Educational material created within the Erasmus+
Strategic Partnerships for Higher Education Programme:
"Online courses with videos for the field of veterinary communication dealing with prevention, diagnosis and treatment of diseases transferable from animals to humans"
Ref. no. 2016-1-RO01-KA203-024732

DIROFILARIOSIS

GUIDE OF MAIN PARASITIC DISEASES TRANSMITTED
FROM NON-HUMAN ANIMALS TO HUMANS –
DIROFILARIOSIS IN HUMANS AND ANIMALS

This project has been funded with support from the European Commission.
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"Ion Ionescu de la Brad" University of Agricultural Sciences and Veterinary Medicine, Iasi (Romania) is the beneficiary of the Erasmus+ project Online courses with videos for the field of veterinary communication dealing with prevention, diagnosis and treatment of diseases transferable from animals to humans 2016-1-RO01-KA203-024732

GUIDE OF MAIN INFECTIOUS DISEASES TRANSMITTED FROM NON-HUMAN ANIMALS TO HUMANS – DIROFILARIOSIS IN HUMANS AND ANIMALS

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2016-1-RO01-KA203-024732
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DIROFILARIOSIS IN HUMANS AND ANIMALS

The generic name “dirofilariosis” joins together all the helminthoses produced by de species of the *Dirofilaria* genus Railliet & Henry, 1910 (*Spirurida: Onchocercidae*) in humans and animals. The *Dirofilaria* genus comprises around 50 species but only 27 have been validated, divided in two subgenera: *Dirofilaria* (5 species with cardio-vascular affinity) and *Nochtiella* (22 species with subcutaneous and conjunctival tropism). Of the validated species (table 1), only six have proved zoonotic potential (*D. immitis, D. repens, D. tenuis, D. ursi, D. striata* and *D. spectans*). Due to the frequency with which they were diagnosed and to their zoonotic potential, *D. immitis* and *D. repens* are considered the most important and consequently the most studied.

Table 1. Valid species of the Dirofilaria genus (Dantas-Torres, F., Otranto, D., 2013)

	Genera, Sub-genera and species	Definitive host (Families)	The spreading area
Dirofilaria	D. ailure (Ryjikov and Románova, 1961)	Procyonidae	China
	D. freitasi (Machado de Mendonca, 1949)	Bradypodidae	Brazil
	D. immitis (Leidy, 1856)	Canidae, Felidae, Hominidae, and many others	Cosmopolitan
	D. lutrae (Orihel, 1965)	Mustelidae	USA
	D. spectans (Freitas and Lent, 1949)	Hominidae (single case), Mustelidae	Brazil
Nochtiella	D. acutiuscula (Molin, 1858)	Canidae, Caviidae, Felidae, Tayassuidae	South America, USA
	D. bonnie (Vogel and Vogelsang, 1930)	Muridae	Java
	D. cancrivori (Eberhard, 1978)	Procyonidae	Guyana
	D. corynodes (Linstow, 1899)	Cercopithecidae	Africa, Thailand
	D. genettae (Baylis, 1928)	Felidae, Viverridae	Nigeria
	D. granulosa (Linstow, 1906)	Felidae	Africa, Asia

	D. incrassata (Molin, 1858)	Bradypodidae, Procyonidae	Brazil and Central America
	D. linstowi (Dissanaike, 1972)	Cercopithecidae	Sri Lanka
	D. macacae (Sandground, 1933)	Cercopithecidae	Indochina
	D. macrodemos (Eberhard, 1978)	Bradypodidae	Guyana, Panama
	D. magnilarvata Price, 1959	Cercopithecidae, Hominidae, Hylobatidae	Malaya
	D. minor (Sandground, 1933)	Felidae	Vietnam
	D. pagumae (Sandground, 1933)	Viverridae	Indochina
	D. panamensis (Eberhard, 1978)	Bradypodidae	Panama
	D. repens (Railliet and Henry, 1911)	Canidae, Felidae, Hominidae, Viverridae	Europe, Asia, Africa
	D. sachsi (Shoho, 1974)	Bovidae	East Africa
	D. striata (Molin, 1858)	Canidae, Felidae, Hominidae (single case), Tayassuidae	Brazil, Venezuela, USA
	D. subdermata (Mönnig, 1924)	Erethizontidae	North America, South Africa
	D. sudanensis (Linstow in Schipley 1902)	Felidae, Hyaenidae	Sudan
	D. tawila (Khalil, 1932)	Cercopithecidae	Africa
	D. tenuis (Chandler, 1942)	Hominidae, Procyonidae	North America
	D. ursi (Yamaguti, 1941)	Felidae Ursidae Hominidae	Asia, North America

