

ABSTRACT

The growing interest of the scientific community in mosquitoes as vectors is driven by the expansion of their geographical distribution, a phenomenon influenced by climate change, globalization, uncontrolled urbanization, and alterations in local ecosystems. These factors increasingly facilitate interactions between vectors, animal hosts, and human populations, thereby raising the risk of emergence and re-emergence of vector-borne diseases.

Mosquito-borne infections represent a significant challenge in veterinary medicine, particularly due to the often nonspecific or absent clinical signs in the early stages of disease. Furthermore, the simultaneous transmission of multiple pathogens or co-infections involving other vectors (e.g., ticks) complicates both diagnosis and treatment, necessitating an interdisciplinary approach and heightened epidemiological surveillance.

The doctoral thesis entitled "**Diagnostic, Prevention, and Control Alternatives in Canine *Dirofilariasis***" is structured according to current academic standards and is divided into two main parts: the first part, titled "**State of Knowledge**", comprises 21 pages and provides a synthesis of relevant literature, while the second part, "**Original Research**", presents the results obtained during the doctoral studies and consists of 61 pages. In addition to these two main sections, the thesis includes a table of contents, acknowledgements, an introduction, an abstract, a bibliography, a list of abbreviations, a list of figures, and a list of tables. The thesis comprises 39 figures and 25 tables, and references 116 bibliographic sources.

The first part, "**State of Knowledge**," is structured into three chapters and presents information regarding the epidemiology of *Dirofilaria* infection, the evolution of dirofilariasis in Europe and Romania, clinical aspects, diagnostic methods, and therapeutic approaches.

Chapter One, entitled "*Recent Advances in Dirofilariasis Research in Europe*," is divided into six subchapters and begins with a description of key aspects related to the epidemiology of *Dirofilaria* infection, including the life cycle, development in definitive hosts and mosquito vectors, vector species and transmission pathways, as well as vector competence. Additionally, risk factors associated with *Dirofilaria* spp. infections are outlined.

Chapter Two, entitled "*The Evolution of Dirofilariasis in Europe*," is structured into two subchapters that focus on the spread and progression of *Dirofilaria immitis* and *Dirofilaria repens* infections across the European continent.

Chapter Three, entitled "*Recent Advances in Dirofilariasis Research in Romania*," provides a synthesis of recent literature addressing the influence of climate change and other contributing factors on the transmission dynamics and geographic distribution of *Dirofilaria* spp. infections. It also presents the clinical manifestations of *D. immitis* and *D. repens* in dogs, along with current diagnostic methods and both prophylactic and curative options used in veterinary practice for the control of dirofilariasis.

The second part of the thesis, entitled "**Original Research**," consists of four chapters, each presenting in detail the results obtained during the research period.

Chapter Four, titled "*Serological Study on the Prevalence of Dirofilariasis in Dogs in the Danube Delta Region*," includes three individual studies that investigated the serological testing of dogs using three different methods: an immunochromatographic antigen test (CaniV-4), an ELISA antigen test (PetCheck Heartworm, IDEXX), and an antibody detection test (Anigen Rapid FeliCheck-3).

The primary aim of this study was to update and deepen the epidemiological knowledge regarding *Dirofilaria* spp. infections in dogs from southeastern Romania—specifically Tulcea County—by applying serological methods based on antigen and antibody detection. Additionally, the study sought to evaluate the field applicability of several serological tests in order to improve diagnostic, prevention, and control strategies for dirofilariasis in the context of its geographic expansion influenced by global climate change.

The main objective was to assess the prevalence of *Dirofilaria immitis* infection in dogs from Tulcea County, an ecologically significant region in southeastern Romania.



The first subchapter of Chapter Four, titled “*Diagnosis of Dirofilaria immitis by Antigen Detection (Immunochromatographic Testing) in the Danube Delta Region*,” aimed to update existing data on the prevalence of *D. immitis* infections in Tulcea County.

