SUMMARY OF Ph.D. THESIS

The Ph.D. thesis entitled *Morphoclinical and Imaging Investigations Utilized in the Diagnosis of Neoplastic Processes within the Abdominal and Pelvic Cavities in Dogs* is composed of two main parts, consisting of a general part and of a part containing personal research, comprising a total of eight chapters. The first part of the thesis, entitled *Bibliographic Study of Neoplastic Processes within the Abdominal and Pelvic Cavities in Dogs* consists of current data of which the origin is the specialized literature, synthesized in three chapters. The second part of the thesis, *Personal Research*, is comprised of five chapters.

The first chapter, entitled *The Origin and the Taxonomic Belonging of the Dog*, presents data concerning the origin of the dog and its place in the biologic systematics, while underlining various aspects of current theories (the most acclaimed ones) about the origin of this animal; basic information concerning the dog breeds are also presented in the same chapter, which are essential in performing several investigations, including the medical ones. The same chapter contains information related to the anatomical planes utilized in the classic imaging examination, concerning the regional and topographic anatomy of the animal. Furthermore, the chapter contains aspects of the directional terminology and of the topographic details that characterize the dog in what concerns its thoracic, abdominal, and pelvic regions.

The second chapter of the thesis, *The Tumors of the Abdominal and Pelvic Cavities in Dogs* contains notions and synthesized data related to the neoplastic processes that evolve in the abdominal and pelvic organs of dogs. Since the data needs to be thoroughly comprehensive, a certain distribution in describing the tumoral pathology was thus utilized: first off, the post-diaphragmatic digestive organs (comprised of the stomach, the intestines, the liver and the pancreas) were described, followed by the urinary organs (the kidneys, the ureters, and the urinary bladder), and by the spleen and lymph nodes; the genital organs (comprised of the ovary and uterus in females and the
prostate in males) were subsequently described, followed by the tumors of the peritoneum and other annexes or organs, such as the suprarenal gland.

The third chapter of the thesis, *The Imaging Diagnosis of Neoplastic Processes within the Abdominal and Pelvic Cavities in Dogs*, describes current data concerning the imaging techniques that are now being applied in the veterinary research and practice, as well as synthesized data about the diagnosis of tumoral processes of the organs within the abdominal and pelvic cavities in dogs. The structure of the chapter is based on the description of certain aspects of the imaging diagnosis of each and every organ, by underlining the particular aspects of the radiologic, ultrasound and CT examinations (or other types of methods). Furthermore, the imaging modifications that can be observed in the case of the neoplastic processes of the studied organs were presented. This chapter represents, in itself, a new material, as the current data in this sense is vague and disparaging, at least in the case of the specialized veterinary literature from Romania.

The second part of the thesis, entitled *Personal Research*, begins with the fourth chapter, which describes the purpose and objectives of the research, along the work hypothesis. The purpose of the research consists of the precocious diagnosis of the tumoral formations within the abdominal and pelvic cavities in dogs, by combining the clinical and imaging information with the paraclinical, blood and histopathological ones.

The main objectives of the studies that were performed during the Ph.D. thesis were planned on time periods, marked by distinct research activities. They consist of the following details:

- The clinical and imaging diagnosis of tumoral processes within the abdominal and pelvic cavities in dogs
- The paraclinical diagnosis of tumoral processes within the abdominal and pelvic cavities in dogs
- Electronic microscopy analysis
- Specific and comparative urography studies
- The review of the relevancy of clinical and laboratory techniques utilized in the diagnosis of formations that suspected of being neoplastic, within the abdominal and pelvic cavities in dogs

The fifth chapter of the Ph.D. thesis is entitled Material and Methods. The studies were performed in three main centers: two research centers consisting of one located in the Faculty of Veterinary Medicine of Iasi and another one, located in the Department of Veterinary Medicine of the Veterinary Radiology Center in the University Federico II of Naples; the third consists of an analysis and data review center within the Faculty of Veterinary Medicine of Cluj-Napoca.

During 2012 and 2014, in both Iasi and Naples, 927 dogs were examined, which presented abdominal modifications and symptoms of the dysfunctions correlated to the the intra-abdominal and intra-pelvic organs. Of all of the dogs that were examined, 117 have presented lesions that were eventually diagnosed as being of a neoplastic nature; 233 lesions were discovered in these patients, which represents the value utilized for the comparative ratio between the laboratory and imaging examination results; the higher number of tumoral lesions compared to the number of patients can be explained by the presence of multiple tumoral processes in the same patient, mostly due to the process of metastasis.

