

ABSTRACT

CONTRIBUTIONS TO THE STUDY OF NEUROPATHIES IN RUMINANTS AND SWINE

The researches of this work have two basic purposes: to specify the topography of some lesions and determination of their nature, and in addition, the necessity of a greater knowledge about the cellular and tissue responses triggered in the nervous system of different physical, chemical and biological aggressors, in order to complete the gaps in comparative neuropathology .

The localization of lesions in the nervous system is remarkably precise and its knowledge is essential for diagnosis and treatment. For this purpose, the confrontation of clinical and laboratory data is essential (obtained in particular by methods like angiography, scintigraphy and ventriculography) with data on various nervous centres and nerve fascicles morphology, knowledge come over time to a remarkable degree of perfection that allows use of the stereotaxic methods for some very precise neurosurgical intervention.

The investigations mentioned above, are applied in our country with preference on human patients, in veterinary examination the most common is histology exam. The study of CNS pathology involves some difficulties, namely: NS is an organ where access is difficult, well protected from the actions of external factors; the special sensitivity to anoxia and richness in hydrolytic enzymes determine a very early installation of cadaverous changes; the sensitivity to autolysis (autolytic processes, which can mimic the histological lesions) and mechanical artefacts; in most cases, the diagnosis of certitude requires histological examination.

The PhD thesis includes 279 pages and 426 colour photographs and is divided into two distinct parts: Part I, which contains bibliographic data on the theme and part II, contains the personal research performed during the preparation of this thesis.

In the first part of the thesis, extended on 88 pages, one can find the stage of the knowledge of the central (CNS) and peripheral (PNS) nervous system morphopathology, presented as a synthesis based on 213 articles, manuals and compendiums of neuropathology, and other 29 neuropathology and neurobiology compendiums and journals accessed on internet. The synthesis proves the high level of

neuropathological knowledge in the human medicine, facilitated by the thorough research using modern means of investigation and the satisfactory level of information provided by the veterinary literature in this field. The material is richly illustrated with 156 macroscopic and microscopic color photographs, forming the needed database for the orientation and interpretation of the personal observations and their evaluation.

The first chapter, entitled „Non-inflammatory and non-tumoral changes of the central nervous system (CNS)”, includes 47 pages, contains the description and illustration of the cadaverous changes, growth disorders, circulatory, dystrophic, necrobiotic and necrotic lesions of the nervous substance and ends with the elementary injuries of the nervous system’s components in human and domestic animals, also referring to the most accepted hypothesis, observations and experimental confirmations on the etiopathogenesis of the morbid processes. The theoretical dissertation is sustained by 98 macroscopic and microscopic demonstrative photographs.

The second chapter, entitled „The morphology of the inflammatory processes in the CNS”, extended on 18 pages and illustrated with 32 colour photographs, is destined to the brief presentation of the inflammatory process according to the classical classifications, in correlation with the topography, extension and typology, using the nomenclature recommended by OMS, specifying the anatomopathologic and histopathologic particularities of recognition and differentiation of the haemorrhagic and haemorrhagic-necrotical, serofibrinous, purulent, tromboembolic, lymphohistiocytic, eosinophilic, fibrous, granulomatous and immune (allergic and demyelinating) inflammations.

The third chapter, reserved for the tumoral changes of the CNS, extended on 15 pages and illustrated with 26 macroscopic and microscopic demonstrative photographs, contains the description of the oncopathies, thorough fully studied in human, yet not completely known in animals, whose identification is based on the morphological and evolutionary similarities. Knowing them is absolutely necessary to establish the onco-epidemiology and to establish the interspecific oncologic relationships. The thesis presents the classifications and the different types and subtypes of tumors, expansive capsulated and not capsulated, and infiltrative, circumscribed and diffuse, the most frequent of them.

The original part of the thesis, extended on 191 pages, illustrated with 270 colour photographs, is destined to the personal research, carried out between 2002-2009 on CNS from many species of ruminants and pigs from livestock overseen and monitored for TSE, dispatched to the Sanitary Veterinary and Food Safety Laboratory from Iași in order to diagnose the rabies and the viral respiratory or nervous infections. The CNS was selected due to the request for elucidate the morbid episodes with nervous symptoms and different etiology. Additionally, the cadavers sent to the laboratory to specify the cause of death were also examined.

