PhD THESIS

Epidemiology, chemoprevention and therapy of gastrointestinal nematodoses in sheep from Transylvania

(ABSTRACT)

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ABSTRACT

The increasing global demand for animal products has led to an intensification of sheep farming, a species appreciated for its adaptability and low maintenance requirements (Morris, 2009). However, gastrointestinal nematodoses represent a major challenge for the sheep sector, significantly affecting animal health and economic profitability (Quadros and Burke, 2024; Rufino-Moya et al., 2024). The negative economic impact stems from both diminished productivity and the expenses related to antiparasitic treatments.

Recent changes in parasite epidemiology, against the backdrop of climate change, management intensification, and the excessive use of antiparasitic drugs, have led to an increase in parasite resistance (Mavrot et al., 2015; Taylor, 2012). This resistance, arising through genetic mutations or progressive adaptations, develops over the years within farms (Jackson and Coop, 2000; Menzies et al., 2010). In Romania, parasite control is predominantly based on standardized treatments without prior epidemiological evaluation, which contributes to increased selective pressure (Höglund and Gustafsson, 2023).

Research findings highlight that underdosing and improper application of treatments lead to the loss of efficacy of antiparasitic molecules (Kenyon and Jackson, 2012). Parasites develop resistance to multiple synthetic molecules, and strategies based only on chemicals are increasingly questioned (Riviere et al., 2009). The use of selective treatments, targeting individuals instead of mass treatment, is recommended to maintain therapeutic effectiveness (Van Wyk, 2001; Cabaret et al., 2009).

In the context of sustainable agriculture, plant extracts emerge as a promising alternative. Studies support the efficacy of natural compounds such as tannins, terpenoids, and alkaloids against nematodes, without the risks associated with synthetic products (Burke and Miller, 2020). In addition to economic and ecological advantages, these products align with the growing demand for organic farming (Hoste et al., 2015).

This research was designed to respond to the pressing needs in the veterinary field, with the main objective of evaluating the situation of endoparasitism in sheep raised in extensive systems in the Transylvanian region. The study aimed to identify the prevalence and intensity of parasitic infections, assess the efficacy of commonly used anthelmintic treatments, and determine the level of parasite resistance to the most frequently used antiparasitic molecules.

The PhD thesis is organized into two main sections: the first, entitled "Literature review," provides a synthesis of the specialized literature, and the second, named "Personal contribution," presents the results obtained during the doctoral studies.

The first part is structured into 3 chapters. **Chapter I.1** presents the main sheep breeds, both native and imported, raised in Romania. The morpho-productive characteristics of the Țurcană, Țigaie, and Merinos de Palas breeds, as well as imported

breeds such as Suffolk, Ile de France, Texel, and Friesian, are described. **Chapter I.2** offers a detailed description of gastrointestinal nematodoses in sheep. It analyzes the etiology, morphology, and main genera involved: *Haemonchus*, *Teladorsagia*, *Trichostrongylus*, *Cooperia*, *Nematodirus*, *Bunostomum*, *Oesophagostomum*, *Chabertia*, *Strongyloides*, and *Trichuris*. It details the epidemiology, life cycle, pathogenic mechanisms, clinical signs, diagnostic methods, and the main molecules used for the treatment of gastrointestinal nematodoses in sheep. **Chapter I.3** is dedicated to the methods used for evaluating anthelmintic resistance. The main techniques are presented and analyzed: the fecal egg count reduction test (FECRT), the larval development test (LDA), the egg hatch assay (EHA), the larval paralysis test (LPA), and molecular techniques (PCR).

The second part includes the author's own research and is structured into five chapters. Chapter II.1. investigates the epidemiological situation of endoparasitoses in sheep from the Transylvania region. Chapters II.2. and II.3. include research regarding the phenomenon of antiparasitic resistance (*in vivo* and *in vitro* tests). Chapter II.4. assesses the efficacy of plant extracts against gastrointestinal strongyles in sheep, and the final chapter, II.5., presents a comparison between morphological and molecular methods for identifying strongyle species.

