

## SUMMARY

The PhD thesis, “*Research on the diagnosis and treatment of bitch and cat mammary gland disorders*”, is organized according to the general rules, in two main parts. The first part is dedicated to the current state of knowledge and condenses up-to-date information regarding the morphophysiology and pathology of the mammary gland in canids and felines, and the second part of the thesis presents the personal contributions in the addressed topic.

For writing the thesis, 321 bibliographic sources were consulted from the national and international specialized literature, the data presented being supported by 25 tables and 76 figures.

The first part includes two chapters that briefly describe the structure of the mammary gland, the particularities of the sexual cycle in the female dog and cat and provides an up-to-date synthesis on mammary gland conditions in these species. This contains original tables and diagrams, designed to facilitate quick viewing and understanding of the information.

The second part of the thesis includes 115 pages, structured in 8 chapters.

Chapters 3 and 4 describe the aim and objectives of the thesis as well as the institutional framework where the research was carried out. To achieve the proposed goal, the following general objectives were established:

- evaluation of the prevalence of the main mammary and genital diseases in canids and felines in two different geographical areas;
- identification of the bacterial prevalence in the milk secretion of bitches with mastitis, as well as the evaluation of their antimicrobial susceptibility profile;
- the analysis of the main clinical signs and the response to therapy in patients with pseudopregnancy, with the aim of identifying new approaches to make therapy more efficient, as well as to prevent recurrence;
- analysis of clinical, histopathological characteristics, as well as hematological and blood biochemical parameters, with the aim of identifying the main risk factors in the occurrence of mammary tumors in canines and felines;
- establishing the relationship between the immunohistochemical expression of Ki-67, PCNA, p53, VEGFA and different histological types of canine mammary tumors;
- the identification of possible hematological biomarkers with value in the prognosis of mammary tumors in both canids and felines;
- analysis of the response to chemotherapy in dogs and cats with mammary tumors;
- the development of an application for monitoring patients with mammary diseases focused on streamlining the communication between the veterinarian and the owner, in order to reduce the number of cases lost from the study (*lost to follow up*) and the abandonment of therapy, the final objective being to increase the quality of life for these patients.

Chapter 5, entitled “*Investigations on the prevalence of the main mammary and genital conditions in canids and felines*”, aimed to study the main reproductive conditions with which felines and domestic canids are diagnosed. In order to establish the prevalence of the main genital and mammary pathologies in these two species, the data recorded for two years, in two veterinary clinics specialized in the treatment of pathologies of the genital apparatus, was analyzed. In

females, diseases of the mammary gland were followed in particular: tumors of the mammary gland, mammary hyperplasia, mastitis and nervous lactation.

The results showed that, in both centers, a significant percentage of the total registered patients were clinically healthy individuals who came for convenience interventions (ovariohysterectomy, orchidectomy, gestation diagnosis). From the pathological category, the highest percentage was represented by females, the main conditions identified being: pyometra, mammary tumors and false lactation, while in males, balanoposthitis, prostate hypertrophy and cryptorchidism were noted.

Chapter 6, entitled “*Research on diagnosis and therapy in non-oncological diseases of the mammary gland*”, comprises two sub-chapters: the first aimed at identifying the bacterial prevalence from milk secretion of canines with mastitis, as well as the evaluation of their antimicrobial susceptibility profile. The study revealed that the etiology of mastitis was predominantly bacterial. Gram positive species were identified: *Staphylococcus aureus*, *Staphylococcus haemolyticus*, *Staphylococcus pseudointermedius*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, as well as Gram negative species: *Pseudomonas aeruginosa* and *Escherichia coli*. The most frequently isolated species in this study was *Staphylococcus aureus*, showing the highest sensitivity to Enrofloxacin and Trimethoprim/Sulfamethoxazole. An unusual finding was the identification of *Aspergillus fumigatus*, suggesting a pathogenesis based on exogenous contamination, in which spores adhered to the mammary gland lesions due to an immunocompromised organism. The second sub-chapter evaluated the current symptomatology of patients with pseudopregnancy, as well as the response to current therapy, with the aim of identifying new approaches to make therapy more efficient, as well as to prevent recurrence. The results obtained by us indicated that although after 6 days of treatment, reduction in the volume of the mammary gland occurred in a large number of patients, a significant percentage of the cases required continuation of the therapy up to 12 days to stop the milk secretion. We believe that even in veterinary medicine, greater emphasis should be placed on the curative action given by increasing physical activity, changing the living environment, basically, on removing the female from the state of depression, similar to the pathology encountered in women, in which the success of the treatment is based on a multilateral collaboration between psychiatrists, psychologists and gynecologists.

