ABSTRACT

Keywords: cytology, effusion, tumoral effusion, serous cavities, carnivores, large ruminants, small ruminants, cytologic examination, histopathology, immunohistochemistry.

The PhD thesis entitled ***"Research regarding the exfoliative cytology of the internal serous cavities in animals"*** was developed in the Doctoral School of the University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad" Iași over four years of study, during the period October 1, 2009 - October 1, 2013 and it is structured in accordance with the current legal provisions in two main parts: Part I, entitled ***"The current stage of knowledge"*** includes 52 pages and represents 25% and Part II entitled ***"Personal contributions"*** which is spread over 154 pages and represents 75%.

In Part I, divided into three chapters, are summarized the informations from the consulted literature related to thesis subject, informations that were subsequently used to interpret the data obtained in the second part. This part is illustrated by 2 selected figures suggestive to detail the summary information.

In the first chapter– ***„The morphology of the internal serous cavity in animals"*** is presented the normal morphology of the serous cavities in domestic studied animal species where are described the anatomical, histological-structural, ultrastructural-cytological aspects referring directly to the cavity fluids.

Chapter II- ***„The morphopathology of the serous cavities in domestic animals"*** presents the morphopathology of the serous cavities in association with lesions of the hollow organs, the etiology, the pathogenesis and their macro - and microscopic manifestation.

Chapter III- ***„The methodology of cytology examination of the liquids from internal cavities"*** presents specific data on all necessary steps obligatory to be completed in veterinary medical practice when the cytological examination is required in a wide range of diseases in animals.

Part II is structured in five chapters (chapters IV-VIII) and includes: chapter IV- ***„The aims and objectives of the research”,*** chapter V- ***„Research on the cyto-pathology of the internal serous cavities in animals”;*** chapter VI- ***„The immunocytochemistry method applied for the differential diagnosis between carcinoma, mesothelioma and thymoma”;*** chapter VII- ***„Correlations between cytological and histopathological results in the examined animal species”;*** chapter VIII- ***„Summary tables”,*** the ***Final conclusions*** summarizing the personal research.

The work was performed on the private households cases and on the intensive system growth cases from Iași and on the cases presented in the Faculty of Veterinary Medicine Iași Clinics, in the Necropsy and Pathology Laboratory, Faculty of Veterinary Medicine Iași and Cytology and Pathology Science Laboratory of the National Veterinary School of Alfort, France, Ecole Nationale Veterinaire d'Alfort (ENVA).

In chapter IV - ***"Aims and Objectives of the research"*** the choice of the subject is justified. The main purpose of the research focuses on analyzing and classifying by cyto morphopathologie issues the effusions collected from animals, lesions which are associated with the serous cavities.

The main objectives of the thesis and the corresponding activities were established in agreement with the scientific manager of the thesis and were represented by:

- ✓ Evaluation of the biochemical and cytological aspects of the cavitary effusions respectively pleural, pericardial and abdominal collections that through the formation mechanism and their position allow the cavitary puncture technique.
- ✓ Evaluation of the sensitivity and the specificity of the cytological examination of the effusions: for that purpose the cytologic and histopathological results were compared. The cytological exam was performed on the effusions collected intravitam and the histopathological examinations were perfromed on the pieces of damaged organs associated with effusions collected from dead or euthanized animals.

The histopathology is characterized by high accuracy due to conservation of the lesions architecture and the possibility of observing the complexity of the disease process and the report of the lesions in relation with the surrounding tissues.

The cytological examination performed on the sampling cells provides information on fine changes in cell morphology, the emergence of abnormal cells in the studied effusion and

helps evaluating the mixing and the cell predominance which is characteristic for certain pathological processes.

- ✓ The attempt to establish a fast and accurate etiologic diagnosis through the appropriate processing of samples in order to highlight the eventual animated etiological agents (such as bacteria and parasites).
- ✓ The introduction of the serous cavities puncture technique into the practice of cytological diagnostic. This allows to determine the effusion nature, the orientation towards a possible diagnosis of the pathological process providing the opportunity of the appropriate therapeutic decisions to the clinician, in a short time and with minimal complications for the patient.
- ✓ The establishment of the correlations between the histopathological, immunocytochemical and cytology aspects in animals.
- ✓ The correlation between the obtained results with the data cited in the literature in order to better understand the normal and pathological cytology aspects of the serous cavities in animals.

