Ph.D. THESIS

Respiratory Strongylidosis in Sheep from Valea Șieului, Transylvania: Epidemiology, Pathological Assessment, and Evaluation of New Therapeutic Molecules

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ABSTRACT

Small grazing ruminants are hosts to a variety of parasites, primarily due to persistent contact with their intermediate hosts, as well as with parasite eggs and larvae(TAYLOR et al., 2007; FAO, 2020). Clinically and economically, the most impactful parasitic diseases in sheep include coccidiosis (*Eimeria* spp.), fasciolosis (*Fasciola hepatica*), haemonchosis (*Haemonchus contortus*), echinococcosis (*Echinococcus granulosus*), and parasitic pneumonia (ANDERSON, 2000; CHARLIER et al., 2020; JACOBSON et al., 2020). The complex of pulmonary strongylosis, the basis of parasitic pneumonia, typically involves the following genera of nematodes: *Dictyocaulus, Protostrongylus, Muellerius, Cystocaulus*, and *Neostrongylus* (DEPLAZES, 2019).

The prevalence of lungworms in sheep is influenced by lush vegetation, which, in the case of the Protostrongylidae family, creates a favorable habitat for terrestrial gastropods (acting as intermediate hosts) (TAYLOR et al., 2007; PAVLOVIC et al., 2010; DEPLAZES, 2019). Furthermore, as seen with *D. filaria*, younger animals are more susceptible to infestation, often occurring in the second half of the first grazing season, while older animals develop lasting immunity against reinfestation (TAYLOR et al., 2007).

Respiratory diseases have a significant impact on the sheep industry, both in terms of economic losses and animal welfare (CHACKRABORTY, 2014). Clinical signs can vary depending on the species involved, and are often nonspecific. The main findings include coughing, stunted growth, weakness, diarrhea in young animals, and reduced productivity in adults (BALLWEBER, 2022). Coinfections with various bacteria or viruses are common, though fatalities are rare (PANUSKA, 2006; CASWELL & WILLLIAMS, 2016).

The costs associated with treatment and production losses can have a significant economic and social impact on the livestock industry (CHACKRABORTY, 2014). Therapeutic options for parasitic pneumonia in sheep typically include oral administration of ivermectin, oral or subcutaneous administration of moxidectin, and eprinomectin spot-on (BURDEN & ELLIS, 1997). While ivermectin and moxidectin effectively reduce lungworm infestations, concerns remain regarding withdrawal times and larval excretion in feces (MOLENTO et al., 2006).

Globally, numerous studies have been conducted on the incidence and prevalence of pulmonary strongylosis in sheep and goats; however, there remain unknown aspects related to the specific pathogenicity of the etiological agents, their regional biological characteristics, and the optimization of diagnostic and prophylactic-therapeutic methods (GEURDEN & VERCRUYSSE, 2007; HORA, et al., 2014; DEPLAZES, 2019).

The introductory study focused on certain epidemiological aspects of the evolution of protostrongylid infections in a flock of 535 sheep, of the Țurcană breed and crossbreeds, the clinical and morphopathological picture, the therapeutic value of some classical preparations, and the establishment of hematological and blood biochemical constants in infested sheep and after deworming, within a disease outbreak in sheep from Valea Șieului, Bistrița-Năsăud, Transylvania.

During the years 2018-2019, it was revealed that muelleriosis is more widespread than protostrongylosis, with the seasonal dynamics of muelleriosis characterized by an increase in infestation during the autumn-winter period and regression in the spring-summer.

The clinical picture is characterized by a chronic or subclinical course in cases of mild infestations, where pulmonary disorders manifest as macrobronchitis, lobar pneumonia, and bronchopneumonia, accompanied by anemia and weight loss. The morpho-pathological examination 28 sacrificed sheep revealed various lesions, predominantly pulmonary, including focal bronchopneumonia, nodules with various developmental stages, atelectasis, and bronchiole obstructions with the presence of adult parasites.