ETIOLOGY

The first description of *Dirofilaria repens* was made for veterinary medicine by Bonvicini in Italy in 1910. He had found this parasite in a dog from Bologna. Later, in 1911, the same worm was studied in France by Railliet and Henry. The first mention of *D. immitis* was done by the noble Francesco Birago in the 17th century when he identified in the heart of his hunting dog a filarial worm which he erroneously described as *Dyoctophyma renale*.

In 1937, Faust proposed the division of the Dirofilaria genus into the *Dirofilaria* subgenus with affinity for the cardiovascular system (*Dirofilaria immitis*) and the *Nochtiella* subgenus with predilection in the subcutaneous tissue (*Dirofilaria repens*). Currently, the most studied parasites of dogs are: *D. immitis* (Leidy 1856) and *D. repens* (Railliet and Henry, 1911) (*Spirurida: Onchocercidae*), which cause cardiopulmonary and subcutaneous dirofilariosis, respectively. Both species are viviparous and microfilariae spread in the bloodstream of their definitive host, therefore having zoonotic potential (Otranto et al. 2013).

Recent studies show the presence of an endosymbiont, *Wolbachia pipientis*, a Gram negative bacterium belonging to the Rickettsiales Order, which resembles otherbacteria of the same order (*Ehrlichia spp., Anaplasma spp*.). Inside the parasite, this bacterium plays an important role in the parasite embryogenesis as well as triggering immunological reactions. The study of this endosymbiont provides better knowledge of the parasite's biology and the pathological mechanisms determined by these filaria, as well as important aspects in the treatment of filariasis (Dingman et al., 2010, Belanger et al., 2010, McHaffie et al., 2012). The presence of vectors in the lifecycle of the *Dirofilaria spp*. is determined by global climate change (Genchi et al., 2001; Sassnau et al., 2014).

MORPHOLOGICAL DESCRIPTION

Dirofilaria immitis has a smooth, whitish cuticle, with only the male showing striae and ridges on the ventral face of the last caudal spindle. Males measure 12-18 cm in length and 0.6-0.9 cm in width, and the tail (figure 1) resembles a corkscrew (ESCCAP, 2012). The spicules are unequal, with many spirurids, the left one is 300-355 µm long and the right one 175-226 µm (Fülleborn et al., 1912; Vogel et al., 1927). Adult females are 25-31 cm long and 1-1.3mm wide.

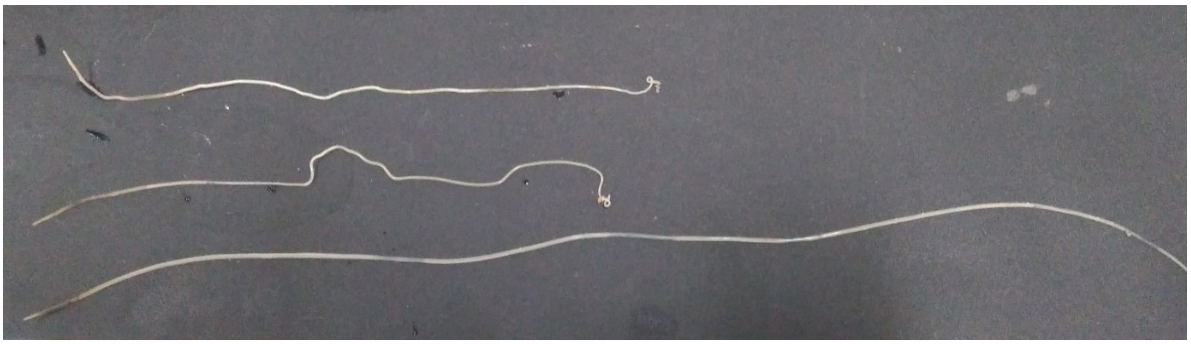

Figure 1. Two males (up) and one female (down) of *Dirofilaria immitis*.