Chapter VI presents the analyzed material submitted to the anatomoclinical, necropsic, microscopic examination and to other laboratory researches. The material was represented by the CNS fragments collected from 10904 cattle, 3187 sheep, 77 goats, 223 cervidae, 1 buffalo and 595 swine. For every case observation sheets with a detailed case history and/or necropsy papers and case studies were

elaborated, the macroscopic lesions were photographed and 5-15 fragments from several levels of the CNS were collected. The methods were many and different according to the purpose and included: classical and special techniques of brain sampling, biopsy material handling methods for making impressions and permanent sections, conservation of duplicate tests fixation, processing (section at 5µm) and of staining the sections in HE, HEA, PAS, MGG, Pappenheim, Congo Red, Mann and Gomori methenamine silver staining. Some materials were submitted to the electronic microscopy study of the viral inclusion bodies. Also, bacteriological exams were performed on usual and special aerobic and anaerobic culture media, serological (Elisa enzyme immunoassay to detect specific antibodies for the Infectious Bovine Rhinotracheitis - IBR, and the Aujeszky disease virus), virological (identifying the antigen using the fluorescent antibodies test - IF, virus isolation by inoculating embryonated eggs), toxicological exams, experimental infection on animals and the fast test TeSeE Bio-rad to determine the prionic protein PrP^{res} from the nervous tissue of the domestic and wild ruminants were performed.

In chapter VII are presented and illustrated the personal histopathologic findings on CNS regarding the fundamental pathologic changes of the nervous tissue (the elementary lesions of the nervous cells, of the nervous fibres, of the cerebral vessels, circulatory disorders, dystrophic disorders with finality in necrobiosis and necrosis and inflammatory processes). These changes are generally overposed to those mentioned in the neuropathological literature and were also described the conditions of their record.

The nevrax cytopathology is dominated by neuronal lesions, nonspecific and specific, which is an ensemble of alterations that might occur during a wide range of aggressions: traumatic, toxic, infectious or of vascular nature.

The elementary lesions are referring to the responded adaptive changes and pathological changes of the pericarions and expansions, glial and ependymal cells. The stick cells, gemistocytes (illustrated by original microscopic images), lipophages and granular bodies, the chromatolysis, the atrophy, tumefaction, ischemic and colliquative necrosis of the neurons, fatty-pigment bodies and nonspecific inclusions are described with priority. The liquefying necrosis of the nervous cells, also called Nissl's severe cellular disease develops upon lesions of cellular tumefaction during the evolution of some exogenic toxicosis, septic or carential state. The liquefying alterations of the cytoplasm are directly connected to the pericellular and neuropil oedema, and perivascular oedema. The lesion was observed in one cow with anaerobic sepsis.

The most frequent an important degenerative modification is the vacuolar dystrophy of the neurons, glial cells and neuropil, which is the essential lesion for the TSE diagnostic, used along the specific clinical symptoms the diagnosis of scrapie in sheep, which will further be confirmed in the National Reference Laboratory for TSE from IDSA Bucureşti. The vacuolar dystrophy is the dominant component in the "spongiosus status" syndrome, has a polifactorial etiology, but is not characteristic for TSE, in the lack of further investigations.

Within the circulatory disorders are distinguished three types of oedema: vasogenic, toxigenic and oncotic, whose recognition is helpful in the etiopathogenetic orientation of this morbid process.

The most important are chapters VIII-XII, reserved to some diseases with a very different etiology, for every morbid entity noting the natural background where it occurred, the affected species, symptoms, the evolution and the end of the disease, the methods used for diagnosis, the pathological and microscopic lesions, differential diagnosis, finally adding a review of citation of comparative pathology.

