ABSTRACT

The doctoral thesis entitled „Morphoclinical and imaging investigations of intraabdominal and mammary tumoral processes and pulmonary metastases in cats" was conceived with the aim of studying mammary neoplastic processes in cats, as well as the derived neoplastic processes. The original character of this work results from the approach to clinical and imaging aspects related to mammary tumors in feline patients, as well as the microscopic analysis performed on these complex neoplastic processes and their metastases.

The topic of the doctoral thesis is a descriptive one, concerning the elaboration of a clinical and imaging diagnostic protocol based on modern methods that can be applied as early as possible during the disease. The aim of the research is to update, analyze and apply the data available in the literature, with emphasis on the importance of the clinical and imaging examination and the complementary paraclinic examination in establishing a certain diagnosis and a prognosis, for subsequent implementation of adequate therapeutic measures.

The thesis is 178 pages long and comprises two parts, a general part and a personal research part, totalling eight chapters. The first part consists of the bibliographical study on the given topic and is systematized in three chapters, and the second part structured into 5 chapters comprises personal research with the goal of establishing the clinical and imaging and laboratory diagnosis of neoplastic formations in cats. The results are supported by a number of 117 figures, 27 graphics and 4 tables.

Chapter 1, entitled *„Morphopatogenesis of mammary and abdominal tumoral processes in cats*" provides a synthesis of data available on the mechanisms of cancer development and on the transition from normal cells with controlled growth to neoplastic ones, presenting the factors and mechanisms underlying these uncontrolled pathological transformations.

Chapter 2, entitled „*Metastasization of tumoral processes in cats"* brings into discussion the data provided by specialized literature regarding the tumoral metastasization process, a complex biological process of extension beyond the primary malignant tumor, taking place over several stages starting with the detachment of a cancerous cell from the primary site and the active or passive mobilization through adjacent anatomo-histological structures and continuing with the remote dissemination in other tissues or organs.

Chapter 3 of the thesis – „*Diagnostic techniques for mammary and abdominal tumoral processes in cats*" describes up-to-date information on imaging techniques currently applied in veterinary practice and research, as well as synthetic data on the diagnosis of mammary and abdominal tumoral disease. The structure of the chapter is based on the reproduction of aspects related to imaging examination of each individual organ, with emphasis on particular aspects of the radiological, ultrasonographic or CT examination, as well as aspects related to pathological anatomy exams, as well as histological and electronomicroscopic ones.

The second part of the thesis, entitled *„Personal research"*, is structured into four chapters, presenting and discussing the results yielded by the research performed over the four years of doctoral studies. It starts with the **4th chapter** describing the aim and goals of the research, along with the working hypothesis. The aim of the research is represented by the early diagnosis of neoplastic processes in feline patients by using clinical and imaging data in conjunction with paraclinical, histopathological and electronic microscopy data.

The main goals of performed studies were pursued in a clearly structured manner in time, achieved by specific research activities:

- Performing the techniques of clinical and imaging examination as completely as possible, systematically, based on an examination record and the periodic reassessment of patients.
- Taking cytological and morphopathological samples for reaching a certain diagnosis.
- Using specific diagnostic techniques such as electronic microscopy.
- Statistical interpretation of resulted data.
- Assessment of characteristics of tumoral lesions including the assessment of etiological, typological and evolutional factors, in order to establish the prognosis.
- Analyzing personal results, performing comparative analyses and reaching relevant conclusions.

Chapter 5 of the thesis is entitled *„Material and methods*" and describes the institutional frame for carrying out doctoral research as well as the biological material and clinical, imaging and microscopic methods used. The doctoral studies were performed over a four-year period, within three veterinary units, through the collaboration of several specialists in the area: the Faculty of Veterinary Medicine of Iași, the Faculty of Veterinary Medicine of Cluj-Napoca and the Faculty of Veterinary Medicine of Cordoba, Spain.

