ABSTRACT

The pathology of wild animals is a relatively recent study subject in the international scientific world, fact that becomes even more obvious if compared to the interest given to domestic species. The scientific and economic value of these studies is no subject of doubt: the sylvatic fauna of Romania is an important source of income, superficially exploited due to the fact that the biological needs and the sanitary status are merely known. Wild species are the reservoir of some disease common to domestic animals and the source of some zoonosis, so clearing out as many aspects as possible concerning their pathology is a major interest point.

The species of wild ruminants included in this study – the red deer (*Cervus elaphus*) and the roe deer (*Capreolus capreolus*) – have important livestocks in the North and North-Eastern area of Romania, the populations being generally bigger than the optimum livestock calculated for the particular hunting areas. Conservation and surveillance programs, food supplementation offered during wintertime lead to an increase in number and biological value of the populations. Even in this context, the mortality percent and the risk of disease transmission between wild animals and domestic livestock that graze freely in the forest is a reality, so a clear image on the sanitary status is essential.

Clearing some of these aspects is the main objective of this doctoral thesis.

The first part of the thesis, „*Literature review*", is made of 3 chapters that synthesize the main data of the speciality literature concerning the biology of the main wild ruminant species from North and North-East Romania (chapter I), the macroscopical and histological etiomorphopathology of the species (chapter II) and the main morbid entithies that affect them (chapter III).

The second part of the thesis, „*Personal research*", is composed of 2 chapters: chapter IV describes the aim and objectives of the research work, the materials used and the working methods applied, while chapter V describes the results of the research performed, including discussions, interpretations, analysis and partial conclusions. The conclusions synthesize the research work. The thesis is documented through 90 figures, 9 tables, 12 graphics and 206 bibliographic references.

Investigations were performed on 138 roe deers and 35 red deers. This study material was represented by animals prelevated during organized hunting sessions, or animals extracted from the population due to severe flows that exclude them from reproduction.

Necropsic examination was performed whenever possible; tissue and organ samples were prelevated for histopathological examination. Particularities of lesions due to death consequent to shooting were always kept in mind.

Most of the samples received in the laboratory were frozen; samples were prelevated in less than 4 hours after death and frozen immediately. Afterwards, they were transported at the Pathology Department of the Faculty of Veterinary Medecine Iasi. Some of the samples were fixed in 10% formaldehyde.

Necropsy was performed according to a well established work protocol: opening of serous cavities, *in situ* inspection of organic systems, evisceration and examination of all organs.

In most of the cases, the transportation of the whole carcass to the Pathology Laboratory was impossible, so necropsic examination was performed on the field. The organs that showed macroscopical lesions were sent to our laboratory, either fresh or frozen.

Histopathological examination was performed on permanent histological slides obtained through paraffine-embedding method, according to the following steps: prelevation, fixation, paraffine embedding, microtome sectioning, staining, mounting and microscopical examination.

Fragments chosen for prelevation depended on the lesional context and on the aim of the investigations. Representative areas at the edge of the lesions were preffered, so the tissue fragment includes both normal and modified tissues.

Approximate size of the samples was of about 1 cm^3: 15 mm in length, 10 mm wide and 5-7mm thick, so the fixative substance quickly penetrates the sample from all directions. After a 3-4 hours fixation, the samples were modelled with sharp blades, their final thickness being of approximately 4-5 mm; afterwards, they were introduced in fresh fixative.

Fixation was performed in glass or plastic containers with a volume of 100-200 ml, in which the volume of the fixative must be approximately 20 times bigger than that of the sample.

The universal fixative used was 10% formaldehyde solution. It is the chemical substance of choice due to its good ability to coagulate proteins and to its penetration speed, tolerance and long time frame for which it ensures preservation.

In order to extract calcium salts, Bouin liquid was used, composed of 15 parts picric acid, 5 parts formaldehyde and one part glacial acetic acid. Bouin liquid can be used for fixation immediately after sample prelevation, since previous fixation is not necessary.

Paraffine embedding has 4 phases: dehydration, clarification, paraffine impregnation and inclusion.

Histological sectioning was performed with the paraffine microtome that offers special conditions for obtaining 5-6 μm thick sections.

Sections were stained in Hematoxylin-Eosin- Methyl blue (HEM), May-Grunwald-Giemsa (MGG) and Periodic Acid – Shiff Fuxine (PAS) methods.

Wild ruminants livestocks from the counties included in this study had a continuously ascendent tendency, in most cases overpassing optimum livestock calculated according to the characteristics of the hunting area. For Botosani county, red deer livestocks remained below the optimum values.

The necropsies that were performed didn't allow the identification of general disease, the animals that were examined presenting an apparently good health status. Histological examinations showed some organic lesions, more or less severe, that were presented in the thesis structured on different organic systems.

The cardiovascular system showed myocardic sarcocystosis associated to areas of congestion and interfibrillar microhaemorrhages. A chronic fibrous pericarditis case was identified too. Miocardopathies were somewhat more frequent, manifested as degenerative lesions of the myocardic fibers to the stage where they were replaced by hyaline. In areas with older lesions, interstitial fibrosis phenomena were noticed.