Chapter II.1. The study was designed as a comparative investigation between the spring and autumn seasons to highlight potential variations in the prevalence and intensity of endoparasitic infections in sheep. A total number of 1304 fecal samples were collected and analyzed, 652 from each season, taken individually from the rectum, originating from 20 flocks located in the counties of Cluj, Sălaj, and Mureș. By using both quantitative and qualitative diagnostic methods (flotation, sedimentation, Baermann technique, and McMaster method), the study aimed to correlate the season with the intensity of egg shedding, providing a comprehensive view of the dynamics of parasitic infections in the extensive sheep farming system in Transylvania.

The results showed an extremely high overall prevalence of parasitic infections (95.70%), with most samples characterized by multispecies infections. Among the identified etiological agents, gastrointestinal strongyles had the highest prevalence (77.1%), followed by coccidia of the genus *Eimeria* (65.66%) and *Strongyloides papillosus* (15.49%). Other species had lower prevalence rates: *Dicrocoelium dendriticum* 12.31%, *Moniezia* spp. 7.58%, *Dictyocaulus filaria* 5.67%, *Muellerius capillaris* 4.14%, *Protostrongylus rufescens* 3.13%, and *Trichuris ovis* 1.81%.

The seasonal analysis revealed significant differences, particularly regarding the prevalence and intensity of strongyle infections, which peaked during the spring season.

Additionally, a questionnaire was used in the study, addressed to the veterinarians responsible for the investigated flocks, aiming to identify the antiparasitic substances used in recent treatments. The data obtained highlighted a high frequency of benzimidazole use (especially albendazole – 40%), followed by ivermectin (15%), the ivermectin+clorsulon combination (15%), levamisole (12.5%),

oxyclozanide (12.5%), and fenbendazole (5%), also underlining the lack of molecule rotation in most flocks.

Chapter II.2 of the thesis presents an in vivo study conducted in Cluj County on sheep from five flocks raised in an extensive system, aiming to evaluate the efficacy of commonly used synthetic anthelmintic molecules in Romania. The study tested five active substances (albendazole, fenbendazole, levamisole, doramectin, and eprinomectin), as well as two of their combinations (doramectin+albendazole and doramectin+levamisole). To assess the efficacy of these treatments, the fecal egg count reduction test (FECRT) was applied, with samples collected on days 0 and 14 of the experiment for the McMaster test and determination of the number of eggs per gram of feces.

The results showed a very high efficacy for the combination doramectin+levamisole, which achieved an average reduction rate of 99.4%. Levamisole administered individually also had a high average efficacy of 95.6%. In contrast, macrocyclic lactones [doramectin (76.2%), eprinomectin (69%)] and benzimidazoles [(albendazole (57.8%), fenbendazole (62.6%)] showed reduced efficacy, suggesting a high degree of resistance among gastrointestinal strongyles in sheep.

Morphological identification of L3 larvae before and after treatment was used for the evaluation of the distribution of strongyle species. Following morphological identification of L3 larvae from day 0, seven genera/species were identified: *Haemonchus contortus* (26.11%), *Teladorsagia circumcincta* (12.17%), *Trichostrongylus* spp. (21.26%), *Cooperia* spp. (33.03%), *Oesophagostomum* spp. (5.29%), *Chabertia ovina* (0.97%), and *Bunostomum trigonocephalum* (0.58%). Among these, *Haemonchus contortus* showed the highest degree of resistance, with a significant increase in prevalence post-treatment.

The study confirms the widespread resistance of gastrointestinal strongyles to several classes of anthelmintics commonly used in Romania, highlighting the need for integrated control strategies and continuous monitoring of antiparasitic treatment efficacy.

Chapter II.3 includes an in vitro study carried out to evaluate the efficacy of four anthelmintic molecules frequently used in Romania: albendazole, levamisole, doramectin, and eprinomectin against gastrointestinal strongyles in sheep. The research was conducted on two flocks from Cluj County (T1 and T2), and the tests applied were the egg hatch assay (EHA), larval development test (LDT), and larval paralysis test (LPA). These methods take less time than *in vivo* tests and are useful for early detection of resistance.

The results obtained indicate a significant degree of resistance to albendazole, with efficacy below 50% in T1 and slightly above 60% in T2 at the discriminatory concentration, both in the EHA and LDT. Resistance to macrocyclic lactones was confirmed in T1, where efficacy was considerably lower compared to T2, wherethe efficacy value was 100% for doramectin and 96.8% for eprinomectin. Levamisole

showed high efficacy (83.4%-89.2% in LDT and 82.4%-94% in LPA), but not high enough to exclude resistance.