The chapters in the Part II begins by describing the work material and methods used for research, all the images of the second part being original, made in the laboratories where the research was conducted. The data presented in the Personal Contributions are supported by 31 and 200 figures inserted tables. The reference list summarizes 222 titles domestic and international literature. The data content in this work is reflected in scientific papers published in symposia with international participation organized by the Faculty of Veterinary Medicine from Iași, Cluj, Timișoara and București. Bacteriological, cytological, immunocytochemical, necropsy and histopathology examinations were performed, and samples of tissues, organs and luminal fluid samples were collected for the proper performance of the mentioned examinations.

The cytological examination of effusions supposed for the beginning to obtain them by puncture of the internal serous cavities. The research was performed on 208 effusion samples taken from animals of all species included in this study, which were analyzed quantitatively and qualitatively. The sampling technique was performed by puncture of the serous cavities, which are a less traumatic technique and of real interest in making a correct diagnosis. It was strictly respects and did not require general anesthesia except for some cases when the local anesthesia was performed. The preferred site differed by species and it was trimmed and

disinfected with iodine to prevent contamination. The effusion samples collected from 100% of the cases were processed in a short time interval after sampling in order to avoid the occurrence of changes in vitro and the cellularity degradation (cytolysis, changes of cell morphology). The studied samples were chemical and microscopical analyzed by physical characteristics. The identified effusions are grouped in a first phase by the determined biochemical parameters (NTCN/µL and PT g/dl) to realize a registration respecting the classification, the hierarchy and the cytomorphological criteria for identifying lesions associated to effusions in the veterinary pathology of which: circulatory disorders, inflammatory and tumor processes.

In section 5.3.1 - ***„Biochemical studies of the effusions identified in animals"*** the determination of the total protein concentration of the liquid (PT g / dl) it was wanted, for this purpose a refractometer and a drop of liquid were needed.

In section 5.3.2 - ***„The cyto-pathological study of the effusions identified in animals"*** the determination of the quantification of nucleated cells in effusions (NTCN/µL) using the manual method and the classical Burker-Turk haemocytometer counting chamber and also the automatic hematology analyzer MS9 from the laboratory of Cytology and Pathology ENVA.

Subsequently, specimens were classified based on the parameters in the 3 ethiopathogenetic classes: transudate, modified transudate and exudate, following the methodology recommended by French literature in accordance with known reference values.

Thus, a total of 132 samples were quantity measured, of which 15% falled in the transudate category, 57% of samples were modified transudate and 28% as exudates.

Transudates were characterized by low cell and protein content. Transudate and modified transsudatele arose due to noninflammatory passive extravasation (passive effusion / Chylous) and due to neoplasia. Modified transsudates have a moderate cell concentration, the protein concentration being in accordance with the reference values with some exceptions where the large number of cells is specific to the chylous effusion type. The identified exudates presented high concentrations of protein and a high number of cells due to the inflammatory non-infectious irritable and necrotic processes.

In sections 5.3.4. - ***„The cyto-pathology of the pleural cavity",*** 5.3.5. - ***„The cyto-pathology of the pericardial cavity"*** and 5.3.6. - ***„The cyto-pathology of the peritoneal cavity"***- are described the pathological processes of the pleural, pericardial and peritoneal

cavities diagnosed on corroborating data from anamnesis, clinical and macroscopic, cytology and histopathology examinations in the cases from our study.

In 5.3.4. section - ***„The cyto-pathology of the pleural cavity"*** are presented and illustrated the circulatory, inflammatory and tumor-associated disorders of the pleural effusions as it follows: circulatory disorders are represented by the presence of hydrothorax in 16%, hemothorax in 4% and chylothorax in 3%; inflammatory lesions were classified as: serous/sero-haemorrhagic pleurisy in 6.9%, sero-fibrinous/fibrinous in 7.4% diffuse / focused purulent in 2%, granulomatous in 2% and gangrenous in 1%, the sero-fibrinous/fibrinous pleurisy having the highest incidence. Pleurisy were often detected by macroscopic, histopathological and cytological examinations being common for most species.

The effusions associated with tumor processes encountered in this chapter are the pleural effusion associated with lung carcinoma diagnosed both in dogs and cats, effusions associated with possible mesothelioma, effusions associated with lung adenocarcinoma metastasis of the mammary gland in cats. Most frequently the histopathological examination confirmed the diagnosis obtained by cytological examination.