The microfilaria sizes are: 301.77 ± 6.29 average length and 6.30 ± 0.26 mean width. The maximum and minimum dimensions fall within the following ranges: 180-340 µm in length and 5-7 µm in width (Taylor et al., 1960a). The microfilaria does not present a sheath, the anterior extremity is tapered and the posterior one is straight with a sharp tail (Magnis et al., 2013).

Dirofilaria repens adults have a white cuticle with different longitudinal and transversal striations and ridges. Adult females measure 10-17 cm in length and 0.46-0.65 mm in width, while males are 5-7 cm long and 0.37-0.45 mm wide. The adult nematodes are located in subcutaneous connective tissues and intramuscular interstices, where they are difficult to detect, they migrate sometimes and produce a subcutaneous nodule of about 1 cm in size (Genchi et al., 2011). The microfilaria of *Dirofilaria repens* measure 325-375 µm in length and 6-8 µm in width. In the microscopic examination, the larvae of *Dirofilaria repens* exhibit an anterior obtuse end, the tail resembling an umbrella handle, and the absence of the cephalic hook (Magnis et al., 2013). The microfilaria aspects of both species are shown in Figure 2.

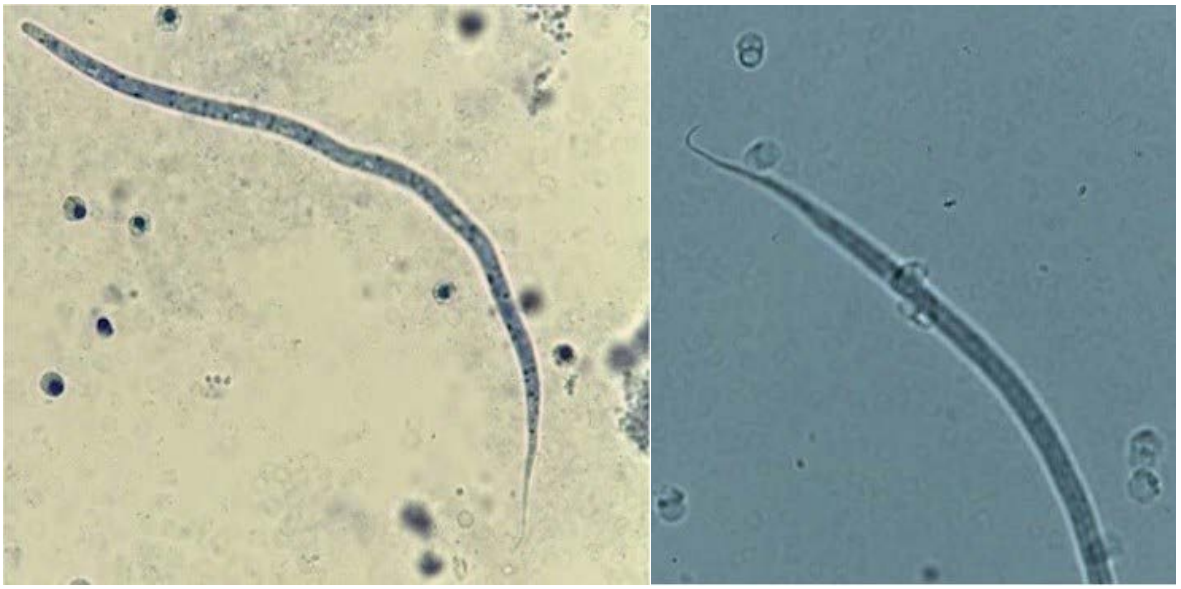

Figure 2 1st stage microfilaria of *D. immitis* (left) and *D. repens* (right) isolated from canine blood using Knott technique. Note the shape of the tail of *D. repens* first stage larva, resembling an umbrella handle. Light microscopy, 1000x