In the context of serological diagnosis of *Dirofilaria immitis* infection, the choice of testing method is a critical factor, as the accuracy of the results has a direct impact on both therapeutic decision-making and the correct interpretation of epidemiological data. Accordingly, the second study, entitled “*Diagnosis of Dirofilaria immitis by Antigen Detection (ELISA vs. Immunochromatographic Testing) in the Danube Delta Region*,” employed two complementary serological diagnostic methods: PetCheck® Heartworm Antigen, an ELISA-based test, and Anigen Rapid CaniV-4, an immunochromatographic point-of-care test, in order to evaluate their effectiveness as exploratory tools for assessing the seroprevalence of infection in this ecologically sensitive region.

The comparison between the two serological methods—PetCheck® ELISA and the CaniV-4 rapid test—revealed complete concordance in results, highlighting both the reliability of rapid tests in veterinary practice and the relevance of ELISA-based assays for large-scale epidemiological investigations.

The third study, entitled “*Diagnosis of Dirofilaria immitis by Antibody Detection in the Danube Delta Region*,” was conducted in the absence of a commercially available immunochromatographic rapid test specifically designed to detect anti-*Dirofilaria immitis* antibodies in dogs. The objective of the study was to test canine blood samples using one of the available feline antibody detection tests, thus providing a complementary alternative to antigen-based tests commonly used in veterinary practice.

Blood samples were tested for the qualitative detection of *Dirofilaria immitis*-specific antibodies using the Anigen Rapid FeliCheck-3 kit. This methodological choice is supported by the interspecific morphological similarities of the parasite, which theoretically allow the test to be applied to other host species, in the absence of species-restrictive specificity.

The present study evaluated 45 blood samples collected from dogs in Tulcea County between April and May 2024. All dogs included in the study had been previously tested a few months earlier for the qualitative detection of *Dirofilaria immitis* antigen using the CaniV-4 immunochromatographic test. All 45 samples had tested positive: 6 dogs had not yet started treatment, 20 were in the third month of therapy, and 19 were nearing the completion of the treatment protocol.

The novelty of the study lies in demonstrating that the FeliCheck-3 test, although originally designed for cats, can be successfully used in dogs, but exclusively in untreated cases, offering the possibility of diagnosing dirofilariasis through serological detection of antibodies.

Chapter 5 of the thesis, entitled “*Prevalence and Parasitological Diagnosis of Dirofilaria immitis and Dirofilaria repens Infection in Dogs from the Southeastern Region of Romania*,” aims to assess the current prevalence of *D. immitis* and *D. repens* infections in the canine population of southeastern Romania, using the **Knott’s test** as the diagnostic method.

The study was conducted on a group of 220 dogs from four localities in Tulcea County, Romania: the city of Tulcea, and the communes of Niculițel, Somova, and Murighiol. The results of the modified Knott’s test allowed the detection of microfilariae in 25 out of the 220 samples analyzed, corresponding to an overall prevalence of **11.36%**. Among these positive cases, 21 were attributed exclusively to the presence of *Dirofilaria immitis* microfilariae, while 4 cases indicated co-infection with both *D. immitis* and *D. repens*. Age group analysis revealed the highest infection prevalence among adult dogs aged 4–7 years, followed by the 1–3 years group (young dogs), 8–12 years (seniors), and 13–18 years (geriatric).

These findings, obtained through the application of the modified Knott’s test, support the notion of a significant increase in the prevalence of cardiopulmonary dirofilariasis in southeastern Romania.

Chapter 6, entitled “*Molecular Diagnostic Methods for Dirofilariasis in the Danube Delta Region*,” aimed to update the current knowledge regarding the prevalence of dirofilariasis in dogs from southeastern Romania using **multiplex qPCR** for pathogen detection. In October 2023, a total of 84 samples were collected from dogs housed in shelters and private households. The analysis was performed at the Laboratory of Parasitology and Parasitic Diseases and the Molecular Biology Laboratory within the Faculty of Veterinary Medicine in Iași.