The subchapter entitled Study Methods comprises data concerning the examination techniques that were utilized, depending on the category of the performed examinations; the imaging examination, the laboratory examination and the anatomo-pathological examination.

The imaging examination comprises detailed descriptions of technique aspects, applied depending on the examined organ: radiography, ultrasound and computer tomography. In the case of the Faculty of Veterinary Medicine of Iasi, the cases were studied once they arrived at the Service of Radiology and Medical Clinics. The Service of Radiology of FMV Iasi has two radiology systems: an immovable Eltex 400 and a movable Intermedio basic 4006, as well as a system of digitalization/archiving of radiological films obtained in a classic fashion. The service owns an ultrasound system
Esaote AU5 (color Doppler) with multiple transducers (fig 5.5). The Medical Clinics of FMV Iasi owns a clinical space especially destined for the examination of pets, as well as an Aguilla ultrasound system.

The Radiology Center within the Department of Veterinary Medicine of Naples owns an immovable radiology system, complete with high frequency, with a CR system Agfa C-30, a single-layer CT system GE Prospeed and an ultrasound system GE Logiq 400 (fig 5.5).

Several blood and urinary analysis were performed in the course of the laboratory examination. Blood samples were taken from 56 patients, which were suspected of having formations or neoplastic processes. The blood samples were performed from the saphenous vein, cephalic vein and jugular vein. The blood was collected in vacutainer tubes containing anticoagulant (EDTA) for the haematological examinations, and in cloth-activator tubes for the serological examinations. The samples were processed by a medical laboratory with a veterinary medicine department, containing the following two specific pieces of apparatus: ADVIA 2120i for the haematology and COBAS 6000 for the serology.

The study material for the urinary examinations consisted of the dogs that showed dysuria, physical modifications of the urine and suspicions of formations that were observed during the imaging examinations of the urinary apparatus.

The methods consisted of the biochemical analysis of the urine (the protein was analyzed with a band test and refractometry and using the pH) and the microscopic examination of the urinary sediment.

The anatomo-pathological examination consisted of the collection of samples that were gathered either by puncture, biopsy or necropsy. The samples were then on analyzed using the microscopic examination and the electron microscopy.

The histological and cytochemical studies were performed within the laboratory of Pathological Anatomy and the laboratory of Cellular Biology, Histology and Embriology from the Faculty of Veterinary Medicine of Iasi. The permanent histological samples were conserved using the classic paraffin sectioning method. The transmission
electron microscopy research (TEM) was begun by analyzing the samples in the laboratory of electron microscopy of the Faculty of Veterinary Medicine of Iasi. The electron microscopy investigations were performed with the help of the transmission electronic microscope *Hitachi* of the Macromolecular Chemistry Petru Poni Institute. Obtaining the electron microscopy images concerning the structure of the tumoral cells was performed based on the following phases: the collection, the fixation, the dehydration, the infiltration and the inclusion, the encapsulation, the modeling, the sectioning, the contrasting, the examination and the photographic phase.

The end of the chapter *Material and Methods* consists of examination charts that were inserted thereon, that were performed on patients with intra-abdominal and intra-pelvic tumors, which demonstrate various and veridical aspects of the case study.

The sixth chapter of the thesis, entitled *Results and discussions*, starts with the subchapter 6.1, entitled *The Relevancy of the Imaging Examination in the Diagnosis of Neoplastic Tumors within the Abdominal and Pelvic Cavities in Dogs*; the diagnosis value of each imaging method was analyzed through the study of the obtained results.

During the research, 83 of the cases were examined using radiography, 106 cases were examined using ultrasound and 33 cases were examined with the help of the CT. Numerous patients were diagnosed by using successive imaging techniques. The radiological examination acts like a director, and frequently leads the examiner towards other, more complex methods. This technique was applied either directly or using a contrast substance (as is the case with 70.94% of the total number of imaging examinations). This method had a medium towards a high degree of diagnosis relevancy in the case of gastric and intestinal tumors, in the case of splenic neoplastic modifications and in the case of urinary neoplastic afflictions (by using the contrast-based examination).

The ultrasonography was the most frequently utilized method while diagnosing tumoral processes of the studied organs (90.60% of the total number of imaging examinations). The method has a high diagnostic value, and is rarely completed by a radiologic or a CT examination. The importance of the method is even higher when
anatomo-pathological samples need to be gathered, particularly in the case of ultrasound-guidance.

