SUMMARY OF THE Ph-D THESIS
LABORATORY STUDIES REGARDING THE CENTRAL NERVOUS SYSTEM IN SHEEP, FOR SUPERVISION AND DIAGNOSIS OF NERVOUS DISEASES

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Summary

Infectious, traumatic, toxic or degenerative factors from other organs, can induce pathognomonic lesions in the central nervous system structures (e.g., rabies, scrapie) or cyto-histological changes in the organization of complex nervous tissue and cause pathological processes characterized in particular by changes in neurons and glial cell elements at that lesions associated with diagnostic significance in conjunction with epidemiological data and other laboratory investigations.

The histopathology is aimed at highlighting the key structural changes in the morphological and histological appearance, in order to identify the mechanisms that generate local disturbances and their impact on the whole body, correlating clinical manifestations of sick animals (when they were captured) with lesions detected.

Structural changes, highlighted by macroscopic and microscopic examinations, are leading to the interpretation of injuries caused by pathogens or aggressors, prioritizing their contribution to the development of lesions in dynamic or cumulative effect when it exists.

Nervous forms are frequently reported in sheep, but also very uniform in clinical manifestation and pathology. For this reason, examination of histopathological changes of the central nervous system to determine the frequency, preferential localization, the predominant type of lesion, or the discovery of specific etiological injuries have practical importance in differential diagnosis.

Transmissible spongiform encephalopathies, along with other major zoonotic disease localized to the nervous system in sheep, rabies, are diseases for which laboratory investigations of the central nervous system in sheep are mandatory – specified in the program of supervision, prevention, control and eradication of animals diseases, those transmissible from animals to humans, developed the "National Sanitary Veterinary Authority, Bucharest".

Since 1990 the European Commission adopted a series of measures to protect human and animal health, the risk of contamination with transmissible spongiform
encephalopathies (TSEs). These measures were based on the safeguard provisions of the Directives on veterinary control measures.

Given the magnitude of risk posed by certain TSEs, the Commission provides for increased monitoring programs for goats and sheep, following detection of cases of bovine spongiform encephalopathy (BSE) in a goat in 2005 and three unusual cases of TSE in sheep, from which BSE could not be excluded.

Research structure. The thesis comprises 248 pages and is structured in accordance with current legal provisions; the content includes a number of 121 figures (pictures, graphs, charts) and 23 tables. In Part I, are the most important and conclusive data in the literature concerning the histology, physiological and cytopathological regarding the nervous tissue and aspects of transmissible spongiform encephalopathies in small ruminants - prion etiology, pathogenesis, clinical and laboratory diagnosis. Part II includes personal research, laboratory studies aiming to monitor scrapie (Chapter II.1), histopathological study (Chapter II.2) of the central nervous system, conducted on a fixed number of brain samples in order identify changes/damages of the nerve tissue, other than those specific to transmissible spongiform encephalopathies and finally to confirm two outbreaks of scrapie (Chapter II.3). The bibliography includes 201 titles of scientific papers from domestic and foreign literature.

Laboratory studies to monitor the scrapie

Chapter II.1. Evaluates the results of laboratory investigations towards scrapie, comparing each diagnostic method, in terms of sensitivity and specificity, ie detection and quantification of abnormal PrP<sup>res</sup> prion protein accumulation in the nervous tissue, resistant to proteinase K, by immunoassay tests and highlight the presence/absence of lesions caused by the conversion of PrP in PrP<sup>res</sup> and its accumulation, by histopathology.

The study looked at lab tests on brain samples from sheep, for supervision and / or monitoring of transmissible spongiform encephalopathies in small ruminants in Cluj County, between years 2004-2010.

During the period under study, we examined a total of 1540 sheep, observing the "target" groups established by legislation on TSEs.
Thus, a total of 610 samples came from dead sheep, 594 samples from emergency slaughtered sheep (in this category enrolling and sheep slaughtered for diagnostic confirmation or for the need of elimination from the population, as those with a diagnosis of infectious epididymitis, Maedi-Visna) and a total of 336 samples came from clinically healthy sheep slaughtered for human consumption (traditional slaughter for family consumption).

Following the technical details of the Program for the eradication and monitoring of transmissible spongiform encephalopathies, for the brain samples collected from dead and from the emergency slaughter animals (except those unfit for histological processing) rapid screening was performed and histological examination according to specific procedure.