The comparative analysis of efficacy between the two flocks revealed statistically significant differences, reflecting variability in treatment susceptibility. The study confirms the presence of multiple resistance in gastrointestinal strongyles. The results highlight the need for continuous monitoring of antiparasitic treatment efficacy and the adaptation of therapeutic protocols based on the local epidemiological context.

Chapter II.4. The aim of this study was to evaluate the *in vitro* antiparasitic efficacy of five alcoholic plant extracts: *Allium sativum* (garlic), *Tagetes patula* (marigold), *Cucurbita pepo* (pumpkin seeds), *Satureja hortensis* (savory), and *Artemisia absinthium* (wormwood) against gastrointestinal strongyles in sheep, in the context of the search for natural alternatives to synthetic anthelmintics, especially for use in organic   farms.
The results showed that all tested extracts exhibited a certain degree of antiparasitic efficacy, but with significant variations. The alcoholic extract from *C. pepo* seeds demonstrated the highest antiparasitic efficacy, with complete inhibition of egg embryonation and larval development at high concentrations (50 mg/ml) and maintained its effect even at the lowest tested concentration (48.8 - 63% at 1.56 mg/ml); the low DL50 values (1.63 - 1.64 mg/ml) confirm its high efficacy. Garlic and wormwood also had pronounced activity, while savory and marigold showed moderate to low effects.

The DL50 value confirmed these observations, indicating the high efficacy of *C. pepo* and *A. sativum* extracts. The study suggests that these plants, especially pumpkin and garlic, may represent promising options in alternative parasite control strategies, contributing to the reduction in the use of synthetic molecules and the risk of developing anthelmintic resistance.

Chapter II.5. The aim of this chapter was to evaluate the diversity of gastrointestinal strongyle species in sheep from Transylvania using morphological and molecular methods, to improve diagnostic accuracy and develop effective antiparasitic control strategies.

The study included 20 sheep flocks, from which fecal samples were used to produce larval cultures to obtain third-stage larvae. The larvae thus obtained were identified by morphological methods and polymerase chain reaction (PCR). Morphological identification revealed 8 species/genera of strongyles (*H. contortus*, *Trichostrongylus spp.*, *C. curticei*, *T. circumcincta*, *Oesophagostomum spp.*, *C. ovina*, *Nematodirus spp.*, and *B. trigonocephalum*), while molecular methods revealed the presence of 15 species (*H. contortus*, *T. vitrinus*, *T. colubriformis*, *Oe. venulosum*, *C. ovina*, *C. curticei*, *T. circumcincta*, *O. ostertagia*, *Oe. columbianum*, *T. axei*, *N. fillicolis*, *N. spathiger*, *B. trigonocephalum*, *C. oncophora*, and *O. leptospicularis*), demonstrating superior sensitivity. *Haemonchus contortus* was the most prevalent species, detected in 85% of the flocks, both by morphology and by PCR. The average number of species

identified per flock was 3.95 through morphology and 5.25 through PCR. The results highlight the limitations of morphological diagnostics and the advantages of molecular methods, which enable rapid, accurate, and comprehensive identification of mixed infections, supporting their integration into routine veterinary practice for optimal management of strongylidosis in sheep.

