenchyma, pancreatic atrophy (usually partial), pancreatic sclerosis or chronic pancreatitis.

The therapeutic management of patients with exocrine pancreatic insufficiency is directed, mainly, to the replacement and supplementation of the enzyme deficiency, but also to the supplementation with cobalamin, of course, along with the therapeutic management of concurrent conditions. Thus, the enzyme deficiency was corrected daily, with pancreatic enzyme supplements (in powder form), being a treatment that is usually administered throughout life (lifelong treatment), in a dose of approximately one teaspoon/10 kg, mixed with food, simultaneously with the administration of a diet, low in fat, with a high degree of digestibility. After 7 days of drug treatment, associated with the administration of a suitable diet, there was an improvement in clinical signs, a decrease in flatulence, burborisms, but also a reduction in fecal volume and frequency of defecation. The use of antimicrobials is an adjunctive strategy frequently applied to dogs with exocrine pancreatic insufficiency.

Chapter VI, the last chapter of the second part of the doctoral thesis is entitled *"Studies regarding the clinical and therapeutic approach in chronic pancreatitis in dogs"*.

This study describes the clinical, clinicopathological and ultrasonographic findings/observations in the group of canine patients with chronic pancreatitis and provides evidence that chronic pancreatitis is a clinically important pathology as it

presents a series of clinical consequences that have led to the development other pathologies, such as exocrine and/or endocrine pancreatic insufficiency (diabetes), in some cases.

Thus, sometimes chronic pancreatitis can be discovered accidentally, when patients present clinical signs common to other diseases. In this study 8 canine patients out of the 14 selected for this study had other concomitant diseases at the time of presentation to the veterinarian (because they developed exocrine pancreatic insufficiency and diabetes mellitus).

For this study, 14 dogs with chronic pancreatitis were selected, belonging to different breeds, with different ages, who presented with anorexia, repeated vomiting (common, incoercible), even with a hemorrhagic appearance, moderate/severe dehydration, intense abdominal pain, generalized weakness, diarrhea (even melena), fever, and rarely jaundice. Apart from clinical coordinates, paraclinical coordinates are particularly important for establishing a definite diagnosis, namely, the serum dosage of cTLI (canine trypsin-like immunoreactivity) and cPLI (canine pancreatic lipase immunoreactivity). Thus, in the canine patients who developed, in addition to chronic pancreatitis and exocrine pancreatic insufficiency, the serum concentration of cTLI (canine trypsin-like immunoreactivity) was <2.5 ng/mL, while in the other patients it exceeded the values of >35 ng /mL, which confirms chronic pancreatitis. In patients with chronic pancreatitis, leukocytosis was recorded (10.6±1.4 thousand/mm3 blood), based on the significant increase in the percentage of neutrophils, which represents a statistically significant increase ($p<0.05$), compared to of the group of clinically healthy patients. The finding of varying levels of serum triglycerides in canine patients with chronic pancreatitis is due to the concomitant loss of exocrine function, leading to maldigestion of fats.

During the ultrasound examination, the pancreas showed an irregular contour, inhomogeneous echostructure, obviously increased echogenicity (chronic sclero-infiltrative pancreatitis), up to an aspect, much increased in volume with diffuse echogenic infiltrations, which changes the characteristic echogenicity and echostructure. More than 50% of cases had ultrasound abnormalities of the bowel and mesentery adjacent to the pancreas, including thickening of the small bowel and/or stomach wall with loss of stratification.

In the case of the group of patients with chronic pancreatitis, the dominant therapeutic goals were: rapid restoration of fluid and electrolyte balance, successful management of vomiting, pain management and administration of specific diet. In the same context, the support and restoration of pancreatic function is essential, in the case of patients with this pathology.

Pain management consisted in the use of analgesics, such as buprenorphine (0.01-0.05 mg/kg/day administered i.v., s.c., or i.m. every 6-8 hours), or fentanyl (2-10 micrograms/kg/h, infusion, after initially, it was administered in a dose of 2-4 micrograms/kg). The dose of fentanyl was decreased on a case-by-case basis, and analgesia was improved by the addition of lidocaine (0.02-0.04 mg/kg/min) and/or ketamine (2-7 micrograms/kg/min). Dietary treatment for patients in this group consisted of administering food with high digestibility and low fat content, in small and frequent meals.

The innovative character of this study consists in the detailed monitoring, in canine patients, of the physiopathological mechanisms responsible for a defective

digestion but also for a deficient absorption/assimilation, along with the monitoring of the clinical and nutritional consequences, in order to establish a definite diagnosis and subsequently, in in order to ensure appropriate therapeutic and dietary management.