PhD THESIS

The Interdisciplinary Approach to Ovine Coenurosis: Integrating Tomography, Hematological Analyses, Electroencephalography, and Neuroclinical Evaluation for Early Diagnosis and Advanced Therapeutic Management

(SUMMARY OF THE PhD THESIS)

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INTRODUCTION

Cenurosis is a parasitic disease of helminthic etiology caused by the larval form *Coenurus cerebralis* of the cestode *Taenia multiceps*. It has an endemic distribution in regions characterized by extensive sheep farming. The parasite has a heteroxenous life cycle, with dogs and other wild carnivores serving as definitive hosts, shedding eggs through feces, while sheep act as intermediate hosts, ingesting the eggs along with contaminated food or water. The oncospheres released in the intestine penetrate the circulatory system and migrate to the central nervous system (CNS), where they develop into the larval stage, resulting in the development of coenural cysts.

The pathogenesis of the disease is directly correlated with the progressive development of coenural cysts in the CNS, leading to compression phenomena and cerebral tissue degeneration. The consequences include progressive destruction of nervous tissue, cerebral edema, perilesional inflammatory reactions, and the onset of a specific neurological syndrome. The main clinical signs include circling, head rotation, ataxia, paresis and paralysis, hyporexia or anorexia, progressive cachexia, aberrant behaviors (such as a tendency to collide with obstacles), and, in severe cases, death. The progression of the disease depends on the size and location of the cyst, with variable incubation periods, while in young animals, the prognosis is guarded to severe.

The pathophysiological impact of coenural cysts on the body's homeostasis is complex, involving both neurological disorders and systemic dysfunctions. The localized inflammatory response contributes to the development of intracranial hypertension and secondary cerebral ischemia. At the same time, affected animals exhibit metabolic disturbances, severe malnutrition, and immunosuppression, predisposing them to secondary infections and increased susceptibility to other pathogens.

Clinical diagnosis is based on the identification of characteristic neurological symptoms, but etiological confirmation requires the use of advanced imaging techniques and complementary tests.

Computed tomography (CT) and angio-CT examinations allow the localization and morphological characterization of cysts, highlighting compressive effects and associated structural changes. Imaging studies performed on *Țurcană* breed rams have demonstrated the presence of large cysts with a significant impact on cerebral architecture. Electroencephalography (EEG) is an additional investigative method useful for detecting abnormalities in brain electrical activity induced by the presence of cysts, particularly in cases with atypical neurological symptoms.

Blood tests may indicate indirect signs of infection and inflammation, such as leukocytosis, eosinophilia, or changes in inflammatory markers. Post-mortem examination confirms the presence of coenural vesicles, enclosed by a thin membrane, containing clear fluid and numerous scolices adhered to the internal surface.

Epidemiologically, the prevalence of cenurosis varies significantly by region, being higher in Italy (1.3% to 28.2%) and lower in other European countries such as Ireland, the United Kingdom, France, and Greece. In Romania, epidemiological studies are insufficient, although the disease is sporadically diagnosed based on clinical signs and post-mortem examination. The associated economic losses are considerable, including a 15-20% decrease in body mass, reduced wool production, emergency slaughters, and the exclusion of carcasses from the food chain.

The main risk factors for the persistence and spread of the infection include the absence of systematic deworming of dogs, uncontrolled access of dogs to carcasses of infested animals, grazing on contaminated pastures, poor hygiene conditions in shelters, high livestock density, and the lack of biosecurity measures. In endemic areas, disease control requires the adoption of integrated strategies, including periodic deworming of dogs, proper management of carcasses, restricting dog access to pastures, improving hygiene conditions, and educating farmers about the parasite's biological cycle and prevention methods.

The modern approach to the diagnosis and control of cenurosis involves the use of advanced imaging technologies such as computed tomography (CT), angio-CT, and electroencephalography, combined with blood tests to detect potential systemic inflammatory reactions. Although these methods provide a detailed characterization of the disease, their high costs limit widespread applicability. Optimizing prevention strategies combined with active epidemiological monitoring are essential solutions for reducing the impact of this condition on the livestock sector and preventing significant economic losses.