Rapid screening method was performed by BIO-RAD TeSeE, which is the validated method of LSVSA Cluj histopathology laboratory, and accredited by National Accreditation Body of Romania (RENAR.). It is an immunoassay method which uses two monoclonal antibodies that allow the qualitative determination of abnormal prion protein PrPRes, resistant to proteinase K, in the central nervous tissue after distortion and protein concentration.

Histopathological, for each sample 20 sections were examined from the spinal bulb, midbrain, cerebellum, cerebral cortex and spinal marrow, processed by paraffin technique and hematoxylin eosin stained.

Thus, out of 1540 samples undergo brain examination by rapid tests, a total of 541 were subjected to histopathological examination for evidence of lesions characteristic to scrapie, as follows: 330 samples of brain taken from cadavers (dead sheep on farms, households, on pasture, during transport, slaughter, or in case of accidents, regardless of their nature), 208 brain samples taken from emergency slaughtered sheep (in which ante-mortem clinical examination established the presence of nervous symptoms, or that cannot move, recumbent, unable to stand or walk without assistance; sheep identified by the physician, with ante-mortem clinical signs of disease, regardless of disease: infectious diseases, etc.) and a number of 3 brain samples taken from sheep slaughtered for human consumption (family).
Of the 1540 tests immuno-enzymatic tests in 1527 showed optical densities below the threshold (cut-off), thus recording the first negative test results. A total of 13 tests were initially located just below the optical density threshold and were retested in duplicate from the original homogenate. For all 13 cases the results were negative for both duplicates.

The 541 brain samples, processed through paraffin for histopathology, revealed no specific lesions of transmissible spongiform encephalopathies, in none of the examined fragments of nervous tissue.

Following these tests (rapid screening, histopathology) has been established that the brain samples analyzed in 2004-2010 came from sheep free of transmissible spongiform encephalopathies (classical scrapie, atypical scrapie).

**Histopathological study**

Chapter II.2 presents the results of a histopathological study of the central nervous system of a fixed number of brain samples (100) processed histologically for monitoring scrapie, following the analysis of incidence changes/injuries (other than the specific TSE) in the central nervous system, the "target" categories of examined sheep, their classification according to the predominant histological features, development of a comparative scale assessment, of the distribution and frequency of microscopic lesions in various parts of the nervous tissue examined, establishing a correlation between changes/lesions identified and health and/or any distance damage. The study identified changes / lesions of different types of nervous tissue in 73% of the examined cases, grouped according to the prevalence of degenerative histopathological aspects, vascular, non-inflammatory or inflammatory cell reaction.

Briefly, the results of microscopic examination, identified degenerative changes of the nerve cell body (changes in the nucleus, cytoplasm), vascular disorders (edema, congestion, hemorrhage, proliferation of endothelial cells, vascular thrombosis), cellular responses (infiltration with mononuclear infiltration of granulocytes , proliferation of glial cells, the macrophages, giant cells present), the presence of viral inclusions, images of cell death (chromatolysis, satellitosis, neuronophagia), metabolic disorders with accumulation of pigment, presence of parasitic type granulomas.
Making a descending hierarchy of the types of changes/lesions in different segments of the central nervous system for the 100 reviewed cases, were found the following:

The most frequent injuries were the vascular type, the result of microscopic examination is a total of 177, in which images of edema were encountered 84 times, 53 times of congestion, hemorrhage 8 times, in 29 images the vascular endothelial cell proliferation was captured, and 3 images of thrombosis.

In the hierarchy, are degenerative changes perykarions with a total of 93 images of "chromatolysis" (45 images that often could be interpreted as nonspecific indicator of degeneration combined with loss of tinctorial affinity), granular and vacuolar degeneration (31) and neuronal atrophy with loss of cytoplasmic volume and reduced in volume (17).

Cellular reactions were found in total of 84, and consisted of mononuclear infiltration (37), granulocyte infiltration with neutrophils (16), gliocytosis (14), infiltration with eosinophils (13), and proliferation of macrophages (2) presence of giant cells (2).

Viral inclusions were captured by two times, intracytoplasmic located. Were captured: 28 images with cell death with satellitosis (11), neuronophagia (9), and tigrolysis (8).

Melanic pigment accumulation was found 3 times in the leptomeninges.

Analyzing the distribution of types of changes/lesions on the sheep categories, the dead and the emergency slaughter represents 97% of the study material, as following:

In both sheep categories, vascular lesions predominated, followed by degenerative changes of perykarions.