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LIFE CYCLE

The lifecycle of *Dirofilaria sp*. is of the two-host type and it is spent between a vertebrate (definitive host) and an arthropod vector (mosquitos from the *Culicidae* family). The species of some genera, such *as Aedes, Culex, Culiseta, Mansonia, Ochlerotatus, Coquillettidia and Anopheles (Aedes aegypti, Ae. albopictus, Ae.notoscriptus, Culex vexans, Cx. quinquefasciatus, Cx. tritaeniorhynchus, Cx erythrothorax, Culiseta incidens, Cu. inornata, Coquillettidia richiardii, Anopheles maculipennis* group) were found to be competent vectors for *Dirofilaria immitis* (Cancrini et al., 2003, 2006, Fuehrer et al., 2016, Loftin et al., 2015, Smith et al., 2013, Vezzani et al., 2005, Lai et al., 2001, Konichi E., 1989, Yildirima et al., 2011, et | al.,1992). The period of adult development of *Dirofilaria immitis* and *D. repens* in the definitive host is relatively long (7-9 months) compared to other nematodes (McCall et al., 2008).

The first stage microfilaria (L1) are ingested by the mosquito vector when feeding on a definitive host. Within 8-10 days (Venco et al., 2011) microfilaria migrate in the Malpighian tubes and molt to L2. The second molting process occurs three days later and L3 have to leave the Malpighian tubules in another 2 days to became infective in the mouthparts of the mosquito. The infective L3 is 1mm long and grows to 1.5mm after being inoculated in the definitive host`s subcutaneous connective tissue (Cancrini and Kramer, 2001 ; Taylor et al., 1960 ; Manfredi et al., 2007).

The development of L1 to infective L3 inside the mosquito depends on the environmental temperature and is favored by the presence of the *Wolbachia pipientis* symbiont. The development process occurs in 10-14 days at a temperature of 27º C and 80% humidity (Orihel, 1961). The number of infested larvae is limited by antigenic recognition and vector-based humoral and cellular defense mechanisms (Castillo et al., 2011).

The infection with L3 of the definitive host is performed during mosquito feeding, when about 10 larvae can be inoculated in a single feeding session. In subcutaneous connective tissue, adipose tissue and muscle tissue of the definitive host, *D. immitis* larvae (L3) develop actively for 70 days. During this period two moltings take place (L4 and L5 are 1-2 cm long) until the pre-adult stage. These stages are able to migrate into the vascular system and from here to the heart and lungs where they localize and undergo final maturation, and become capable of reproduction within 120 days post-infection (McCall et al., 2008; Manfredi et al., 2007).

Dirofilaria immitis is located in the pulmonary arteries, with a predilection for the caudal

lobes, but also in the right ventricle, the right atrium and occasionally in the cava vein. Adult females begin to produce the first larvae (L1 microfilaria) after 6-9 months post-infection. Adult longevity in the host may be longer than 7 years, and the microfilaria's lifespan more than 2 years (Venco et al., 2011). Adults of *D. repens* remain in the connective tissue, the abdominal cavity and the muscular fascia of the definitive host (Genchi et al., 2011). The prepatent period in the dog is 6-9 months, when new microfilaria are released by the adult female (Venco et al., 2011). After infesting a host, the microfilaria continues to live in the blood for several months, up to 3 years. Adults can live for 4 years or more at the site of inoculation. *Dirofilaria repens* can be located in the subcutaneous tissue in the nodules and may also invade the ocular region (Paes-de- Almeida et al., 2003; Mircean et al., 2017). Incidentally, both filarial species can also be found in other anatomical regions other than those described above (Pampiglione et al., 2000; Theis et al., 2005).