STRUCTURE OF THE THESIS

The thesis is structured into eight chapters, each dedicated to a clearly defined topic.

Chapter 1 analyzes cenurosis, a parasitic infection caused by Coenurus cerebralis, the larval stage of Taenia multiceps, which affects the central nervous system of herbivorous animals. The infection is transmitted through the ingestion of eggs shed by dogs, the definitive hosts of the parasite. Diagnosis is established using advanced imaging techniques (CT, MRI), while prevention includes dog deworming and proper management of infected carcasses.

Studies by Tas et al. (2024) introduce a minimally invasive surgical technique for the removal of cysts, significantly reducing metabolic stress and intracranial

pressure. Rahsan et al. (2018) highlight granulomatous inflammation and neuronal destruction at the histopathological level. Other research, such as that by Gazioglu et al. (2017), demonstrates the use of computed tomography and molecular analysis for the diagnosis of coenurosis in animals and the economic impact of the disease.

Studies by Deplazes et al. (2019) address cysticercosis and coenurosis, with the risk of transmission to humans through the consumption of contaminated meat. Recent cases in Europe and Africa underline the prevalence of these infections and the associated risks.

Human coenurosis is extremely rare, and the majority of cases are caused by Taenia multiceps. However, a few cases of central nervous system coenurosis caused by Taenia serialis have been reported. These cases have been identified in France (Bonnal et al., 1933), Nigeria (Cannon, 1942; Tappe et al., 2016), Canada (Benger et al., 1981), Senegal (Debrie et al., 1982), and Ivory Coast (Collomb et al., 2007). The case presented by Yamazawa et al. (2020) is particularly remarkable, as it is the first case of human neurocoenurosis caused by Taenia serialis. The patient, a 38-year-old man, was initially diagnosed with lymphoma, but through mitochondrial DNA analysis, the disease was identified as neurocoenurosis caused by Taenia serialis. Treatment with albendazole and surgery led to a full recovery.

CNS coenurosis, caused by Taenia multiceps, presents symptoms similar to neurocysticercosis, such as headaches, personality changes, and seizures (Lescano and Zunt, 2013; Garcia and Del Brutto, 2005). Genetic tests, such as PCR, are essential for differential diagnosis, and research emphasizes the importance of accurate diagnosis and public health measures for the prevention of zoonoses.

Chapter 2 addresses the anatomy of the sheep skull, including sagittal and transverse sections through the head, with a detailed description of the brain, muscular, vascular, and nervous structures, organized into distinct layers. The anatomical differences between the sheep brain and the human brain are highlighted, particularly the disproportionate size of the olfactory bulb in sheep, which provides a much sharper sense of smell, essential for survival. The chapter also discusses the ability of sheep to recognize human faces, a behavior comparable to that of primates, with important implications in neurobiological research, especially in studies related to Huntington's disease.

Chapter 3 proposes a working hypothesis for investigating coenurosis in sheep, through the application of advanced diagnostic methods, including computed tomography (CT), biochemical blood analysis, plasma spectrofluorometry, and electroencephalography (EEG), correlated with therapeutic intervention through the puncture of the Coenurus cerebralis cyst. The combination of these techniques enables early diagnosis, effective treatment, and complete reintegration of affected sheep into

the flock, having a significant impact on improving animal health and farm productivity.

Chapter 4 presents a study on the evaluation of coenurosis in sheep using computed tomography (CT), aiming to employ this technique for accurate and early disease diagnosis. CT imaging was performed using a Siemens Somatom Scope multidetector scanner with 16 slices, applying helical scanning with parameters of 130 kVp, 250 mAs, and a slice thickness of 1 mm.

For contrast imaging, a Mallinckrodt LF Dual Head injector with Iohexol (Omnipaque) was used, administered intravenously to visualize parasitic structures. Images were acquired 45 seconds after contrast administration in three anatomical planes: axial, coronal, and sagittal, and were reconstructed using a soft tissue algorithm, providing fine details of parasitic cysts and tissue lesions. The study highlights the advantages of CT, such as non-invasive diagnosis and disease progression monitoring, contributing to a better understanding of pathology and treatment effectiveness. The study was conducted on 10 sheep with neurological symptoms suggestive of coenurosis, and anesthetic procedures were performed following a standardized protocol.