In the case of brain samples from emergency slaughtered sheep, the cell responses were recorded for all cell types (mononuclear, neutrophil granulocytes, glial cells, eosinophils). The presence of macrophages and giant cells, parasite-like was found equally in both categories.

Images of cell death were more common in emergency slaughter sheep, compared with histological preparations from corpses.
Melanic pigment accumulation was seen in histological preparations from corpses, but was not seen in preparations obtained from emergency slaughtered sheep.

Parasitic granulomas were found equally in the two categories of sheep.

On histological examination, from sheep slaughtered for human consumption, were met: chromatolysis type images (2), congestion (2), infiltration with mononuclear (2), edema (1), satellitosis (1), melanosis (1).

Statistical evaluation of location within the main types of microscopic lesions in the central nervous system examined segments revealed the following:

Degenerative changes of perykarion, primarily affecting the spinal bulb (22.31%), then about equally cerebral cortex (19.83%), cerebellum (19%), pons (18.18%) and quadrigeminal tubercles (16.53%). In a much smaller extent, were affected neurons in the hippocampus or cervical spinal marrow.

There is an increased incidence of non-inflammatory lesions consisting of edema, congestion, hemorrhage, especially in the quadrigeminal tubercles (29.37%), bulb (22.03%), cerebrum (19.21%), cerebral cortex (12.99%) and pons (11.30%), while their prevalence is much lower in cervical spinal marrow (3.38%) and hippocampus (1.69%).

If inflammatory lesions, spinal bulb was the most affected, the proportion of 34.14%, then quadrigeminal tubercles, in a proportion of 24.39%, cerebellar cortex and cerebral cortex at a rate of 19.31%, respectively, 12, 50%, much lower percentage of inflammatory lesions were located: in the hippocampus up to 4.55%, spinal marrow and pons, each with 3.41% and 2.27%.

Melanic pigment deposition was identified in 100% of cases, with localization in leptomeninges, in the cerebellum and cerebral hemispheres.

There is no statistically significant difference, in terms of frequency of localized changes/lesions in the nervous tissue segments examined. Bulbs and quadrigeminal tubercles are mostly affected, while the hippocampus and spinal marrow are affected in the slightest degree. Cerebral cortex and cerebellum are changed approximately with the same frequency. The pons stands the presence of degenerative changes and non-inflammatory injuries of perykarion and to a much lesser extent inflammatory lesion occur.
Vascular lesions were predominant in 41% of cases, followed in the hierarchy by inflammatory lesions, with a rate of 16%. These include inflammation of parasitic nature (8%), bacterial inflammation (6%) and inflammation of viral nature (2%). For 11% of cases, changes were identified whose etiology could not be determined and were attributed to diseases and pathological conditions in the visceral organs, such as metabolic, infectious and/or parasitic. Accumulation of melanic pigment was observed in 3% of cases and changes in cerebrospinal fluid in 2% of cases. No changes were observed in nerve tissue in the central nervous system segments examined in 27% of cases.

Non-inflammatory edema was diagnosed as the predominant change in 37 cases, and consisted of images showing the enlarging of Wircoff-Robin space, sometimes with the presence of poorly colored liquid with vacuolar appearance, capillaries or arterioles having a reduced lumen due to transudation. Based edema, is the vascular endothelial injury with a severity that increase vascular permeability. The fluid will disperse between cells in response to hydrostatic pressure from the movement of the brain and from other tissues.

They found differences in susceptibility to edema, between gray and white matter and the vulnerability of different areas was also different and consisted of images of intracellular edema, hypertrophy translated into astrocytes, oligodendrocytes accompanied sometimes by the reaction of the white matter (nucleus increased, less dense, hypochromic) vasogenic edema (intercellular) without major changes in the gray matter, but white matter, which provides a low resistance to passage of edema fluid, is textured with vacuolar appearance.

Frequently, there was swelling in the ependymal channel epithelium, epithelial cells are invaded by liquid, cell nucleus pushed to the apical pole, sometimes pyknotic, other times the epithelial cells were detached, and the substance surrounding nerve had spongy appearance.

Cerebellum, particularly in the area of Purkinje cells, was the seat of the displace and compress of the cells. Often, the swelling was accompanied by a discreet gliocytosis and/or lymphocytosis.
Hemorrhage, has prevailed in four cases, the issues encountered by diapedesis, bleeding without specificity in the topographic distribution with perivascular remaining erythrocytes, with or without hypertrophy and endothelial cell nucleus, and bleeding per rexis with bilateral and symmetric location in the cortex brain. For two of these cases, the bacteriological examination was positive for Clostridium spp, and another case, showed this massive infestation of Oestrus ovis larvae that were in the circulation that reached skull. Images of edema and hemorrhage were not accompanied by inflammatory changes.