Chapter VIII (subsection 8.1.), regarding the histopathologic aspects in the encephalomyelitis produced by the enterovirus type 1 in pigs is based on studies performed on 62 cadavers of weaning and young pigs from several livestock with conclusive clinical symptoms of Teschen disease, illness with maximum receptivity at 3-4 months of age and 80% mortality of sick animals. The macroscopic lesions were discrete and sometimes consisted in meningeal congestion, seldom petechial haemorrhages in the cerebellous cortex, circulatory disorders in major organs, cerebriform aspect of intestinal mucosa, gastric mucosal folding in some corpses and rarely stomach ulcers.

The histological examinations revealed in enterovirus type I encephalomyelitis the presence of cerebellar and spinal proliferative meningitis, polio- and leuko- cerebellitis lesions overposed on congestions, microthrombosis, microhemorrhages, oedema and malacia. Productive inflammatory lesions, of a smaller intensity, are also present in the cerebral trunk, spinal bulb and the cerebral cortex. Important lesions for the diagnostic are in the lumbar marrow, more severe in the gray substance than in the white substance, consisting in neuronal degenerations, diffuse mononuclear infiltrations, glial nodules, lympho-plasmocytic perivascularitis and foci of malacia. Personal observations bring completion to the description of the disease by signalling polio- meningocerebellitis and spinal polio-meningomyelitis and defining their role in diagnosis, and electronmicroscopy examination revealed multiple intranuclear viral inclusion bodies.

Rabies (subsection 8.2.) was diagnosed in nine adult cattle, one calf and one pig, using 3 laboratory tests: FAT, histological and experimental infection on white mice. In bovines were histologically identified non specific circulatory disorders, and also perivascular ring-shaped haemorrhages, considered typical for rabies infection, small or medium degenerative lesions of the Purkinje and ganglion cells of Ammon's horn, and minor glial-type proliferative lesions.

In ruminants, animals particularly susceptible to rabies infection, rabies nodules are very small and rare and the stages of microgliosis, satellitosis and neuronophagy are discrete, proportional to the degree of neuronal alteration, lower than in other species (carnivores) while inclusion genesis can be remarkable.

In the cattle investigated the meningoencephalitis lesions were discrete or absent.

The genesis of the corpuscles is always present in the cerebellum (although not all sections) and inconstantly in Ammon's horn and in cerebral trunk. The intracytoplasmic Babeş-Negri inclusion bodies appear in the perikaryons and in the cells' expansions, are acidophilic, surrounded by a thin halo, single or multiple, unequal, round- and oval- or blackberry-shaped, homogenous or with basophilic Volpino grains, sometimes symmetrical (placed "in mirror"). In calf, the specific inclusion bodies were present both in the cerebellum and in Ammon's horn.

The inclusion genesis is inversely proportional to the degree of neuronal degeneration and inflammatory changes in nervous tissue.

In pig, the regressive lesions and inflammation hyperplasia of neurons located in Ammon's horn, cerebral trunk, cerebellum and meninges are broader and expressed as perivascular muffs, diffuse gliosis, glial nodules with the stages of satellitosis and neuronophagia, leptomeningitis and choroiditis. The inclusions being absent in the examined histological sections, the diagnosis was established based on FAT tests and mice experimental infection. It is the first description of rabies in pig.

For diagnosis of IBR-IPV infection, serological, bacteriological and histopathological investigations were performed in 8 cattle and a calf, demonstrating the respiratory complications with *Staphylococcus ssp.* and *Pasteurella spp.* in 6 cases and with an anaerobic sepsis in one case. The dominant pathological lesions were exsudative tracheobronchitis and bronchopneumonies of different morphological types (purulent), associated with extensive pulmonary emphysema, petechial haemorrhagies, degeneration of parenchymal organs in adults and hyperemia of the snout ("the red nose disease"), parenchymal keratitis, necrosis, erosions and ulcerations of the superior digestive tract, in calf.

The histopathologic examination of the brain from 2 bovines infected with IBR without complications, demonstrated lesions of nonsuppurative meningoencephalitis, lesions more severe in the bulb and the Ammon's horn, as gliosis, glial nodules and perivascular lymphohistiocitar muffs of different thickness. The acidophilic intranuclear viral inclusion bodies were observed in the neurons from the cerebral trunk and in Ammon's horn and in the glial cells from the perivascular muffs.

