Summary

The doctoral thesis titled **"RESEARCH REGARDING FILARIOSIS IN DOGS IN MOLDOVA"** was founded on the increasing number of filariosis in dogs in the last years. On account of the zoonotic potential of these tapeworms I deemed it useful to research and improve the morphological, serological, and molecular methods for diagnosing filarosis, especially that of dirofilariosis and the relevance of applying these methods in diagnosing diroflariosis in dogs and humans in Moldova.

The thesis is comprised of two parts according to the standard requirements: the first part which is dedicated to a study of the subject matter along with up to date information from the scientific literature and the second part, which encompasses own research.

The outcome of the work is illustrated using an original iconography, containing clear and relevant explanatory figures and tables.

In the first part, titled "**Current Knowledge**", a bibliographical study regarding the geographical spread of filariosis is made, with mentions being made of the ecology and epidemiology of these vector-borne helminthiases, in dogs and in humans. The information obtained following this study was later used in order to elaborate, interpret and compare the results obtained by my own research.

The first chapter, titled "The Importance of Filiariosis in Dogs" is used to make a short description of the main types of filariosis that develop in dogs, to describe some important data regarding the epidemiology and geographical spread of dirofilariosis in dogs, current data regarding the use of heat treatment in diagnosing dirofilariosis in dogs, as well as the short description of the biological cycle of species which determine the evolution of this zooanthroponosis.

Chapter II, titled "**The zoonotic aspect of dirofilariosis: importance of dirofilariasis in humans**", depicts an overview of existing data regarding the clinical aspects of pulmonary and subcutaneous dirofilariosis in humans, describing the epidemiology, diagnosis and geographical spread of dirofilariosis.

A subchapter presents the clinical aspects of infestation with *Dirofilaria immitis* and *Dirofilaria repens*, as well as the locations characteristic to each species, while another subchapter presents the epidemiologic aspects of the disease, depicting - according to the data found in the literature - the endemic nature of this disease in the Mediterranean Basin, until around the 2000s,

following this period, there being a recorded expansion to other areas that were considered non-endemic.

In another subchapter the diagnosis methods used in humans are described, taking into account the zoonotic nature of the disease. Essential procedures that must be followed in order to confirm the disease are described, namely adequate sample collecting and accurate identification of the pathogen, which is also true for diagnosing the disease in animals.

Invasive methods, such as the biopsic puncture of the parasitic nodule, as well as alternative methods of molecular, immunehistochemical or serological (ELISA and Western Blott) diagnosis are described, taking into account the possible antigenic interference with other helminths in humans, such as the ones belonging to the *Toxocara, Ascaris and Echinococcusgenera.*

The reasoning behind this approach lies in the fact that the professional literature is reporting an increase in the number of cases, however, this does not actually depict the true evolution of the disease in rural and urban areas and a reevaluation of this information is necessary - which we have attempted with this scientific investigation. It is necessary to continue investigations for both humans and animals, as the epidemiological cross-over we carried out demonstrates that there are areas where dirofilariosis in humans is present, however there are no related cases that spread from animals and vice versa.

The second part, ”**Own Research**” is comprised of 6 chapters that depict and discuss the results of the carried out investigations.

Chapter III, ”**Geographic Distribution of canine heartworm (*Dirofilaria immitis*) infection in stray dogs in eastern Romania**” is a study carried out in eastern Romania in order to evaluate the prevalence and geographical distribution of the infection caused by Dirofilaria immitis in dogs. Plasma samples were collected from 458 dogs from shelters in 8 counties that have undergone serological testing for the presence of the *D. immitis* antigen. The present study revealed negative results in Botoșani, Suceava, Neamț, Bacău and Vrancea counties. Also, 45 blood samples from dogs in a shelter from the municipality of Galați were examined, using the modified Knott method and the multiplex-PCR technique (the polymerase chain reaction). Immunoenzymatic testing showed a prevalence of the infection caused by Dirofilaria immitis of 8,9%. The values for optical density (OD) that were obtained for the positive samples varied between 0,217 and 2,683. Geographic Information Systems (GIS) have been used in order to overlap the distribution maps of the *D. immitis* prevalence and the predictive maps based on the necessary optimal temperatures needed for the extrinsic incubation

period of dirofilariasis. The highest prevalence recorded by this study was established in the central-eastern region up to the northern border of the country, namely Galați county (60%), followed by Vaslui county (12%) and Iași county (7,7%). Of the 45 samples examined using the Knott method, 23 were positive for circular microfilariae (51,1%), while 19 were positive for *D. immitis* and 4 were positive for both *D.immitis* and *D. repens*, using the multiplex-PCR test. The high prevalence of *D. immitis* in the south-eastern region (Galați - 42.2%), also using the multiplex-PCR method, provided a strong support for the results obtained by using serological testing. The present study confirms the capacity of Geographic Information Systems to identify the distribution and epidemiology of dirofilariasis in different geographic areas, as was already confirmed by using the empirical epidemiological data obtained at continental, national and inter-regional levels.