Comparative therapeutic evaluation in muelleriosis revealed that Febantel had the highest efficacy, followed by ivermectin, while tetramisol was ineffective. In sheep with protostrongylid infections associated with other helminthoses, pre-therapeutic examination revealed changes in hematological and blood biochemical constants, indicating a state of anemia. Under the influence of Febantel and ivermectin, there was an increase in red blood cell count, hemoglobin, serum albumin, and cholesterol levels, while total serum protein and gammaglobulin levels decreased. No correlated clinical changes were observed.

The main objective of the second study was to evaluate the prevalence of pulmonary pathologies in sheep based on lung examination, following slaughter, and to present the morphological characteristics of lung lesions induced by parasites, bacteria. Furthermore it also aimed to descibe the macroscopic and microscopic aspects of tumor formations. In this study, 2784 sheep lungs were examined post-mortem in

a slaughterhouse from Transylvania. Macroscopic examination identified lesions that were classified into seven categories, and 30 lung samples were collected for histological evaluation. Parasite species identification was performed using the Baermann method on 42 fecal samples and lung fragments, while the identification of bacteria associated with lung lesions was performed using microbiological methods.

Macroscopic lesions were identified in 56.42% of the analyzed lungs. Verminous pneumonia was the most frequently identified lesion (31%), either as a single lesion or in association with echinococcosis (26.5%); echinococcosis as a single lesion was identified in 21% of the examined cases. Other notable lesions included bacterial pneumonia (7.2%) and lung tumors (1.84%). Additionally, in 8 cases of lung tumors, concomitant verminous pneumonia lesions were identified. Of the 42 samples analyzed larvoscopically, *Muellerius capillaris* was predominantly identified (15 cases), followed by *Protostrongylus rufescens* (6 cases), *Dictyocaulus filaria* (4 cases), and mixed parasitic infestations (7 cases). The main bacteria identified in bacterial pneumonia cases were *Pasteurella pneumotropica* and *Vibrio metschnikovii*. This study highlights the high prevalence of parasitic and bacterial pneumonia, indicating a significant health concern in sheep populations in Transylvania. It also emphasizes the importance of antiparasitic treatment and the need to improve the management of bacterial respiratory diseases in sheep populations.

In sheep, various studies reported that both ivermectin (administered orally) and moxidectin (administered orally and subcutaneously) effectively reduce lungworm infestation and larval excretion in feces (REHBEIN & VISSER, 2002; PAPADOPOULOS et al., 2004). Therefore, the objective of the third study was to evaluate the efficacy of two modern molecules in the therapy of primary pulmonary helminthiasis in Transylvanian sheep. The study was conducted on two flocks of sheep from Bistriţa-Năsăud county,from different localities, namely from the village of Ruștior and the village of Herina. A total of 144 sheep were examined in each flock. The study included three groups/flock of sheep, each consisting of three subgroups, each subgroup comprising 12 sheep of different ages. For each group of 12 treated sheep/age category, 12 control sheep were used. Statistical analysis was performed using SPSS software. Two macrocyclic lactones were chosen for the experiment, namely eprinomectin (4'-(epiacetylamino)-4'-deoxy-avermectin B1), the commercial product Eprecis, 20mg/ml, and a combination of Ivermectin and Clorsulon, known commercially as Ivomec Plus.

The comparative use of the two commercial products in the treatment of pulmonary strongylosis in sheep—Ivomec Plus and Eprecis—demonstrated a superior efficacy of Ivomec Plus, observed both 12 days post-therapy and especially 28 days after therapy. The results confirm that the treatment of parasitic pneumonia in sheep can be effectively conducted using substances from the macrocyclic lactone group. Furthermore, it was demonstrated that the main pulmonary strongyles were represented by *Dictyocaulus filaria, Muellerius capillaris, Protostrongylus rufescens,* and *Cystocaulus ocreatus*. Additionally, supplementary parasitic forms were detected, from the following genera: *Eimeria, Dicrocoelium, Moniezia, Nematodirus, Trichuris*, and *Strongyloides,* along with strongyle eggs. The efficacy of Ivomec Plus therapy was superior to that of Eprecis in pulmonary strongylosis in sheep across all age groups.

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