In the study, a 1.5-year-old sheep presenting clinical signs suggestive of coenurosis underwent a CT scan following a standardized general anesthesia protocol. Imaging reconstructions in the axial, coronal, and sagittal planes revealed the presence of a single parasitic cyst. Volumetric, density, and compositional analysis in correlation with adjacent structures revealed significant structural changes in the bone, leading to the decision to surgically puncture the cyst to evacuate its contents. Post-drainage, the cyst volume was reduced to 0.71 cm^3, with a mean Hounsfield Unit (HU) value of 28.6, suggesting an increase in fluid density. Additionally, a secondary, smaller cyst (0.28 cm^3) with an HU value of 14 was observed, indicating the formation of new cystic structures. The primary cyst membrane showed hyperattenuation, with HU values ranging from 17 to 71, enabling detailed visualization of the cyst's shape.

Computed tomography (CT) imaging has proven to be an essential diagnostic tool in the evaluation of coenurosis in sheep, providing an accurate method for identifying and characterizing parasitic cysts, as well as assessing their impact on brain structures.

Hounsfield Unit (HU) measurements enabled the differentiation of cysts, highlighting a predominantly fluid content and the presence of protoscoleces, supporting the applicability of CT in diagnosis and therapeutic planning.

The administration of a contrast agent significantly enhanced the visibility and characterization of cysts, and post-intervention analysis revealed a substantial reduction in cyst volume and sclerosis processes in the cyst membrane.

The observed changes in brain structures, particularly at the level of cerebral lobes, suggest the onset of functional recovery, although the risk of residual brain lesions remains. In light of these results, the integration of computed tomography into

diagnostic and therapeutic protocols for managing Coenurus cerebralis infections is recommended, as it has the potential to improve diagnostic accuracy and the effectiveness of clinical interventions.

Chapter 5 highlights the fact that, up to this point, no specific EEG research has been conducted for coenurosis in sheep, and the literature lacks information on EEG characteristics associated with cerebral cysts in animals.

There are no published studies directly analyzing the neurophysiological changes caused by the presence of coenurotic cysts in sheep, making this research a pioneering study in the field.

By performing EEG recordings in this context, the study provides a significant contribution to understanding cerebral electrical activity in coenurosis, opening new avenues for investigating parasitic brain pathologies in veterinary medicine.

Chapter 6 presents investigations into the biochemical differences in blood between sheep diagnosed with coenurosis and a control group, using standard analyses and fluorescence spectroscopy.

The research included 16 sheep from a farm in Baia Mare, of which 7 exhibited neurological symptoms confirmed by computed tomography. Symptomatic animals were anesthetized following a standardized protocol, and blood samples were collected and analyzed at the Faculty of Veterinary Medicine in Cluj-Napoca. Plasma was exposed to spectrofluorimetric analysis to determine kynurenic acid levels, and biochemical differences between the groups were statistically evaluated using the Welch's t-test. The results contribute to understanding the metabolic changes associated with coenurosis in sheep.

The biochemical analysis revealed significant changes in hepatobiliary function in sheep with coenurosis, particularly increased levels of Gamma-Glutamyl Transferase (GGT) ($p = 0.001$) and Alkaline Phosphatase (ALP) ($p = 0.013$), suggesting potential liver stress and secondary parasitic infections.

Copper levels were significantly lower in affected sheep ($p = 0.025$), while cholesterol showed a significant reduction ($p = 0.005$), though without clinical relevance. Creatine kinase (CK) levels were elevated in both groups without statistical differences, indicating possible muscular or neurological stress.

Mineral variations (calcium, phosphorus, magnesium) were insignificant and likely influenced by the animals' diet. Spectrofluorimetric analysis revealed normal kynurenic acid (KYNA) levels in one sheep without neurological symptoms, supporting its potential as a biomarker for evaluating coenurosis.

The results suggest that liver dysfunction and metabolic imbalances may be involved in the disease's pathogenesis, though the analyzed biochemical markers are not specific for diagnosing coenurosis.