The additional lesions produced by the association of the IBR-IPV virus with the anaerobic septicemia was bacterial embolism, vascular wall necrosis with intramural and perivascular emphysema, invading oedema, demyelination and malacia foci.

The retraction of the cell body and cytoplasm homogenisation, the condensation of the nucleus with perinuclear space enlargement, chromatin arrangement in circular and semicircular layered filaments, under the cariolema, are aspects of the cortical hypercytchromia from the beginning of apoptosis, the images being a novelty for the veterinary pathology.

Scrapie of sheep was suspected for the first time in our country in 2002, on two sheep, based on the neurological clinical signs, with the prevalence of paralytic stage and on histopathological observations seen in the context "status spongiosus" syndrome. Further investigations were performed on 17 sheep from the same owner, who reacted positively to rapid ELISA assay (BioRad), the diagnosis being set based on electrophoresis and western blotting by the National Reference Laboratory for Transmissible Spongiform Encephalopathies of Institute for Diagnosis and Animal Health Bucharest, and immunohistochemically confirmed at the World Reference Laboratory for TSE VLA, Weybridge, United Kingdom, validated by IDSA. Histological lesions identical to those seen in the first two cases correspond to those described in foreign literature and consist of vacuolation of neurons, of extensions and neuropil, associated with variable gliosis and other changes of the nervous substance.

Chapter X refers to the morphopathology of some bacterial diseases, microbiologically diagnosed in the laboratory, the author's comments are outlined in subsections for listeriosis in sheep, acute septicemia with *Erysipelothrix rhusiopathiae* in pig and anaerobic enterotoxic syndrome in young pigs.

Listeriosis was studied on 5 sheep, on CNS segments (pons, medulla oblongata, cerebellum, quadruplet bodies, cerebral cortex, mesencephalon). The histopathological lesions of listeriosis are characterized by the association of proliferative lesions with suppurative ones. Extravasation begins by diffuse infiltration of neutrophils as cellular aggregates or becomes microabscess, very rarely encapsulated abscess. The lymphohistiocytic proliferation is followed by satellitosis around the degenerated neurons, neuronophagies and the formation of the glial nodules and perivascularitis. The enzymatic liquefaction of the nervous substance is recognized by microcavities and deposits of cholesterol resulted by demyelination in the suppuration areas. The other signs are of meningitis, peri-ependymitis, plexichoroiditis and pyogenic encephalitis.

The acute swine erysipelas, diagnosed in 4 cases by specific laboratory methods, manifested microscopically by vascular lesions, represented by hyperaemia, septic thrombosis, oedema, microhaemorrhages, DIC, capillary embolisms with free and phagocytated germs. Endothelial swelling and vacuolation, degeneration and fibrinous necrosis of small arteries and arterioles, the invasion of the vascular walls by leucocytes, the leukocytoclastic vasculitis and the fibrosis are characteristics for the evolution of the immune periarteritis nodosa.

The degenerative-necrotic parenchymal lesions consist of central chromatolysis, ischemic necrosis, vacuolation of neurons, glial cells and neuropil, apoptosis and focal leucomalacia. The inflammatory lesions consist in mixed cell infiltration, lymphocytes, macrophages and glial cells, with satellitosis, glial nodules and fasciculate dendrogliosis. Phagocytic activity against bacteria, erythrocytes, lipids resulted from demyelination and the apoptotic bodies are very active.

Anaerobic dysentery of piglets (anaerobic enterotoxic syndrome) was histopathological investigated in 53 pigs, out of which only 5 were infected with *Clostridium perfringens*, the diagnostic being set by isolation on specific culture media and confirmed by positive experimental infection on white laboratory mice and Guinea pigs, VF supernatant producing dermonecrosis. The pure infection produced congestive-haemorrhagic and dystrophic lesions in cerebellum, pons, mesencephalon and the cortex. Microscopic lesions in cases of simple infection with *Clostridium perfringens* are dominated by the porosity and cerebral lacunarism. The fine, spongy vacuolisation is observed both in the neurons and glial cells (especially in astrocytes). In mixed infections involving *Clostridium perfringens* and aerobic Gram-positive and Gram-negative germs, sponginess is complicated by meningitis and polymorphous inflammatory cell infiltration, more marked in the cerebral trunk.