Molecular screening revealed 42 positive cases out of a total of 84 samples (50%). Specifically, the prevalence of *D. immitis* infection was 73.9%, *D. repens* 2.3%, and co-infection with both *D. immitis* and *D. repens* was identified in 23.8% of cases.

With regard to the treatment and prevention of dirofilariasis, **Chapter 7**, entitled “**Prophylactic and Curative Therapeutic Alternatives Used in Dirofilariasis in the Danube Delta Region**,” presents two individual studies that focused on prophylactic and curative treatments. In the **prophylactic treatment** study, two veterinary antiparasitic products—**Nexgard Spectra** and **Simparica Trio**—were used, selected for their broad-spectrum antiparasitic action and proven efficacy in preventing *Dirofilaria immitis* infection, as well as other common vector-borne and gastrointestinal parasitoses in dogs. The primary objective of this study was to evaluate prophylactic efficacy and identify any associated risks.

The research was conducted between June 2023 and December 2024 at the SC DMA Vetland SRL Veterinary Clinic in Tulcea, in a geographic context characterized by a high risk of *Dirofilaria* spp. transmission, due to the proximity of the Danube Delta. For comparative evaluation, the study sample consisted of 300 dogs, randomly assigned into two equal groups. All 300 dogs included in the study underwent a standardized paraclinical testing protocol, which comprised three types of tests. The first was **serological testing** using the Caniv-4 diagnostic kit, which yielded 300 negative results, followed by **complete blood counts (CBCs)** and **biochemical profiling**.

Following the completion of the treatment cycle—consisting of six consecutive monthly doses, with the final dose administered in December 2024—blood samples were collected to assess potential cumulative paraclinical changes induced by the prophylactic treatment. The results of this final evaluation generally indicated good tolerability of the tested products, with hematological and biochemical parameters remaining within physiological limits for the majority of the dogs. The only notable alterations were observed in the group of senior dogs (8–12 years old) treated with **Nexgard Spectra**, where elevated levels of alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were recorded.

In conclusion, although **Nexgard Spectra** and **Simparica Trio** proved to be safe and well tolerated by most dogs in the study, **periodic monitoring of liver function parameters is recommended**, particularly in senior dogs undergoing long-term prophylactic treatment.

Study no. 2, entitled “**Curative Treatment**,” had as its primary objective the evaluation of the efficacy of an alternative therapeutic protocol for the treatment of canine dirofilariasis, commonly referred to as the “**slow kill**” **method**. This protocol involves the sequential and combined administration of several classes of active substances, aiming to gradually reduce the parasitic burden, avoid acute adverse reactions typically associated with classical adulticide therapy, and support the function of affected organs. The therapeutic regimen included an **antibiotic**, **macrocyclic lactones**, **hepatoprotective agents**, and **glucocorticoids**.

Through this therapeutic approach, the study aimed not only to assess the efficiency of parasite elimination over time, but also to evaluate the impact on hematological and biochemical homeostasis, as well as the medium-term safety of such a strategy compared to standard treatment protocols. The “**slow kill**” protocol proves to be a viable therapeutic option in cases where conventional adulticide treatment with melarsomine is unavailable, contraindicated, or difficult to administer.

A total of 220 dogs were examined in the study, consisting of 124 females and 96 males, aged between 7 months and 18 years. Blood samples collected from these animals were tested for the presence of circulating microfilariae using the modified Knott’s test, resulting in 25 positive cases. Two months after completing the therapeutic protocol, all 25 dogs were retested using the Caniv-4 antigen test. The results showed complete negativity (25/25), indicating effective parasite elimination and therapeutic success of the applied protocol.

Very important is the fact that the choice of treatment protocol should be individualized, taking into account the clinical condition of the patient, the availability of therapeutic products, economic considerations, and the capacity for long-term monitoring of the patient.

The final chapter, Chapter 8 – “**Final Conclusions**,” summarizes the main findings of the conducted research, providing an integrative perspective on the results obtained within this doctoral thesis.

