

Summary

The habilitation thesis entitled “**Gastric pathology in dogs and cats: the role of *Helicobacter* spp. infection, molecular mechanisms, prognostic markers and clinical significance**“ represents, succinctly and documented, the certification of my qualifications to supervise the scientific work of the PhD students in the field of veterinary pathological anatomy.

The thesis includes the most important achievements/ results of my professional, academic, and scientific activities in the field of veterinary pathological anatomy, since 2011, the year of getting the PhD in the field of Veterinary Medicine, up to the present.

During the period from 2011 to 2024, my scientific activity took place within the Department of Pathological Anatomy at the Faculty of Veterinary Medicine from Cluj-Napoca, the University of Medicine and Pharmacy from Cluj-Napoca, the Synevovet Laboratory from Bucharest, and in other research laboratories from different countries (e.g., Portugal, Italy, Israel), addressing various research topics with practical applicability in the field of veterinary medicine: naturally occurring conditions (tumors and infectious diseases) in animals and experimental studies (toxicological pathology, chronic gastritis caused by infection with *Helicobacter* spp., nanomedicine in tissue regeneration, and beneficial effects of various natural and synthetic molecules on different tissues/ organs).

The thesis is structured in three parts: **part I** includes information about my education and professional training and specialization, teaching/ academic activity, and international recognition; **part II** presents the most important results of the scientific activity in my research field (gastric pathology in companion animals); **part III** presents the plan for the development of my university career.

Regarding my scientific activity (part II of the thesis), the results obtained from the studies on gastric pathology in companion animals, especially in dogs, are structured into three chapters, each chapter containing 2-4 subchapters. The results of these research activities have been presented at various national and international conferences and published in numerous BDI and ISI journals with impact factor, as well as in three scientific books.

The first chapter, entitled “*Studies on the Helicobacter-related inflammatory gastric pathology in companion animals*“, includes two subchapters. The objectives of these studies were to evaluate the role of *Helicobacter* infection in the development of gastric lesions in companion animals and to update the information regarding the classification, morphological aspects, diagnosis, and pathogenesis of the gastric diseases in dogs.

In subchapter 1.1, the results obtained from an inter-institutional study (universities from Romania, Portugal, and the United Kingdom) regarding the identification, classification, standardization, and updating of data on the main gastric pathologies encountered in dogs are presented; this study was based on reviewing both the scientific literature and our own archives. Thus, through this study, a new classification of inflammatory, preneoplastic, and neoplastic gastric lesions encountered in dogs was made, and where possible, gastric diseases were compared with equivalent lesions described in humans. Additionally, it was noted that the gastric lesions found in dogs exhibit several gross, histological, and immunohistochemical similarities to those present in human patients. However, there are significant differences in terms of etiology, predisposing factors (e.g., genetic factors),

and various molecules involved in the development of gastric pathologies in both dogs and humans.

Subchapter 1.2. presents the original results of a study about naturally occurring gastric pathology associated with different species of *Helicobacter*. This study is entitled “*Pathological significance of Helicobacter spp. in gastric mucosa in dogs*“ and it was conducted in collaboration with the Department of Pathological Anatomy of the University of Porto. For this study, gastric biopsies from 69 dogs were used. The samples were evaluated by histopathology, immunohistochemistry, and molecular techniques (PCR). Our results showed that gastric infections with various non-*Helicobacter pylori* helicobacters (*H. heilmannii*, *H. felis*, *H. bizzozeronii* and *H. salomonis*) are frequently found in dogs, often causing mild to moderate gastritis. However, a specific relationship between inflammatory gastric lesions and *Helicobacter* spp. infection could not be established in the evaluated dogs.

Chapter 2, entitled “*Morphological, immunohistochemical and molecular features of non-neoplastic polypoid gastric lesions in dogs*,” includes 2 subchapters, and the main objectives were a) to evaluate the morphological and molecular aspects of spontaneous gastric polyps in dogs, b) to identify some predisposing factors involved in the development of these lesions, and c) to assess the potential markers of their malignancy.

In subchapter 2.1, “*Macroscopical and histopathological features of non-neoplastic gastric polyps in dogs*,” 15 cases of naturally occurring hyperplastic and inflammatory gastric polyps, were described. Following this study, it was observed that these polypoid lesions were developed in dogs showing severe or moderate chronic antral gastritis. Secondly, features of

epithelial metaplasia and dysplasia were identified within the gastric polyps, suggesting the potential risk of neoplastic transformation. Furthermore, the study opens up new research opportunities regarding the role of *Helicobacter* infection in the development of chronic gastritis and gastric polyps in dogs.