Subchapter 5.1 – *„Biological material"* records statistical data on the patients taken into study. Between October 2012 and August 2016, 76 feline patients weighing between 2 kg and 5.2 kg were examined. Of these, 72 patients were females, with a percentage of 94.45% and 4 patients were males, representing 5.55% (Figure 5.1.1), with ages ranging from 7 to 20 years. From a standpoint of age category, the adult group (7-10 years) comprises 16 patients (21.05%), the senior group (11-14 years) comprises 46 patients (60.53%), and the geriatric group (15 years and over) comprises 14 patients (18.42%).

Subchapter 5.2 – *„Working methods"* describes the manner of examination of the investigated cases. Feline patients were subjected to clinical and imaging assessment, and some of them were furthermore subjected, depending on case, to other paraclinical methods in order to obtaining a diagnosis as precise as possible. Of the additional methods used we may mention cytological, necropsy, histopathological and electronic microscopy examination, as well as occassional blood tests.

The clinical examination was performed similarly in all patients, based on a signalment sheet, containing an optimally complete anamnesis. Each animal was

identified and recorded by name, sex, age, weight, reproductive status, clinical signs and medical antecedents, wherever these aspects were known.

The radiological examination was performed according to acknowledged techniques in the field literature, respectively by obtaining images in lateral and ventro-dorsal positions, as specified in the specific protocol, when the physiological state of the animal allowed it. Of the 76 recorded cases, in only three of them the radiological examination could not be performed. Performed radiographs were assessed objectively, considering the visual appearance of mammary suspicious tumoral formations, the imaging aspect of the abdomen and internal organsand the presence of other formations and possible metastases.

Where abdominal neoplasia suspicions occurred and the circumstances made it possible, a subsequent ultrasonographic examination was performed for a more complete assessment and a better visualization of the structure of abdominal internal organs.

Examination by computed tomography was performed in only four patients, according to the techniques described in the field literature. In these cases, the computed tomography technique was required since the suspicion of the existence of tumoral processes was not confirmed by other imaging exams such as radiological and ultrasonographic.

Pathological anatomy samples were obtained from live animals by fine needle punction or during surgery, as well as from cadavers during necropsy examination, followed by microscopic examination.

Chapter 6 of the thesis, entitled „*Results and discussions*", starts with subchapter 6.1 – „*Diagnosed mammary tumoral processes*", presenting the diagnosis of mammary tumoral processes in cats taken into study, from a clinical and imaging standpoint and according to the TNM system (Tumor-Node-Metastasis).

A total of 70 feline patients were diagnosed consequently to clinical and imaging examination with mammary neoplastic formations, of which 68 were females, constituting 97.14% and 2 were males, constituting 2.86%, with ages ranging from 7 to 20 years, of European, Burmese, Siamese, Persian, Russian Blue breeds and crossbreeds.

Patients were examined clinically, based on signalments and anamnesis. All mammary gland pairs were examined carefully and thoroughly. Clinical staging and histological classification of malignant formations in feline patients are similar to those applied in dogs, using the original system proposed by Owens with several modifications (Owens L: *Classification of tumors in domestic animals*, Geneva, 1980, World Health Organization). In the modified system, the stage advances from I to II and then to III as the size increases from the 2 cm small one up to the one between 2 and 3 cm and then to over 3 cm. Stage III of the disease includes T1 or T2 tumors, accompanied by lymph node metastases, and lymph node metastases are not a mandatory criterion for T3 tumors. Stage IV of the disease is represented by any tumor with any lymph node metastasis and remote metastases.

Radiological examination was performed in 68 of the 70 feline patients clinically diagnosed with mammary neoplastic formations, in left and right lateral positioning and

ventro-dorsal positioning. The imaging perspective implied determining locations as precisely as possible, as well as the form, extent of infiltration and morphological aspect (tumoral calcifications) of mammary formations, but also the presence of other suspicions of neoplasia in internal organs.

Following clinical and imaging examination, 127 neoplastic mammary formations were identified and staged, corresponding to the same number of diseased mammary glands and 70 individuals. Individuals with multiple mammary tumors in several mammary glands were identified.

Subchapter 6.2 – „*Diagnosed abdominal tumoral processes*" discussed aspects related to diagnosed abdominal metastases originating from mammary tumors, as well as some primary abdominal neoplastic processes, encountered as isolated cases. Representative cases were presented using radiological, ultrasonographic, computed tomography and necropsy images.