Chapter 7, “*Research on the diagnosis and therapy of oncological diseases of the mammary gland in canids*”, is structured in 4 subchapters.

The first subchapter aimed to analyze the clinical, histopathological characteristics, as well as different hematological and blood biochemical parameters, with the focus on identifying the main risk factors in the occurrence of mammary tumors in canids. The results showed that a significant proportion of bitches with mammary tumors were not spayed, and those that were spayed went through this surgery at older ages (average 9.5 years). The incidence of mammary tumors was observed to increase significantly from the age of 8, reaching a peak at 10 years, and purebreds and small dogs were more prone to the condition. A small percentage, 1.67%, of the tumors examined were identified as benign, while the remaining 98.33% were malignant. The most common type observed was complex carcinoma, with a frequency of 25%. Of all patients, 45% were in stage I ( $T_1 < 3\text{cm}$ ), 15.5% in stage II ( $T_2: 3\text{-}5\text{ cm}$ ), 5.17% in stage III ( $T_3 > 5\text{cm}$ ), 24.5% in stage IV ( $T_{1-3}N_1M_0$ ) and 10.34% in stage V ( $T_{1-3}N_{0-1}M_1$ ).

The second subsection aimed to examine the relationship between Ki-67, PCNA, p53 and VEGFA in different histological groups of canine mammary tumors, by using immunohistochemistry. This study highlighted that the most reliable marker in canine mammary tumors was Ki-67, its expression being associated with tumor grade, while the evaluation of PCNA, p53 and VEGFA is only useful if the data is interpreted in an integrated context.

The third subchapter aimed to identify the neutrophil-lymphocyte ratio for canine patients with mammary tumors and its correlation with cancer stages. Our results show that NLR can provide important information for the assessment of stages in mammary tumors in canines, and can be considered a marker of the cancer extent.

The fourth subchapter, „*Carboplatin and cyclophosphamide therapy for advanced canine mammary carcinomas*”, presents in detail the evolution of canine patients with mammary tumors under treatment with these substances. The administration of carboplatin and cyclophosphamide was well tolerated by the patients. The adverse effects encountered were represented mainly by digestive disturbances and changes in the general condition, both of which had a low degree of severity. The main changes in hematological parameters were represented by leukopenia and thrombocytopenia. The obtained information highlights the fact that, through a correct management of the symptoms that appear (apathy, vomiting, diarrhea), these therapeutic protocols can be applied safely, ensuring optimal medical care. Support programs that include monitoring, information, and counseling services for owners can be critical in improving the quality of life for pets.

Chapter 8, “*Research on the diagnosis and therapy of oncological conditions of the mammary gland in felines*”, includes 3 subchapters.

The first subchapter, entitled „*Multidimensional analysis of feline mammary tumors*”, followed the epidemiology of this condition, analyzing different factors related to the evolution of mammary tumors in cats. The main tumors identified were of a malignant nature, in a much higher percentage compared to other data published so far (94.3% malignant and only 5.7% hyperplastic lesions). In this study, 12 histological subtypes of mammary neoplasia in cats were identified, the most common being tubular carcinoma and tubulo-papillary carcinoma, in percentages of 28% and 20%, respectively. The highest percentage of mammary carcinomas identified (66%) was classified as stage III ( $T_3 > 3\text{cm} / T_{1-2} N_1 M_0$ ), signaling advanced progression of mammary tumors in cats. Regarding the variation of blood parameters in the context of the established diagnosis, the only change identified in a significant percentage of patients was thrombocytosis.