Dirofilariosis is a zoonotic disease that accidentally affects humans, the most important definitive host being the dog (Cancrini et al., 2001). *Dirofilaria immitis*, *Dirofilaria repens*, *Dirofilaria ursi, Dirofilaria tenuis, Dirofilaria striata, Dirofilaria spectrans*) affect the human being as an accidental host (Horst, 2003). The vectors involved in transmitting the disease to humans are anthropophilic mosquitoes of the genera: *Aedes, Culex, Anopheles, Armigeres* and *Mansonia* (Joseph et al., 2011). If so far it has been known that the biologic cycle of dirofilariosis in humans is incomplete (absence of adults and implicitly of microfilaria in the blood), recent studies (Sulekova et al., 2017) show that *D. repens* microfilaria have been found in a nodule subcutaneously in the iliac region, without being present in the bloodstream, though. Usually, *D. immitis* pre-adults end up in a branch of the pulmonary artery and, due to the immune response, they are destroyed and occasionally identified in a lung node (Simon et al., 2005). *Dirofilaria repens* infection may occur with cutaneous or ocular localizations.

Sometimes, infective larvae from a single inoculum can develop at different rates, and the symptoms of parasitism are manifest clinically at long intervals. Orihel et al. 1997 and Lupse et al. 2015 described cases of recurrent human dirofilariosis, probably by exposure to a single inoculum.

EPIDEMIOLOGY

Although infestation with *D. immitis* has been diagnosed in more than 30 mammalian species: wild and domestic carnivores, domestic and wild felines, mustelids, monkeys, marine mammals, rodents and ungulates (Otto, 1975), dogs are most frequently infested with a large number of parasites (Genchi et al., 1988), being the most competent reservoir of infection. Humans and cat are less susceptible hosts to infection due to changes in the process of development of filaria in their bodies (McCall et al., 2008). In cats microfilaremia occurs in 20% of cases (McCall et al., 1992), and adults survive a 2-4 year period in contrast to dogs in which adults of *D. immitis* survive for a period of 5-7 years (Venco et al. al., 2008). Cats are usually infected with a small number of *D. immitis* adults, 6 or less (McCall et al., 2008). Normally, cats are not receptive to *D. repens* microfilaria, but recent studies reveal their presence in the blood (Tarello, 2002). In the natural infection (Figure 3), the number of adults parasiting increases with the dog's age (about 150 parasites / dogs in the endemic areas) (Genchi et al., 1988, Miller et al., 2011, Bolio Gonzales et al., 2007). It is accepted that dirofilariosis occurs in cats in any area where dogs are infected with *D. immitis* (Kramer and Genchi, 2002).

Figure 3. Adults of *D. immitis* removed from the heart and pulmonary artery of a 12-year-old male mongrel dog at necropsy

Numerous studies conducted so far have focused on the identification of the Culicid mosquito species involved in the transmission of dirofilariosis. Thus, it has been shown that most of the species that allow the growth of *D. immitis* and *D. repens* are *Aedes, Culex and Anopheles* (Cancrini and Kramer; Cancrini and Gabrielli). Subsequent studies have

determined the vector species that tend to provide developmental conditions for *D. immitis* and *D. repens*. Thus, the *species Anopheles maculipennis, Aedes aegypti, Mansonia uniformis, Mansonia annulifera , Armigeres obturbans and Aedes albopictus* for *Dirofilaria repens* and the species involved in the transmission of *D. immitis* are of the genus *Culex, Aedes, Anopheles* and *Culiseta*. After the blood meal, mosquito females lay eggs in rafts-shaped groups or solitary eggs on the surface of water, humid soils or in tree hollows. As a rule, the larvae develop at temperatures below 18 degrees Celsius, but they can also adapt to higher temperatures (Cancrini et al., 1988).

Once ingested by the mosquito, the microfilaria are temperature-dependent throughout the development process up to the infective larval stage (L3). Thus, for larvae (L1) it is necessary to reach the optimal temperature within 30 days to get to the infestation stage, a process called extrinsic incubation period (Slocombe et al., 1989; Medlock et al., 2007). The time required for the development of larval stages in the mosquito is influenced by temperature: 8-10 days at 28-30^{o}C, 11-12 days at 24^{o}C and 16-20 days at 22^{o}C.