Of the 76 feline patients recorded in the study, 44 presented metastases, with a relative percentage of 57.9%, and 32 did not present metastases, with a percentage of 42.1%.

Of the 44 recorded cases of metastases, 20 presented metastases exclusively in the thoracic region (45.45%), 8 exclusively in the abdominal region (18.18%), and 16 both in the thoracic and abdominal region (mixed metastases) (36.36%).

Consequently to the radiological exam, suspicious tumoral masses could be visualized located mostly at the hepatic and splenic levels, but also in the urogenital tract region. The ultrasonographic exam provided data on the internal structure of target organs.

In subchapter 6.3 entitled „*Diagnosed pulmonary metastatic processes*" the cases of pulmonary metastases were presented that were diagnosed by imaging and pathological anatomy in feline patients.

The imaging examination was performed in patients with tumoral mammary masses in order to verify the presence of pulmonary metastases, but especially in patients with respiratory signs, regardless of the tumoral clinical stage that they were found in.

As for the frequency of mammary tumor metastases located atthe thoracic level, these are characterized by an increased incidence compared to metastases located in abdominal organs. Thus, with an absolute frequency value of 35 and a percentage of 44.87%, pulmonary metastases are the most frequently encountered in feline patients with mammary tumors.

Consequently to computed tomography examination, pulmonary tumoral masses of small size, starting from 1 mm, could be visualized, unlike radiological images, where only metastatic processes over 5 mm in size could be viewed.

Subchapter 6.4 – „*Microscopic investigations of diagnosed tumoral processes*" provides data on special microscopic investigations, serving to certify the diagnosis of tumoral processes in cats. Within this subchapter, definitive histopathologic and electronic microscopy images are presented.

Cytological examination was performed in 25 of the 76 feline patients, with a percentage of 32.89% of the total (Figure 6.4.1.1), with valid results. For financial

reasons dependent on the owners of the animals, the special exam could not be performed on all individuals taken into study.

The used technique was the fine needle aspiration technique, described in chapters III and V. Patients were first subjected to local trimming and asepsis. Needles with lower gauge values (green needles) were used for more effective cell aspiration. Imobilizarea fermă a masei de puncţionat cu reducerea la maxim a grosimii ţesuturilor de deasupra formaţiunii tumorale reduce riscul diseminării celulelor tumorale. This procedure is minimally invasive and did not require patient anaesthesia, a safe contention sufficing instead.

Histopathological exams were performed postoperatory, after surgical ablation of mammary tumors, but also following necropsy. For histopathological examination, tissue fragments sized approximately 1 cm^3 were harvested from the mammary gland undergoing the neoplastic transformation process or from organs presenting metastases. Specimens were stained using the Hematoxylin-Eosin (HE) stain, thus enabling us to distinguish the tumor type.

Electronic microscopy was performed using the HT7700 Transmission Electronic Microscope of the Petru Poni Macromolecular Chemistry Institute. The steps for obtaining electronic microscopy images are the following: sampling, fixation, dehydration, infiltration and embedding, encapsulation, modelling, sectioning, contrasting, examination and photographying.

Following cytological exams by mammary gland punction, tumoral processes were identified pertaining to mammary adenocarcinomas. These are malignant epithelial tumors developed from glandular structures, in this case mammary ones, which, according to performed studies, constitute a high amount of the neoplastic mammary processes encountered in companion animals.

Samples from mammary tumors were subjected to the specific histopathologic technique and then examined microscopically. Overall, we may mention the diagnosis of malignant lesions such as carcinoma, adenocarcinoma, haemangiosarcoma, and benign ones such as leiomioma, lipoma, adenoma.

Chapter 7 – „*General discutions*" aims for the discussion of results obtained in previous chapters. Thus the subchapter 7.1 entitled „*Considerations on the importance of the clinical and imaging exam in diagnosing mammary and abdominal tumoral processes in cats*" discusses the value of imaging exams in the stages of tumor diagnosis in feline patients.