Chapter IV, titled "***Dirofilariosis* forecast in eastern Romania, based on the ET30 mathematical model**", depicts a study that used a mathematical model based on a climatological forecast of air temperature in order to estimate dirofilariosis in eastern Romania in the next 20 year. Calculation of HDU (Heartworm Development Units) was accomplished using the maximum, medium and minimum daily temperature values, during the interval of the 1st of January 1961 and the 30th of June 2016, from the main weather stations in Moldova, the eastern part of Romania (Botoşani, Bacău, Iaşi and Galaţi, for the hills and plateau areas, and Ceahlău-Toaca for the high mountains area).

The study illustrates the following results: grouping the temperature values by seasons shows that during summer the optimal conditions for the development of the infecting larva (L3) inside the body of mosquitoes is met, for almost all days, while in spring and autumn there aren't 30 consecutive days in which the HDU could accumulate, but the infecting larva (L3) could still develop in these periods as it can be preserved inside the vector's body if optimal temperature days alternates with days of extreme temperatures.

During winter, the accumulation of sufficient HDU units for the pathogen to develop is impossible. The estimations for the year 2035, obtained by using the ET30 model, depicts the same increase in the following 20 years, by a value of 1,1°C. Due to the increase in temperatures in the year 2035, the period of extrinsic incubation of dirofilariasis is reduced by 5% per year.

This significant decrease will be recorded during spring and autumn and will be approximately 6.5%. This represents a decrease of the extrinsic incubation period of dirofilariasis by 1-2 days which leads to a rise in generations of Dirofilariaris in mosquitoes as well as the significant spread of infection in areas that were included in the study. This translates to a rise of prevalence and a possible habitat extension for *Dirofilaria spp.*

In chapter V, titled "**Heat treatment of serum samples from stray dogs naturally exposed to *Dirofilaria immitis* and *Dirofilaria repens* in Romania**" results of applying heat treatment in the serological treatment of dogs suffering from cardiopulmonary and subcutaneousdirofilariasis are depicted. Serum sampling is carried out from a population of dogs which was naturally exposed to infection with *Dirofilaria repens* and *Dirofilaria immitis*. Serum samples were tested for the presence of the Dirofilaria immitis antigen before and after heat treatment. Of the 194 dogs tested for the *Dirofilaria immitis* antigen, 16 dogs (8,2%) tested positive before applying heat treatment and 52 dogs (26,8%) tested positive after applying heat treatment. Of the 108 dogs tested by using the Knott method, 24 dogs (22,2%) were found infected with circular microfilariae. After the subsequent identification of the samples which tested positive after the Knott method, by using the multiplex-PCR technique, the results were as follows: 6 dogs infected with *Dirofilaria immitis*, 12 dogs infected with *Dirofilaria repens* and 5 dogs infected with both species. 50% of the dogs found having circular microfilariae of *D. immitis* tested positive to the antigen detection test both before and after heat treatment, while the other 50% tested positive only after heat treatment. 60% of the dogs with *D. immitis /D. repens* mixed infections tested positive in the antigen detection test both before and after heat treatment, while the rest 40% tested positive only after heat treatment. The *D. immitis* antigen detection test used on the 12 dogs infected only with *D. repensmicrofilariae* resulted in contradictory results. Only two dogs (16%) tested negative both before and after heat treatment. In the case of six dogs (50%) the result of the test turned positive after heat treatment, in the case of four dogs (30%), the result of the test was positive both before and after heat treatment. Following this study we can conclude that false negative result for the *D. immitis* antigen detection test can be reversed by applying heat treatment to the serum sample and the dogs infected with *D. repens* can also have an occult infection with *D. immitis*.

Chapter VI, titled "**Serological investigations concerning human dirofilariosis in Romania and the Republic of Moldavia**", describes the following data: during December 2014 – March 2015, a serological screening was carried out, in which 450 patients participated: 187 patients from Romania (Galați, București, Bacău, Iași) and 263 patients from the Republic of Moldova. The age of the participants ranged between 7 and 78 and of these, 166 were men and 284 were women. The patients' serum was used to identify the IgG antibodies' response to the antigen originating from the extraction of adult *Dirofilaria immitis* and *D. repens*.