The minimum temperature at which the larvae's growth process can be carried out is 14°C (Lok and Knight, 1998; Slocombe et al., 1989; Vezzani and Carbajo, 2006; Medlock et al., 2007; Genchi et al., 2011). Taking into account the period and temperature required for the development of the infesting larva (L3), Slocombe et al. (1989) developed a model that estimates the initial and final period for the transmission of dirofilariosis as well as the number of generations of dirofilaria.

Thus, the complete development of the larva (L3) requires 130 "degrees-days". The extrinsic incubation period is also called "Dirofilariosis Development Units" (HDUs). Another important rule of the extrinsic incubation period is the accumulation of HDUs within 30 consecutive days, the maximum survival time of the mosquito. The literature provides many epidemiological studies that estimate the distribution of dirofilariasis over time as well as the number of generations of dirofilaria in different regions by using the predictive model described above (Slocombe et al., 1989) and the temperatures recorded at meteorological stations (Lok and Knight, 1998, Genchi et al., 2005, 2009, 2011, Vezzani and Carbajo, 2006, Medlocket et al., 2007, Mortarino et al., 2008, Rinaldi et al. ., 2013b; Kartashev et al., 2014; Sassnau et al., 2014; Simón et al., 2014).The capacity of geographic information systems to predict the distribution and epidemiology of dirofilariosis in different geographic areas has already been demonstrated by empirical epidemiological data obtained at continental level (Genchi et al., 2009; and Kartashev et al., 2014), national (Medlock et al. ., 2007; Simón et al., 2014) and regional (Mortarino et al., 2008; Montoya-Alonso et al., 2015). Geographic information systems could become an important tool for managing dirofilariosis in endemic and non-endemic countries. In dirofilariosis, the host-parasite relationship is complex mainly due to the capacity of the two, *D. immitis* and *D. repens*, to infect various vertebrate hosts in which the filaria develop and give rise to different pathologies, as well

as to the presence of the symbiotic bacterium Wolbachia in the larval stages and in the adult stages of both species above. Receptive hosts are exposed to both antigenic, nematode and *Wolbachia* bacteria; the response induced by these antigens correlates directly with the survival or death of the nematode and the inflammatory process developed in dirofilariosis. From an epidemiological point of view, dirofilariosis is considered an emerging parasitic disease of humans and animals. Significant and continuous change in the distribution and prevalence of canine reservoirs hosts is reported worldwide, and these changes in turn alter the epidemiological parameters in the dirofilariosis with humans and cats. Global warming influences the stages of the parasite's lifecycle, and pet management and human intervention in the environment affect vertebrate hosts and vectors, which might explain the substantial increase in the *Dirofilaria* infection.

Despite efforts to prevent infestation, especially in dogs, the disease appears to spread to previously non-endemic areas (Genchi et al., 2007), so many countries are now considered endemic to dirofilariosis (Genchi et al., 2011). The spread of cardiopulmonary dirofilariosis in Europe may be due to several factors such as global warming (Genchi et al., 2001; Sassnau et al., 2014), the presence of vectors and favorable climatic conditions for its development, new species of mosquitoes which are competent vectors of filariasis (Madon et al., 2002; Cancrini et al., 2003; Roiz et al., 2007), the growing number of dogs traveling with their owners, for example on holidays, as well as the increasing role of infection reservoirs, such as jackals and foxes (Tolnai et al., 2014).

Subcutaneous dirofilariosis is considered a widespread zoonosis. The prevalence of this disease seems to be growing, and new cases are reported in South-East, Central and Western Europe, Asia and Africa (Tarello, 2010). The *D. repens* infection is considered an emergent zoonosis in several European countries: France, Italy, Hungary, Russia (Kramer et al., 2007; Genchi et al., 2009), where the main host and reservoir is considered to be the dog. The highest prevalence was reported in dogs in Sri Lanka (60%) and Italy in the Po River Valley (30%), Spain 9%, Greece 22%, Serbia 49.22%, Belgrade 19.26%, Hungary 14%, France 22%. Although there are various specific and sensitive diagnostic methods, effective prophylaxis, dirofilariosis in dogs is still prevalent in large areas (McCall, et al., 2008). This disease affecting animals and humans is more and more frequently detected in Mediterranean countries (Genchi et al., 2005). Spain, Portugal, Italy and France were endemic before 2001 and remain in this situation. However, in these regions, the distribution of cardiopulmonary dirofilariosis is generally reported only sporadically or not reported at all (Morchon et al., 2012). Dirofilaria species have spread to eastern and northeastern Europe, but limited epidemiological information from these countries is available (Genchi et al., 2009, 2011).