The research activities described in subchapter 2.2, named "*Immunohistochemical study of gastric polyps in dogs*," represent a continuation of the previous work (from subchapter 2.1), aiming to investigate the molecular features (COX2, ki67, CDX2, p53) of gastric polyps in dogs, in order to understand better their biological behavior. Our results have shown that, gastric polyps in dogs develop naturally and are benign proliferative lesions.

Chapter three, entitled "*Studies on the identification of new molecular markers for diagnosis and prognosis of gastric adenocarcinoma in dogs*," includes four subchapters, aiming to evaluate the role of new molecules (previously studied in human medicine) in the diagnosis and prognosis of naturally occurring gastric carcinoma in dogs.

Our research described in subchapter 3.1. "*Expression of Sn and Sialyl-Tn antigens in proliferative and neoplastic gastric lesions in dogs*" highlights the following aspects: a) it was the first study to investigate the expression of Tn and STn antigens in different gastric tissues (normal mucosa, gastric polyps, and gastric adenocarcinomas) in dogs; b) the STn antigen was significantly expressed in canine gastric carcinomas; c) the STn antigen could play an important role in both the neoplastic transformation of gastric mucosa and the metastatic process of gastric adenocarcinoma in dogs; d) the study opens up new research opportunities regarding the serological evaluation of the STn antigen in dogs with clinical adenocarcinoma and its

use in clinical practice as a non-invasive biomarker for diagnosis and monitoring the progression of the disease.

Subchapter 3.2. "*Expression of Trefoil Factor 1 (TFF1) in non-neoplastic and neoplastic gastric lesions in dogs*", describes the relationship between the expression of Trefoil Factor 1 (TFF1) in normal, proliferative, and neoplastic gastric mucosa, the clinicopathological characteristics of tumors, and the survival rate of affected dogs. In these studies, we observed that the expression of TFF1 was significantly reduced in canine gastric carcinomas compared to hyperplastic gastric polyps and normal gastric mucosa. Thus, we concluded that TFF1 plays an important role, as a tumor suppressor factor, in canine gastric carcinogenesis. Additionally, the gradual reduction of TFF1 expression may explain the role of this factor in the invasion of the gastric wall by the neoplastic cells and progression of cancer. Due to its similarities with human gastric adenocarcinoma, dogs could serve as a model for studying the gastric cancer in human patients.

In subchapter 3.3. "*Expression of E-cadherin (cell adhesion protein) in gastric carcinomas in dogs*", the results of our original research concerning the role of intercellular junctional proteins (E-cadherin) in canine gastric carcinogenesis and their association with certain clinicopathological features of gastric carcinoma, are detailed. Through this study, we demonstrated an abnormal expression of E-cadherin in malignant gastric lesions in dogs, especially in those that are anaplastic/ poorly differentiated, and the expression was significantly associated with the degree of tumor invasion into the gastric wall and the presence of neoplastic emboli. Thus, E-cadherin could have prognostic value in gastric carcinomas in dogs.

Subchapter 3.4, "*Prognostic value of vimentin and ki-67 in canine gastric carcinomas*" describes the original results of a study conducted on canine gastric adenocarcinomas, where the role of some molecules involved in the epithelial-mesenchymal transition of neoplastic cells and the proliferative index (mechanisms involved in carcinogenesis) was investigated. Secondly, a potential relationship between the expression of these molecules (vimentin and Ki-67) and the clinicopathological features of tumors, and the survival rate of dogs with gastric cancer was evaluated. We concluded that the expression of both molecules was significantly increased in gastric carcinomas compared to normal mucosa, and the evaluation and quantification of vimentin and Ki-67 proliferative index can provide important information about the prognosis of canine gastric carcinoma.

In the third part of thesis, the plans for career development are presented, including both professional/ academic and scientific. Regarding the plan for scientific development, the main objectives are: to increase the international collaborations with prestigious universities and research centers, to win national and international research grants, to increase the number of publications in ISI journals, to participate in various international research working groups, and to learn new diagnostic/evaluation techniques used in research.

Together with my PhD students, we will continue the research activities on non-neoplastic gastric pathology (e.g., follicular gastritis in dogs and cats) and neoplastic gastric pathology (Lynch syndrome - colorectal cancer, gastric lymphoma) in dogs and cats. Since I am a member of various research projects, other than those focused on the gastrointestinal pathology in dogs and cats, I will also involve the PhD students in other topics of

research, such as lung cancer in sheep, urothelial cancer in cattle, mammary cancer in dogs and cats, osteosarcoma in dogs, transmissible venereal tumors (TVT) in dogs, and pathology of non-neoplastic viral infectious diseases in farm animals.