Organopathies of the respiratory system were the most frequent lesions encountered. The predominance of lesions caused by parasitic infestations was noticed. The most frequent parasites encountered belonged to *Protostrongylus spp.*. Incriminated etiological agents were identified based on morphological characteristics and lesions they produced as belonging to *Protostrongylus* species (*Protostrongylus rufescens)*, nematode belonging to Fam. *Protostrongylidae*, that is localised in the alveolae, bronchiolae and small bronchiae and causes chronic bronchopneumonia. On the slides obtained from infested lungs we identified both parasite eggs as well as migrating larvae. In one of the cases, the tissular reaction associated to the presence of the larvae was extreme. Protostrongyl larvae are surrounded by pseudonodules of lymphohistiocytic prolifferation. Areas of vicariant subpleural emphysema are also noticed, adjacent to areas of pulmonary densifications. The presence of parasite eggs and larvae and the simultaneous evolution of an interstitial pneumonia lead to accumulation in the bronchiolar epithelium of necrotic cells, secretions and sometimes of what probably is immune hyaline.

On histologically examined fragments of lungs, transverse sections reveal the presence of strongyl larvae that generated a characteristic local reaction. Thus, the general aspect of lymphoid peribronchic hyperplasia and thickening of interalveolar interstitium is added a focalised infiltration of lymphocytes. The nodule forms a true compact parasitic granuloma, with a central area of structured necrosis where the transverse section of the parasite is also noticed, a

median area of epithelioid-gigantic prolifferation and a peripheric area of prolifferation with hystiocytes, lymphocytes, plasmocytes, fibroblasts and fibrocytes.

Variable quantities of mucus and fibrine were noticed inside the bronchiolae, that sometimes appeared obstructed with secretions. The extremely delicate bronchiolar epithelium shows reversible lesions, with degenerate and exfoliated cells that float free in the airwave.

Another case showed a severe pulmonary congestion, with the ectasy of blood vessels that appear filled with erythrocytes. Bigger or smaller areas of pulmonary emphysema were noticed, associated to the lesion.

Cases of acute catarrhal bronchopneumonia were identified, with hyperemia, mucus hypersecretion, infiltration and laceration of the mucosa by the accumulation of serous exsudate. The more or less accentuated activation of the lymphoid tissue associated to the airwaves was also noticed, either diffuse or nodular, in some cases the diagnostic of lymphohystiocytic bronchopneumonia being more fitted.

The presence of hyaline membranes in wild ruminants must also be mentioned. In areas limitroph to fibrinous bronchopneumonia lesions, the alveolae are filled with hyaline membranes, with the aspect of PAS-positive moulds that contain nuclei of descuamated cells and of cells migrated from interalveolar spaces.

Macroscopical aspects of fibrinous bronchopneumonia were noticed. The lungs are slightly distended, with a characteristic mosaic aspect on the surface of the pleura and on the section surface, a slightly increased consistence, a relatively dry section surface. The docimasy is positive. These lesions are characteristic to fibrinous bronchopneumonia, with all the phases evolving simultaneously in neighbouring territories.

Fibrinous bronchopneumonia identified in the case of pasteurellosis is also associated to fibrinous pleuresia. In some areas, the inflammatory process has already reached the final stage, with predominantly fibroblastic hyperplasia, that causes the thickening and colagenisation of the pleura, that appears folded and covered by a predominantly cellular superficial exsudate.

In the case of an approximately 7 years old roe deer, a form of visceral necrobacillosis was diagnosed, with pulmonary, hepatic and testicular involvement.

Grey-yellowish foci of variable dimensions were noticed on these organs; the diamensions of the lesions were variable but didn’t overcome 2-2,5 cm. When sectioned, a relatively high consistency exsudate appears, with an unpleasant odour.

Histologically, necrotic bronchopneumonia was diagnosed, with structured necrosis areas, where the normal architecture of the pulmonary alveolae and interalveolar tissues can still be identified, and areas where the evolutive process has completely errased the specific structure of the tissue, replacing it by an amorpheous pulverulent mass of cellular debris.

The digestive system showed slight intestinal infestation with oocysts of *Eimeria spp.*.

The liver was the center of an active congestion characterised by excessive accumulation of blood both in sinusoid capillaries and in between the hepatocytes, Remak cordons appearing to be slightly compressed.

In one necrobacillosis case, necrotic, grey-yellowish foci were noticed on the surface of the liver. Necrotic hepatitis was observed in both phases of structured, then unstructured necrosis. In the first case, the persistence of degenerate Remack cordons was noticed, while in the latter, the hepatocytes are completely gone, the plasmalema is fragmented upon disappearance and the nuclei completely disintegrated.

The kidneys were the center of the parietal thrombosis of a renal arthery, with a thrombus in the organisig stage, that caused a 50% reduction of the diameter of the arthery. In another deer, urinifery tubes were affected by granular dystrophy, with the presence of hyaline cilinders, as testimony of the proteinuria. The glomerules showed lesions of membranoprolifferative glomerulonephritis, characterised by hypercellularity due to prolifferation of the glomerular capillary endothelium, thickening of the basal membrane and of the mesangium of the sanguine capillaries. In severe affected areas, interstitial fibrosis was noticed, an indicator of chronic glomerulonephritis.

Another case showed lesions of coagulation tubular necrosis of the renal parenchyma, with severe envolvement of the tubular epithelium and apparition of intraluminal proteic debris, caused by local ischemia or by the intervention of nephrotoxic factors.

The locomotor system, the muscles respectively, were all infested with *Sarcocystis spp.*. The infestations were moderate or severe, the infestation degree ranging between 3 and 21 sarcocysts for each histological section.

Necrobacillosis lesions were also identified at testicular level, expressed by areas of exsudative orchitis that evolves towards necrotic orchitis, with wide spread tubular necrosis.