In 5.3.5. section - ***„The cyto-pathology of the pericardial cavity"*** were identified and illustrated the intracavitary collections, pericardial inflammation and effusions associated with metastasis of the mammary gland in dogs, as it follows: intracavitary fluid collections were the effusion and haemopericardium, predominantly being the pericardial effusion in most species; pericardial inflammation were represented by serous and serous fibrinous pericarditis with a higher incidence of the serous fibrinous pericarditis; tumor effusion was associated with cystic metastasis carcinoma localized in breast in bitch. The cytological results were also completed by macroscopic and histopathological examination, the latter confirmed the lesion diagnosis. The lesions were identified by cytomorphopathologic tehnique as described in the literature.

In 5.3.6. section – ***„The cyto-pathology of the peritoneal cavity"*** the circulatory disorders are represented by hydroperitoneum or ascite in 17% of the cases, haemoperitoneum in 5% of cases, uroperitoneu in 1%, and 1 case of biliary peritoneum.

Inflammatory lesions are classified as serous fibrinous peritonitis (2.5%), fibrinous peritonitis (3.5%), fibrinous haemorrhagic (0.5%), diffuse and focussed purulent (4.5%), peritonitis caused by parasites (3%) , the purulent peritonitis being placed first. Both circulatory disorders and inflammatory processes were identified macroscopically, cytological

and histopathology, and diagnosed in some cases by corroborating the clinical data, history and complementary examinations (bacteriological, radiological).

The effusions associated with peritoneal tumor processes are relatively rare (1.5%), represented by effusion associated with ovarian epithelial formation, splenic mastocytoma and with hemangiosarcoma. The tumor formation origin confirmation was achieved by histopathology in 1% of the cases analyzed. From the 14% of samples containing bacterial germs we identified: anaerobic (*Clostridium spp*), 7% of the cultures, 45% containing aerobic (facultative anaerobes, *Pasteurella spp*, *Arcanobacterium pyogenes*), 24% contain Enterobacteriaceae (*Escherichia coli*), 24% containing *Streptococcus spp.*

The research carried out in Chapter VI – ***„The immunocytochemistry method applied for the differential diagnosis between carcinoma, mesothelioma and thymoma”*** describes the immunocytochemical examinations which were performed by using cell markers to highlight the diagnosis. Thus the study of the immunocytoma marking was applied in the differential diagnosis between carcinoma, mesothelioma and thymoma in 3 cases (cats) with neoplastic effusion which were cytological examined.

The immunocytochemistry method was used for highlighting the tumor cell, epithelial or lymphocytic (T lymphocytes, B lymphocytes) using monoclonal antibodies specific for each class of cells, applied on fresh samples (fresh cells plated blades).

Using the immunocytochemistry method, the malignant tumor cells have been recognized in one case of the three-valued (effusion carcinoma). In 2 cases immunocytoma marking was negative due to blockage by specific antibodies, the intense protein background and the low number of cells from samples, the diagnosis being of malignancy suspicion.

The immunocytochemistry marker with a high sensitivity in making a distinction between the fluids associated with mesothelioma and carcinoma proved to be cytokeratin A1/AE3.

Chapter VII- ***„Correlations between cytological and histopathological results in the examined animal species”*** the sensitivity and specificity of cytological examination of effusions was evaluated.

The data from cytological interpretation were compared with the histopathological findings. The cytological examination was performed on intravital effusion samples such as passive effusion (noninflammatory), active effusion (inflammatory) and effusions associated with tumor processes. The histopathological examination was performed on organ fragments

associated with effusion lesion from dead or euthanized animals, to confirm and/or to refute data obtained by cytological examination.

For 99% of cases the histopathological diagnosis confirmed the results obtained by cytological examinations with one exception, where the cytological examination leaded to adenocarcinoma breast metastasis suspicion, but histological confirmed a simple breast adenoma.

The cytological examination falls into the diagnostic algorithm of pathologies accompanied by cavity collections. The results of the cytological examination contributes to the confidence diagnosis by corroborating the history, clinical, bacteriological and histopathological examinations.

In chapter VIII- „***Summary tables*”** the study population was displayed in form of tables according to the investigated species, serous cavities, clinical data specifications and in particular quantitative parameter values determined for the collected effusions, total proteins (PT g/dl) and total nucleated cells (NTCN/µL¶) ending with etiopathogenetic classification.