Diagnosing mammary and abdominal neoplasias in cats implies going through a multi-step protocol. The foundation of this protocol consists of performing clinical and imaging assessments and clinically staging the neoplastic process.

Thus, the present study employed clinical diagnostic methods based on the examination and clinical staging of mammary tumors, providing statistical data on the topographic categorization of mammary masses according to the diseased glandular pairs. Data provided in the literature concerning the importance of clinical staging is supported, the final purpose being that of informing the owners on the evolution and prognosis available for the animal, as well as establishing an adequate therapeutic conduit mutually agreed upon to be followed.

The clinical and imaging examination serves to orient clinicians with regard to the physiological state of the patient, the tumoral stage and a possible therapeutic procedure, constituting a fundamental examination, indisposable in veterinary oncology.

Subchapter 7.2 – „*Considerations on the importance of the microscopic examination in diagnosing mammary and abdominal tumoral processes in cats. Statistical considerations"* presents statistical values resulted following the assessment and diagnosis of mammary neoplastic processes, as well as their metastases, and the diagnostic value and authenticity of the microscopic examination as illustrated by the thesis study.

Investigations are based on special microscopic exams in over 50% of the patients, more specifically in 41 of these (53.95%).

Thus, 13 patients were subjected to cytological examination – 15.79%, 12 patients were subjected to histopathological examination – 15.79%, 12 patients were subjected to both cytological and histopathological examination – 5.79%, and 4 patients were subjected to both electronic microscopy and histopathological examination – 5.26%. The presented absolute values add up to 76 cases, of which 25 were subjected to cytological examination and 28 to histopathological examination.

In 35 of the patients, examination by special microscopic exams was not possible, amounting to a relative value below 50%, respectively 46.05%.

Within the doctoral study, 76 feline patients were examined using the criterion of mammary tumor localization and of the metastatic processes of the respective tumors.

Of these, 57 individuals had received hormonal treatments during their lives, constituting 75% of the total. Twelve patients had not received such treatments (15.79%) and in seven cases amounting to 9.21% of the total, information was unavailable for various reasons.

As for necropsy examination, of 76 recorded patients, 33 were subjected to this specific examination, amounting to 43.42% of the total. In 28 cases – 36.84%, this was not possible, due to the owners' refusal of necropsy. Necropsy was not necessary in 15 patients due to the absence of demise or euthanasia, amounting to a relatively small percentage of 19.74%.

Depending on the location of primary tumors, 70 of the feline patients taken into study presented mammary tumors, constituting the highest amount in relation to the total, with a percentage of 92.11%. In isolated cases, following necropsy and histopathological examination, 6 cases of primary tumors were found with other locations than the mammary one. Thus, 4 patients presented primary tumors at the hepatic level (5.26%), more specifically hepatic cholangiocarcinoma. Other 2 patients were classified under the pulmonary primary tumor type, mixed bronchoalveolar (bronchioloalveolar) pulmonary carcinoma, amounting to 2.63% of the total number of cases.

Following cytological and histopathological exams, 14 tumoral types were identified, of which 3 benign and 11 malignant.

Benign tumoral types consisted of lipoma, vesical leiomioma and mammary adenoma. The mammary adenoma type was diagnosed subsequently to fine needle

punction of a mammary mass in a male patient. The other two tumoral types were identified histopathologically in mammary masses in two female patients. Expressed as relative values, each one constitutes 3.03% of the total of the tumors.

In the case of malignant tumors, 11 cases of simple mammary adenocarcinoma were identified, recording the highest percentage of 33.33%; 5 cases of solid adenocarcinoma and 5 cases of compact adenocarcinoma, with a value of 15.15%; 4 cases of hepatic cholangiocarcinoma – 12.12%; 3 cases of compact carcinoma – 9.09% and 2 cases of mixed bronchioloalveolar (bronchoalveolar) pulmonary carcinoma constituting a percentage of 6.06%.

Chapter 8 – „General conclusions and recommendations" ends the work with a number of general conclusions and recommendations resulted following the study of mammary tumors in cats and their metastases, as well as the originality elements of the thesis.

Last comes the presentation of the bibliographical list with titles from the Romanian and foreign field literature used during the research.