The CT was utilized in a reduced number of cases (28.20% of the total number of imaging examinations), and only when supplementary details were required aside from the ones revealed by the radiology and ultrasound tests. Furthermore, the CT was used in order to additionally check for any lung metastasis. When it was performed, the method had the highest diagnostic value, as it brought forth important information, including for future surgical interventions.

The subchapter 6.2, entitled *The relevancy of the laboratory examinations in the diagnosis of the intra-abdominal and intra-pelvic neoplastic processes in dogs* consists of the analysis of the diagnostic value of the blood and urinary exams, by observing the obtained results.

The relevancy of the laboratory examinations in the case of the hepatic neoplasms is relatively high, as it is conferred by the carcinoma-type tumor, as shown by the signs of anemia, the increase in the ALT enzyme, as well as the hypoproteinemia and the hypoglycemia. The ratio between the AST and the ALT, with a subunitary value, has an increased diagnostic relevance. Over half of the carcinoma cases had trombocytopenia.

The relevancy of the laboratory examinations in the case of splenic neoplasms is relatively high, as the non-regenerative anemia, combined with the discovered neutrophilia usually direct the examiner toward a diagnosis of hemangioma or hemangiosarcoma.

The relevancy of the laboratory examinations in the case of prostatic neoplasms is relatively high, as the increase in alkaline phosphatase, the hypercalcemia and the increase in ureic acid usually direct the examinator toward a diagnosis of adenocarcinoma, particularly in the case of intense and chronic prostatomegalias.

The results of the urinary examinations have proven their usability while any signs of dysuria were present, and when imaging tests were positive for the urinary organs and for the prostate. With urinary neoplasm suspicions, the microscopic
examination was aimed at identifying the tumoral cells. The relevancy of this type of examination was high, particularly in the case of the transitional cell bladder carcinoma.

The subchapter 6.2, entitled *The Relevancy of the AnatomoPathological Examinations*, comprises of data on tumoral lesions of the intra-abdominal and pelvic organs in dogs. Thus, the post-diaphragmatic digestive organs were affected by neoplasms with 45 lesions, the organs of the urinary apparatus presented 28 lesions, the organs of the genitalia presented 42 lesions, the spleen and the lymph nodes have been detected with 29 lesions, and the peritoneum and other organs and annexes presented 7 lesions.

The systematization of the cases on the affected organs was the following: 5 tumoral processes or formations of the stomach, 11 tumoral processes or formations of the intestines, 44 neoplastic processes or formations of the liver, 6 neoplastic processes or formations of the pancreas, 28 tumoral processes or formations of the kidney, 17 neoplastic processes or formations of the urinary bladder, 41 neoplastic processes or formations of the spleen, 5 neoplastic processes or formations of the lymph nodes, 12 neoplastic processes or formations of the of the ovary, 20 neoplastic processes or formations of the of the uterus, 35 neoplastic processes or formations of the of the prostate, and 9 neoplastic processes or formations of the of the peritoneum and of other organs within the abdominal cavity.

The etiological research was completed with electron microscopy investigations, in the case of liver neoplasms and of tumors metastasized in the mesentery and in the spleen.

The liver carcinoma is characterized by hepatocytes which, in electron microscopy, show numerous Golgi barrels inside the cytoplasm around the nuclei, that have a flat aspect and that contain a finely granular material. The extremities of the sacks are dilated, extended and they constitute macro-vesicles, which have a secretory or condensing role of the hepatocyte within the liver with carcinomatous lesions. Inside the cytoplasm of the hepatocytes, numerous autophagic vacuoles (autophagosomes) can be
observed, which contain mitochondrial debris and therefore trigger a digestion of the
organites within the affected hepatocyte, by autophagy.

The same hepatic lesion is characterized by Kupffer cells that are a part of the
macrophage-monocyte system, at the level of the vascular endothelium of the sinusoid
capillary of the plasmalemma; in the cytoplasm of these structures there are numerous
autophagic vacuoles, which yet again is a sign of the intense digestion performed in the
auto-phagolysosomes. The cytoplasm of the Kupffer cells contains multiple lysosomes;
following an intense activity, the presence of the heterophagosome and later on, of the
phagolysosome makes the digestion more and more active.

In the case of the mesenteric metastasis, the mesothelial cells come in contact
with one another by interdigitation (because of the metastasis, the cytoplasm contains
heterophagolysosomes). There are many plasmocytes in the conjunctive axis of the
mesotheliofascicular tissue of the mesenterium. The plasmalemma of these plasmocytes
presents numerous microvilli that come in close contact with the membrane of the
mesothelial cells, which proves that there’s a cooperation at an immunological level
between these two types of cells.