Attention was paid to central nervous system inflammatory lesions, very important for veterinary pathology since most of them are fatal and that histological examination should guide or determine etiologic diagnosis.

Parasitic inflammation represented half (50%) of the inflammatory identified lesions. Bacterial inflammations were diagnosed in 25% because of Listeria and other purulent inflammation 12.5%. Viral inflammations have been identified in a proportion of 12.5% due to rabies.

Histopathological diagnosis of parasitic encephalitis was correlated, in the case of 6 emergency slaughtered sheep, with nervous signaling disorders, with spins in the riding and balance disorders as a result of compression exerted in the cerebral hemispheres, respectively cerebellum or spinal bulb.

The picture was pathologically correlated with the stage of disease evolution, showing a pronounced polymorphism. We identified the presence of neutrophilic and eosinophilic infiltrate, in addition, in two of the cases, parasitic granuloma with giant cell, vascular-conjunctiva and macrophage reaction with the cytoplasm 'sparkling', the remains of parasites, three of the cases were diagnosed as parasite migration encephalitis, with irregular foci of necrosis, eosinophilic infiltration, the two encephalitis cases were accompanied by leptomeningitis eosinophilia. Damage caused by parasites was found in the bulb, quadrigeminal tubercles, cerebral cortex, deck, hippocampus and cervical spinal bone.

For two dead sheep, veterinary documents did not specify the nervous symptoms, brain samples were processed only for monitoring scrapie, although histopathological study revealed advanced lesions, resulting in giant cell development, multinucleated, with the cytoplasm focused on detritus and loaded with vacuoles and tissue debris, and
the presence of parasitic thrombus.

Bacterial encephalitis, accounted for 37.5% of inflammatory lesions, of which 25% were identified as listeria-like.

At a sheep without clinical signs, slaughtered for human consumption, inflammation was predominantly with monocytes, which indicates an acute stage of infection which, logically, should be accompanied by nervous symptoms.

At the emergency slaughter sheep, because they had clinically abnormal head position, the lesions were located in the spinal bulb and cerebellum, and consisted of foci of liquefaction necrosis with neutrophilic infiltration with monocytes and lympho-histiocytic perivascular sleeves.

A percentage of 12.5% of bacterial inflammation were observed in emergency slaughtered sheep (2 cases), and were purulent encephalitis with massive infiltration of granulocytes neutrophils, leptomenigitis, and the presence of bacteria arranged in nests, in a case with localization in the spinal bulb, and in the second case in the cerebellum, in both cases bacteriological examination was positive for β-hemolytic strains of E. coli. Infiltrating stage, with purulent exudate character were correlated with disease duration, both clinically diagnosed cases manifested by nervous symptoms and prolonged decubitus.

The diagnosis of viral encephalitis, respectively, rabies was established in two cases showed nervous symptoms, which were slaughtered by emergency. In both cases, in addition to lympho-histiocytic encephalitis, Babes Negri corpuscles were identified. Alterative lesions and cell proliferation were moderate compared with clinical manifestations.

Nonspecific encephalitis has been described in five sheep, emergency slaughtered, and 6 dead sheep. Intensity was less relevant and considered to be associated with disease states with locations in other organs. Degenerative changes were identified perykarions (eccentric nuclei, cromatolisis, outlining Nissl granules, pyknosis, neuronal lysis, with changes in astrocytes and capillary endothelium in the vicinity of affected neurons) of the spinal bulb, quadrigeminal tubercle, cerebral cortex and cerebellum, glial proliferation, neuronophagia with microglial phagocytes gathered around the body fragments,
degenerate neural perivascular edema and/or nervous substance, the diapedesis bleeding, presence of eosinophilic infiltrate in the lumen of capillaries and arterioles.

Melanosis was found in two bodies, and a sheep slaughtered for human consumption. Pigment deposition was shown in a manner analogous to other lysosomal storage diseases; a well-defined limiting membrane is not obvious and was identified in cerebellum and leptomeninges of the hemispheres. In one case perivascular melanocytes were present but in no cases melanocytes were identified in the nervous substance.

In two bodies, we found an increase in cerebrospinal fluid, translated by dilating aqueduct, sometimes associated with edema surrounding brain substance.