General Conclusions

- The endoparasitic profile of Țurcană and Țigaie sheep breeds raised in an extensive system in Transylvania highlights a high prevalence of parasitic infections (95.70%), with a clear dominance of mixed infections (86.06%), underlining constant and complex exposure to gastrointestinal and pulmonary parasites in these production systems.
- The highest prevalence was recorded in gastrointestinal strongyles (77,1%).
- The highest efficacy was recorded for the doramectin + levamisole combination (99.4%), followed by levamisole (95.6%).
- Through the FECRT method, a high level of resistance to the main anthelmintic molecules used (albendazole, fenbendazole, doramectin, and eprinomectin) was highlighted.
- The presence of resistance is also emphasized by the in vitro test results, which show a high level of resistance to albendazole, along with partial resistance to levamisole and macrocyclic lactones.
- The tested plant extracts showed variable antiparasitic efficacy, significantly influenced by the chemical composition of the extract and the type of bioactive compounds.
- The remarkable antiparasitic activity of *Cucurbita pepo* and *Allium sativum* extracts highlights the promising prospects of phytotherapy in the context of managing anthelmintic resistance.
  The most prevalent species identified by both morphological examination and PCR analysis was *Haemonchus contortus*.
  • The morphological method allowed the identification of 8 species/genera (*H. contortus*, *Trichostrongylus* spp., *C. curticei*, *T. circumcincta*, *Oesophagostomum spp.*, *C. ovina*, *Nematodirus* spp., and *B. trigonocephalum*), while the PCR method revealed 15 species (*H. contortus*, *T. vitrinus*, *T. colubriformis*, *Oe. venulosum, C. ovina*, *C. curticei*, *T. circumcincta*, *O. ostertagia*, *Oe. columbianum*, *T. axei*, *N. fillicolis*, *N. spathiger*, *B. trigonocephalum*, *C. oncophora*, and *O. leptospicularis*).
  • The average number of species identified per flock was 3.95 using morphological identification and 5.25 using PCR, a result that confirms the higher sensitivity of the molecular method.
  • *Haemonchus contortus* showed the highest prevalence in our study, being identified in 85% of the flocks (17/20), a value observed with both methods.

REFERENCES

1. Burke, J. M., & Miller, J. E. (2020). Sustainable approaches to parasite control in ruminant livestock. Veterinary Clinics of North America: Food Animal Practice, 36(1), 89–107.
2. Cabaret, J., Benoit, M., Laignel, G., & Nicourt, C. (2009). Current management of farms and internal parasites by conventional and organic meat sheep French farmers and acceptance of targeted selective treatments. Veterinary Parasitology, 164, 21–29.

containing legumes as a model for nutraceuticals against digestive parasites in livestock. Veterinary Parasitology, 212(1–2), 5–17.

3. Höglund, J., & Gustafsson, K. (2023). Anthelmintic treatment of sheep and the role of parasite refugia in a local context. Animals, 13(12), 1960.
4. Hoste, H., Torres-Acosta, J. F., Sandoval-Castro, C. A., Mueller-Harvey, I., Sotiraki, S., Louvandini, H., Thamsborg, S. M., & Terrill, T. H. (2015). Tannin
5. Jackson, F., & Coop, R. L. (2000). The development of anthelmintic resistance in sheep nematodes. Parasitology, 120(Suppl), S95–S107.
6. Kenyon, F., & Jackson, F. (2012). Targeted flock/herd and individual ruminant treatment approaches. Veterinary Parasitology, 186, 10–17.
7. Mavrot, F., Hertzberg, H., & Torgerson, P. R. (2015). Effect of gastro-intestinal nematode infection on sheep performance: A systematic review and meta-analysis. Parasites & Vectors, 8, 557.
8. Menzies, P., Andrew, P., Shakya, K., Avula, J., Fernandez, S., Jones, A., Kelton, D., Mederos, A., Guthrie, A., Falzon, L., de Wolf, B., VanLeeuwen, J., Martin, R., LeBoeuf, A., Corriveau, F., & Jansen, J. (2010). Manuel de lutte contre les parasites internes du mouton. Dept. Population Medicine, Ontario Veterinary College, University of Guelph.
9. Morris, T. (2009). Economics of sheep production. Small Ruminant Research, 86(1–3), 59–62.
10. Quadros, D., & Burke, J. (2024). Nutritional strategies for small ruminant gastrointestinal nematode management. Animal Frontiers, 14(5), 5–12.
11. Riviere, J. E., & Papich, M. G. (2009). Veterinary pharmacology and therapeutics (9th ed., pp. 1051–1145). Wiley-Blackwell.
12. Rufino-Moya, P. J., Zafra Leva, R., Gonçalves Reis, L., Acosta García, I., Ruiz Di Genova, D., Sánchez Gómez, A., & Martínez-Moreno, F. J. (2024). Prevalence of gastrointestinal parasites in small ruminant farms in Southern Spain. Animals, 14(11), 1668.
13. Taylor, M. A. (2012). Emerging parasitic diseases of sheep. Veterinary Parasitology, 189, 2–7.
14. Van Wyk, J. A. (2001). Refugia – overlooked as perhaps the most important factor concerning the development of anthelmintic resistance. Onderstepoort Journal of Veterinary Research, 68, 55–67.