The reaction of the lymph node is evident in the splenic metastasis, within the
white pulp, as numerous lymphoblasts are present, which are interconnected with one
another via the microvilli. In the conjunctive tissue of the spleen, there are classic and
interdigitated reticular cells, which show numerous irregular cytoplasmic extensions.

Numerous plasmoblasts with a rugous endoplasmic reticulum and numerous
attached rybosomes can be found in the lymphoid population of the splenic nodule, in the
case of the same type of metastasis. This is a sign of an intense protein synthesis which is
used for freeing immunoglobulines. Multiple macrophages with a kidney-shaped nucleus
and a cytoplasm rich in phagolysosomes can be seen as a reaction to the neoplastic
process.

The aim of the subchapter 6.4, *A Comparative Study of the Urography performed
with Various Contrast Substances in Dogs* was to prove the quality of the three utilized
contrast substances in the relevancy of the intravenous urography, which are currently
being used both in veterinary medicine and in human medicine; furthermore, proving the lack of toxicity of these substances by performing hematological and biochemical analyses of blood samples collected 15 minutes and 24 hours after the procedure was yet another objective of the subchapter.

Three different contrast substances were utilized: Optiray®, Visipaque®, and Iomeron®. The studies were performed on 12 healthy canine patients, and each of the lot was administered a different substance. The work protocol consisted of seriated radiographs that were made immediately after the substance was injected intravenously, and 5 minutes, 15 minutes and 30 minutes following the procedure. Each urography was accompanied by the collection of blood samples, used for hematological and biochemical analyses; collecting the samples was performed before and after the urography, and 24 hours after the procedure. The hematological and biochemical blood values were all within normal parameters before the substance was administered.

The analysis of the imaging examinations has led to real data concerning the aspect of the nephrogram, the ureterogram and the cystogram, as well as real data about the tranist speed of the contrast substances within the formerly mentioned anatomical segments.

The studies in the field of urography prove the superiority of the Optiray (Ioversol) solution, especially in the nephrographic phase, which is why it should be recommended for diagnosing kidney disease, undiagnosed using ultrasound.

The Visipaque (Iodixanol) solution in the performed canine urography was utilized for the first time in Romania. The solution is characterized by a fast transit speed at the level of the kidney, but with a decrease in imaging details. The speed of the Visipaque 320 substance can be a time advantage, as the waiting period is significantly reduced in the case of a confirmation of any ureter-related pathology.

As is the case with the Visipaque solution, the Iomeron (Iomeprol) substance was also utilized for the first time in canine urography, in Romania. Iomeron 300 has an even greater transit speed compared to Visipaque 320, but it triggers a lower quality of the nephrogram. This substance has been shown to have the least performance when it comes
to diagnosing a kidney issue. Nevertheless, the product can be utilized successfully in the case of any ureter-related pathology.

All of these three studies substances have caused no adverse effects and have been at the core of minimal blood modifications, which are completely absent at 24 hours after performing the procedure. All of the three studied substances can be successfully used in performing retrograde cystography, so much so when they’re employed in the course of a double contrast technique, in order to underline the imaging details of tumoral processes at the level of the urinary bladder and of the urethra. The use of urographic techniques is highly recommended both for confirming any tumoral suspicions which might come up following the ultrasound examination, and for supporting and verifying the success of any surgical intervention on the urinary organs of dogs.

The seventh chapter, entitled General Discussions, consists of considerations on the imaging techniques and the anatomo-pathological techniques, as well as epidemiological considerations in regard to diagnosing tumoral processes of the organs within the abdominal and pelvic cavities in dogs. The importance and frequency of a certain type of imaging technique are analyzed, depending on the nature of the studied organ (these results have been analyzed graphically). The results of the anatomo-pathological examinations are presented in the shape of a synoptic picture, which can be used by the viewer in order to understand the exact types of tumors that have affected a particular organ, complete with their percentual distribution. All of these results have been analyzed graphically. In the end, several general considerations are described, with a net epidemiological value. Correlations have been made between the possible factors that might lead to the increase in the incidence of canine cancer, and the factors that make the collection of oncological data in the veterinary field quite difficult. These have a direct influence on the results of the profile research activity.

The tenth chapter, General Conclusions, consists of a combination between the data resulting from the partial conclusions and from the general discussions, as well as some recommendations of a practical nature. Last but not least, it should be underlined that the studies performed during this Ph.D. are fully original.