In summary, the most affected by changes/lesions in nervous tissue were dead sheep, i.e. 40 cases, and emergency slaughter sheep, 31 cases, and of sheep slaughtered for human consumption, although two cases were identified with lesions, should be noted the very small number of histopathological examinations were performed on sheep in this category namely, 3. Moreover, three cases were processed to obtain histological preparations of nervous tissue for microscopic examination of reference, which are received in the pathology laboratory with accompanying documents, certifying the state of clinical healthy animals slaughtered for human consumption, and heads were been submitted for rapid screening by immuno-enzyme test.

Changes/injuries were diagnosed in the nervous tissue from emergency slaughtered sheep, which do not have nervous symptoms or macroscopic changes in central nervous system, and clinically healthy sheep slaughtered for human consumption.

Histopathological study revealed a higher incidence of changes/central nervous system lesions, compared with clinical case studies, which are commonly found in sheep, often asymptomatic or evolving clinical signs are less obvious, leading to their non-reporting by the owner or by veterinarians.

Presence of changes/lesions in the central nervous system, associated with primary lesions in other tissues and organs, indicate that blood-brain barrier exists, but not for any of the pathogenic organism.
Confirmation of two outbreaks of scrapie

Of the 158 brain samples examined in 2011 by immune-enzyme test BIO-RAD, a rate of 39.24%, i.e. a total of 62 samples were histologically processed for evidence of specific lesions of transmissible spongiform encephalopathies (classical scrapie, atypical scrapie).

The first positive diagnosis of scrapie cases, the Pathology Laboratory, specializing in the diagnosis of transmissible spongiform encephalopathies in LSVSA Cluj, in 2011, was the motivation for further research on forms of transmissible spongiform encephalopathies in small ruminants.

In Chapter II.3 we presents the results of the diagnostic protocol adopted in the two positive cases, representing the two "target" categories, comparing the performance results of laboratory tests performed on these positive cases, and epidemiological aspects of scrapie in the Cluj county, in the context of evolution of the disease in Romania.

'Target' categories, represented by the two positive cases were: clinically healthy sheep, slaughtered for human consumption, and emergency slaughter sheep following clinical examination that was showing: nervous symptoms that are seen with ataxia, head support on various objects, generalized tremors, stiff neck, increased self-mutilated pruritus in the upper limbs, ataxia, progressive weakness, lying on the ground.

As a result of framing in the two "target" categories, according to the compulsory work protocol - we proceeded to the taking of samples that were targeted for laboratory investigations.

From sheep with nervous symptoms was taken the whole brain, and the working protocol consisted of nervous tissue sampling, from the obex region, for rapid screening of scrapie by immuno-enzyme test, pons fragments, cerebral cortex, cerebellum for scrapie histopathology, brainstem fragments (loose tissue, preserved by freezing) for Western Blot confirmatory test, and pons fragments for scrapie immunohistochemistry.

From clinically healthy sheep, we collected brainstem by foramen magnum technique, and have taken: nervous tissue sample for scrapie immunoassay test from obex region and brain stem fragments for Western blot confirmatory test.
Western Blot confirmatory test was done by digesting the cellular prion protein (PrP\text{c}), followed by purification and concentration of PrP\text{res} specific for this disease. PrP\text{res} detection was done by electrophoretic migration followed by immunoblotting.

Immunohistochemistry test for scrapie was performed on histological sections using a highly specific monoclonal antibody for PrP\text{res} - monoclonal primary antibody on anti PrP\text{SC} mouse.

Immuoassay tests - had resulted in initial testing, for both samples, optical density values above the threshold (cut-off), resulting in large reports in favor of optical density recorded for those samples compared to cut-off (1.768/0.218 OD/C.OFF for the case with nervous signs, 1.692/0.220 for the case without clinical signs).

To check the accuracy of the results from the initial homogenate, both samples were retested by immuno-enzymatic test in duplicate. In the case with nervous symptoms, the optical density was well above the threshold, resulting in reports over optical densities recorded for the sample, compared with cut-off (3.601, 3.634/0.219). Optical densities were obtained over the threshold (cut-off) even if no clinical symptoms (1.670, 2.306/0.220). The results were interpreted as positive for scrapie.

High value of optical densities obtained in nervous tissue samples taken from the case with nervous symptoms, is due to more increased quantity of PrP\text{res} detected in nervous tissue compared to the amount of PrP\text{res} if present in nervous tissue without clinical signs.

Obtain optical density above the threshold in the clinically healthy sheep slaughtered for human consumption, shows sensitivity and specificity of immuno-enzymatic test for rapid screening in detecting PrP\text{res} abnormal prion protein in infected nervous tissue.