The case of the fourth sheep in Group 2, diagnosed with coenurosis via computed tomography and subjected to surgical cyst drainage, highlights a partial improvement in hepatobiliary parameters. ASAT levels stabilized, and total protein returned to normal, indicating nutritional recovery. However, GGT and ALP levels remained elevated, suggesting persistent liver stress, while creatine kinase (CK) remained high, possibly due to incomplete cyst removal. These findings highlight the necessity for continuous monitoring and potential further interventions.

This study represents the first investigation of plasma kynurenic acid (KYNA) levels in sheep diagnosed with Coenurosis, using fluorescence spectroscopy. To date, no research has been reported analyzing the neuroprotective metabolites of kynurenine (KYN) in this pathology, highlighting the innovative nature of the approach.

The results indicated a significant reduction in plasma KYNA levels in sheep with Coenurosis compared to the control group. This decrease could be correlated with chronic neuroinflammatory processes, which may lead to the activation of the kynurenine pathway and tryptophan depletion, alongside a reduced expression of kynurenine aminotransferase (KAT), an enzyme essential for KYNA biosynthesis.

Furthermore, fluorescence spectroscopy has proven to be a sensitive and cost-effective technique for investigating the biochemical changes associated with Coenurosis, providing a complementary diagnostic alternative to computed tomography (CT).

Considering the absence of other studies on the involvement of KYNA in this neurological condition in sheep, these results indicate the necessity for further research to elucidate the underlying pathophysiological mechanisms and the potential therapeutic implications of kynurenine metabolites.

The study highlighted significant changes in the blood biochemical parameters of sheep affected by Coenurosis, without identifying specific pathognomonic markers that would allow for early diagnosis.

Creatine kinase (CK) activity has proven to be a useful indicator, especially when associated with neurological symptoms. Fluorescence spectroscopy analysis revealed significant differences in the emission profiles of kynurenic acid (KYNA) between the control and experimental groups, suggesting a reduction in KYNA levels in sheep with Coenurosis, associated with neuroprotective effects.

These findings indicate the potential use of KYNA as a diagnostic marker, but further research is needed to understand the underlying mechanisms and to validate it as a specific biomarker. Although computed tomography (CT) remains the primary diagnostic technique, the use of fluorescence spectroscopy provides an economic and sensitive alternative for early diagnosis.

Chapter 7 includes conclusions and recommendations, pointing out the relevance of computed tomography (CT) as a high-precision imaging method for the

diagnosis and characterization of Coenurus cerebralis parasitic cysts, providing a detailed assessment of their impact on the central nervous system of sheep.

Morphometric analysis and tissue density measurement through tomography allow for a clear demarcation of cysts from healthy brain tissue, making it a valuable tool in monitoring the clinical progression of the disease.

In parallel, the identification of kynurenic acid (KYNA) as a specific biomarker and the use of creatine kinase (CK) to assess the progression of the parasitic infection add new diagnostic and monitoring tools for this pathology. However, to validate these techniques and implement them in veterinary practice, further research is required to deepen the understanding of the pathogenic mechanisms involved, as well as to develop more effective therapeutic strategies that contribute to better management of Coenurosis and the prevention of associated complications.

Chapter 8 presents data on the originality and innovative contribution, which is significant in the field of diagnosing and evaluating Coenurosis in sheep, through the application of advanced techniques and original approaches.

The use of morphometric analysis and Hounsfield units (HU) for the detailed characterization of Coenurus cerebralis cysts and their correlation with the symptomatic severity of the infection represents a significant advancement in the objective assessment of the pathology.

The pioneering application of EEG to investigate parasitic brain changes adds an important dimension to understanding the pathogenic mechanisms of Coenurosis and the correlation of EEG changes with cortical compression effects highlights the direct impact of cysts on neuronal functionality.

Additionally, the discovery of kynurenic acid (KYNA) as a specific biomarker for Coenurosis, combined with the use of fluorescence spectroscopy, represents a promising non-invasive approach for the early diagnosis of the condition.

The proposal of creatine kinase (CK) and its brain isoenzyme as additional diagnostic indicators for evaluating the early stages of the disease adds considerable value to veterinary practice.

These contributions open new perspectives for future research and significantly enhance diagnostic and therapeutic approaches in the management of Coenurosis in sheep.

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