The anaerobic enterotoxic syndrome lesions observed in piglets, uncomplicated form, fall in *spongiosus status*.

Chapter XI, entitled „The morphopathology of CNS in some parasitic diseases” is destined to the coenurus infections, oestrosis and sarcocystosis.

Examining the encephalon in 45 sheep and 7 goats, coenurus infections has been diagnosed in meningeal and encephalic localisation, characterized by traumatic encephalitis and fibrinopurulent meningitis. The microscopic lesions consists in migration lesions: haemorrhagic necrotic and suppurative trajectory and lesions of the localisation stage, cystic parasitic granulomas in whose walls are gigantic cells, epithelioid cells, neutrophils and eosinophils PMN, monocytes- macrophages lymphocytes, plasma cells, stick-glia, astrocytes, lipo-macrophages, gemistocytes, granular bodies. At some distance are vacuoles, vesicles and leuco- and polio- encephalomalacia cavities.

The coenurus infection in goat, described for the first time, induces a more ample inflammatory reaction. The cyst cavity contains pus, germs, calcium precipitates, and the peripheral layer of the adventitia contains fibroblasts, fibrocytes, neocapillary and collagen fibers. At a distance, leptomeningitis and dissecting aneurysms occur.

In the supraethmoidal intracranial oestrosis, discovered in 4 sheep, not described histologically in the literature, meningeal lesions are associated to the encephalic ones, consisting in multiples micro-abscesses with polymorphous cells, including eosinophils, plasma cells, granular bodies, gemistocytes and glial cells, purulent perivascular cuffs and fasciculated demyelination. WBC joints are identified also around the ependymal channel penetrating epithelium and reaching the lumen. In the nervous tissue there are many small vacuoles containing oedema fluid and proteic precipitates or microcavities with foamy material representing decrepitude areas soaked with water and various fats from myelin degradation.

Sarcocystosis was diagnosed in 2 sheep from a lot of 24, with lesions in the mesencephalon, pons and cortex, characterized by the tumefaction and proliferation of the endothelial cells, medial fibrinoidosis, thrombosis, oedema and perivascular haemorrhages, gliosis with satellitosis, neuronophagia and glial nodules. The parasitic elements, highlighted in PAS staining, were present in the endothelial cells, in the cytoplasm of the neurons and glial cells, free and as sarcocysts in the white substance. The differential diagnosis concerns toxoplasmosis, whose infecting elements is PAS-negative and produce, granulomas with calcifications and scar aspects in the chronic evolution.

Chapter XII, entitled "CNS Pathology in plant toxicosis" describe plant poisoning with green alfalfa (*Medicago sativa*) with sedge (*Carex* spp) and yellow iris (*Iris pseudocorus*), whose microscopic lesions are not presented in the studied specific literature.

The vegetal intoxication with alfalfa (*Medicago sativa*), plant accumulators of nitrate, was investigated in 10 cows - dead or slaughtered after a foamy indigestion. The anatomopathological exam revealed a softening of the nervous substance, and the histopathological examination revealed micro-vacuolization and progressive liquefaction of the neuronal cytoplasm and nucleus from the cerebral trunk, vasogenic and cytotoxic oedema, multiple, big decrepitude outbreaks, well-defined and confluent areas up to pseudocystitis, lipophages and granulous bodies.

The intoxication with sedge (*Carex* spp.) and yellow iris (*Iris pseudocorus*) was investigated on the cerebral trunk, cerebellum and mesencephalon. The microscopic examination reveals microthrombosis and necrobiosis of the Purkinje cells, gliosis in the cerebellum, the atrophy of the

cerebellous blades and the melting of the granular layer and of the white substance, cytotoxic oedema, sponginess of the white substance from the cerebral trunk and peri-ependymal leukomalacia.

The thesis ends with 35 conclusions and four synthetic tables, one for each studied species, where are listed the researched diseases, the number of cases histopathological investigated, laboratory methods used for diagnosis of certainty, processed sections of the nervous system, microscopic lesions concordant with bibliographic information and personal scientific contributions.