For both samples, immunoblotting yielded specific profiles, with the presence of large patches only on molecular weight protein band of 27-30 kDa, present in the sample treated with proteinase K. Electrophoretic migration resulting profiles, followed by immunoblotting, demonstrating digestion of PrP\text{c}, and disease-specific prion protein transformation into a fragment resistant to proteinase, with low molecular weight by removing amino-terminal end of the protein, the two upper bands correspond to mono-
and di-glycosylated forms (27-30 kDa) and lower band corresponds to non-glycosylated form.

Histopathological examination identified the presence of specific vacuolation, located in neurons perikaryon, and in the gray matter neuropile with symmetric distribution, bilateral. Perikaryons look relaxed, with one or more vacuoles, round or oval, with net and regularly shape, that merging, resulting in spongiform appearance of gray matter. Images are found with neuronal necrosis, neurons with pyknotic nuclei, homogeneous cytoplasm, intense eosinophilia, discrete glial proliferation and perivascular amyloid deposits, no inflammatory reactions were observed. Dorsal nucleus of the vague proved to be the place of choice for specific vacuolation.

Highlighting lesions demonstrate that prions penetration in the central nervous system is followed by conversion of normal PrP<sup>c</sup> protein produced by neurons and glial cells in PrP<sup>Sc</sup>, and its accumulation, followed by characteristic histopathological changes responsible for clinical disorders.

Immunohistochemistry performed on the case with nervous symptoms, in histological sections made from fragments of brain stem, were identified the storage points, PrP<sup>res</sup> granules arbitrarily arranged in several foci or diffuse in neuropile and perikaryons, with different intensity and distribution of immuno-reactivity.

The analysis results show the sensitivity and specificity of the immuno-enzymatic test for rapid screening in the detection of abnormal prion protein PrP<sup>res</sup> in infected nervous tissue by detecting the disease in sheep clinically healthy slaughtered for human consumption, and that there is correlation between the amount of abnormal prion protein PrP<sup>res</sup> accumulated in nervous tissue, and the onset of clinical signs, as evidenced by the high value of optical densities obtained in samples of nervous tissue, taken from the case with nervous symptoms, compared with those obtained in the case without clinical evidence.

The study revealed that scrapie can evolve in the absence of clinical signs, which means that occult prion infection in animals are much more widespread than known clinical cases. Given that the positive diagnosis of the sheep slaughtered for human consumption was made through routine monitoring, emphasizes its importance.
General conclusions

1. Histopathological study, resulted in detection of changes / lesions of the central nervous system in 73% of the cases examined, highlights the importance of examining the tissue with greater attention, both macroscopically and by sampling for histopathological study to identify primary lesions and those associated.

2. Changes / lesions in nervous tissue were diagnosed in emergency slaughter sheep, which showed no nervous symptoms or macroscopic changes in central nervous system, and in clinically healthy sheep slaughtered for human consumption.

3. Factors as: infectious, traumatic, toxic or degenerative, in other organs induce cyto-histological changes in the complex organization of nervous tissue, with the emergence of pathological processes, showing that blood-brain barrier exists, but not for any of the pathogenic organism.

4. Identification of purulent encephalitis in sheep slaughtered for consumption raises serious public health implications by eating products from such animals. The presence of bacterial colonies in the central nervous system structures, points out the need for mandatory investigations of this tissue, with histological/bacteriological investigations performed more frequently than at present.

5. For statistical evaluation of the changes/lesions incidence, in different segments of the central nervous system is resulting the practical importance of the obligation to perform the microscopic examination of brain stem, not only for the presence/absence of specific lesions of transmissible spongiform encephalopathies.

6. It would be appropriate to review the circumstances in which histopathology is said mandatory - in the "Program of the surveillance, prevention, control and eradication of animal diseases, those transmitted from animals to humans."

7. Positive case of scrapie diagnosis in sheep slaughtered for human consumption, in an increasing number of samples collected from this "target" category is an argument for increased monitoring of sheep slaughtered in the traditional system, while the structure of the sheep sector in Romania has its specifics.
8. Without considering the situation of prion infection in Cluj County as a large epidemiological study, several useful aspects to be deducted, provided that, at present, the roadmap for transmissible spongiform encephalopathies of the European Commission states that one of the strategic objectives is to review the eradication measures for small ruminants which by taking into account the existing diagnostic tools and ensuring the protection of the consumers.