The second subchapter aims to determine if there is a connection between the platelet level and the histopathological grade of mammary tumors in the cat, considering the thrombocytosis identified in the previous study. The obtained results indicate that increased platelet values can be associated with a higher histological grade in the case of mammary tumors in cats and can be used as prognostic factor in this pathology. A platelet threshold value of 552 was identified. This threshold value improves diagnostic accuracy, establishing platelets as a clinically relevant biomarker in this context.

The third subchapter, „*Effects of epirubicin and cyclophosphamide therapy on hematologic parameters and associated clinical adverse reactions*”, aims to describe the evolution of feline patients with mammary tumors under chemotherapy. The administration of epirubicin, as well as epirubicin with cyclophosphamide, was well tolerated by patients, the side

effects having a minimal and low intensity. The most common adverse reactions identified in cats treated exclusively with epirubicin were of a digestive nature: vomiting and diarrheal stools. Patients who also received cyclophosphamide had vomiting as the most frequent adverse reaction, and a more pronounced deterioration of the general condition. Hematological parameters showed variable alterations following the administration of these substances, underlining the need for careful and frequent monitoring. This is essential in order to facilitate prompt interventions and appropriate therapeutic adjustments. The group of patients in the epirubicin-only group had a less variable and more predictable hematological profile compared to the group in which both substances were administered, which showed greater fluctuations in parameters.

In chapter 9, “*Research on reducing treatment dropout and improving monitoring of patients with mammary diseases*”, an application developed with the aim of improving the communication between the doctor and the owner is presented. This will provide more accurate monitoring of the patients in order to increase the quality of life. The application is structured in two main components: one intended for medical professionals (*web*) and another intended for animal owners (*mobile*).

The web application comprises 5 modules. *Add patient module*. It allows healthcare professionals to add a patient to the system so that their progress can be tracked in the future. To streamline this process, the patient record can be uploaded directly, so most fields will be pre-filled. *The patient details and management module* is crucial, because it provides an overview of the patient's evolution through a chart in which the events the patient went through (mastectomy, palliative treatment, chemotherapy, monitoring) are highlighted, but also the adverse reactions with which he faced. Moreover, the details of the patient as well as the owner are always available. *The account creation module* is necessary to be able to establish close communication between the medical personnel and the patient. After the user account is created by the doctor, the owner will receive an email and an SMS inviting him to use the mobile application. *The notifications module* requires that, on a recurring basis, the owner is invited to complete a questionnaire selected by the medical staff, which includes questions related to adverse reactions or other information of interest. If an owner does not complete the questionnaire on a given day, they will be notified again on the following days to avoid losing patient data. *The calendar module* provides an overview of all events in a given month, displaying information about patients seen each day.

The mobile application includes 6 modules. After login, *the account management* is the first module presented to the owner, which gives him the possibility to log in, change the patient photo and stop the monitoring if the collaboration is no longer desired. The following two modules (*Open/Closed Questionnaires*) give the owner the opportunity to complete a questionnaire consisting of several questions, in order to be able to transmit to the medical staff information about the patient's evolution in the last period. Even before completing the questionnaire, the owner can also add notes, if he considers it necessary. At the same time, the module offers owners the opportunity to see a history of questionnaires completed in the past. The fourth module is *the chat*, an interaction space that gives owners the opportunity to communicate with each other. The *questions and answers* module is the one through which we prevent the possible ambiguities of the owners and proactively offer a list of questions and answers in the oncology area. *Stop monitoring* is the last module; it will be used when the owner decides that monitoring no longer needs to continue. To complete the step, he must state the reason he wishes to terminate the collaboration, as the reason may be valuable information for healthcare professionals.

The developed application integrates several aspects that make it a valuable tool in modern veterinary medicine, for both clinicians and researchers, as it will ensure a more rigorous monitoring, contributing to the reduction of *lost to follow up cases* and abandonment in therapy, in order to increase the quality of life.

Chapter 10, entitled "*General conclusions*", summarizes the research results, providing a general view of the thesis, as well as a series of suggestions and recommendations regarding canine and feline mammary gland pathologies.

At the end of the paper, the bibliographic sources from the specialized literature, national and international, used during the research are presented.