Taking into account only the counties where the screening was carried out, sero-prevalence was only 6% in the county of Bacău, 7% in the county of Iaşi and 18,2% in Bucharest. Dirofilariosis sero-prevalence for patients from the Republic of Moldova was 9.1% (n-236)

The t test for independent samples showed that there is no statistically significant difference of the average age between the two groups. Therefore, both patients who tested positive to the IgG anti-*D. immitis* infection (M=50,31 years old) and those who have tested negative (M=47,10 years old) are of close average age. This means that age does not represent a predisposing factor for the transmission of the *D. immitis* infection.

Seeing as how there is no recorded data concerning dirofilariosis in dogs in the Republic of Moldova and this study confirms the presence of dirofilariosis in humans by way of immunological methods, serological studies and epidemiological investigations are needed in order to confirm and identify the parasitosis prevalence in dogs and implicitly the true state for zoonotic risk posed by this disease.

The results of the t test for independent samples also showed that the average age doesn't differ significantly from a statistical point of view between the group tested positive for gG anti-*D. repens* and those without a *D. repens* infection. Therefore, we may state that age is not a predisposing factor for the *D. repens* infection either.

By analyzing the relation between the sex and Dirofilaria infection presence, we can observe the balanced distribution between the two sexes. Thus, there is no higher prevalence in the case of one of the two sexes, sex not being a predisposing factor for infection in humans.

. Study results confirm the zoonotic effect of the disease on the territory of both Romania and the Republic of Moldova. Considering that the reservoir for the disease is comprised of microfilaria-positive dogs and that the disease is transmitted by mosquitoes, there is a need for programs monitoring the vector and the dogs in both territories: Romania and the Republic of Moldova, in order to reduce the zoonotic risk of the disease.

Chapter VII, titled "**Investigations regarding the clinical features and onchocercosis diagnosis in dogs in Romania**", describes the first account of dog infestation with *Onchocerca lupi* in Romania. This study describes the clinical picture of three dogs infected with *Onchocerca lupi*: a female German Shepherd, 4 years old, from Târgovişte, a male crossbreed, 5 years old, from Târgovişte and a male crossbreed, 6 years old, from Iaşi. The patients were brought to the clinic because of ocular disorders with excessive eye discharge. During eye examination all patients suffered from the following eye lesions: the first case: moderate conjunctivitis, with the presence of white,

lean worms on the surface of the conjunctiva, in the inward angle of the eye, blepharospasm, and lacrimal secretions which were mucopurulent, with an agglutination effect on the fur. On the outward angle of the LE (left eye) a subconjunctival nodule of approximately 0,5 cm in diameter was discovered; the second case: mucopurulent secretions, moderate conjunctivitis and a conjunctival mass of 1,2 cm in diameter. The RE (right eye) did not present abnormal aspects and the general state of the patient was good. the third case: conjunctival hyperemia, mucopurulent secretions and vascular episcleral congestion. The presence of a anepiscleral nodule was observed in both eyes, featuring the presence of various individual elevated masses, originating from under the bulbar conjunctiva (bilaterally situated, in the lateral or dorsolateral limbus). The nodules were surgically removed, two weeks apart, first from the RE, then from the LE. Two months later, a recidivation was observed at the LE. The dog was brought back on the basis of photophobia, pronounced conjunctival hyperemia and purulent secretions. After a careful examination of the bottom of the inferior conjunctival sac, a long, spiral-shaped worm was extracted under local anesthesia.

During microscopic examination it was revealed that the worms extracted from the two dogs were adult females, with a double uterus containing numerous microfilariae without their sheath. The adult females featured a relatively thick epidermis, made up of two distinct layers. Concisely, the outer layer featured cuticular crests shaped like equally distanced round protrusions, having identical size and shape, and the inner layer featured striations, with one under each crest and one between the adjacent crests, specific to the *Onchocerca lupi* species. The present study confirms the spreading of the species in eastern Europe. Parasitosis is extremely important for both dogs and humans, seeing as how its zoonotic risk has been confirmed by other authors in some regions of Europe and the United States of America.

Chapter VIII comprises the **General Conclusions** which can be drawn after completing the studies that are part of the present thesis.

The thesis ends with the presentation of the bibliography containing the Romanian and foreign titles from the scientific literature that were used during the research process.