The prevalence of *Dirofilaria spp*. infections in dogs and humans in the Balkan Peninsula suggests that ecological factors, the climate and an abundance of vectors favor the full development and transmission of the infection (Tasic-Otasevic et al., 2015). However, in Romania the prevalence and distribution of *Dirofilaria spp*. infections in the dog are still unclear. The highest figures on prevalence range from 3.6% to 14% in Tulcea County, 3.3% in the south and southwest regions of the country (Mircean et al., 2012). Another study conducted in several areas of Romania demonstrated a seroprevalence of 23.7-35% for *D. immitis* (Coman et al., 2007), while information on *D. repens* was recorded only in the western regions (Ciocan et al., 2010, 2013) and the south (Tudor et al., 2013). In a recent study by Ionica et al. (2015), the seroprevalence of *D. immitis* infection was 7.1% in the eastern and southern parts of Romania. The highest prevalence of cardiovascular dirofilariosis was found in the central-eastern part of Romania, with a value of 60% recorded near the northern border of Galati County, followed by Vaslui County (12%) and Iasi County (7.7%). The prevalence of co-infections in the southeast is 8.8% (Ciuca et al., 2016).

The diffusion of this disease is increasingly fast, covering new endemic regions. Even if the pathology of dirofilariasis is known, it will still be a priority topic for veterinary research due to the zoonotic implications and the increased incidence of this disease in humans and animals (Simón et al., 2012).

Dirofilariosis has an uneven spread across the globe, being found in tropical, subtropical and temperate areas. The disease is strictly related to the concomitant or successive existence of the definitive and intermediate hosts in the same area. As development in the intermediate host is only possible in cases where the ambient temperature is above 14°C, limited spread is understandable at higher latitudes (Dărăbuș et al., 2006, Genchi et al., 2007, Cosoroabă et al., 2008). In recent years, a large number of native cases have been reported in dogs in new areas of Europe, such as Germany, Slovakia, the Czech Republic, Hungary, Romania, Ukraine, Russia, Austria, Switzerland, northern France and the Netherlands as a consequence of climate change, but also of the increase in the number of pet travel. Dogs living in rural areas are more exposed to the mosquito attack. Canine dirofilariosis is found especially in southern European countries, although the parasite was also diagnosed in northern France as a consequence of autochthonous infestation (Genchi et al., 2005, Genchi et al., 2007). The largest endemic area in Europe is the Po River valley in northern Italy, where the prevalence of Dirofilaria spp. infection is between 40 and 80%, largely due to the absence of chemoprophylaxis (Genchi et al., 2005). Imposing quarantine in the case of parasitosis is not effective because of the appearance of microfilaria in the blood within 9-10 months after the infested mosquito feeds on the definitive host.

In Romania, seroprevalence of *Dirofilaria repens* was reported at 16%, and at 6% for *D. immitis* (Ilie et al., 2012). Molecular biology tests showed the prevalence for *D. immitis* to be 2.7% and 15% for *D. repens*. Increased prevalence of cardiovascular dirofilariosis may be the consequence of the growing canine population and lack of prevention measures. In addition, the infestation values with *D. immitis* are directly influenced by the density of the mosquito population, the exact species and probability of multiplication, but also by climatic and environmental variables (temperature, humidity, precipitations, vegetation and presence of watercourses) (Madon et al., 2002; Cancrini et al., 2003; Roiz et al., 2007).
Clearly, on the basis of previous epidemiological studies, there is the zoonotic risk of this parasitosis (Darchenkova et al., 2009; Genchi et al., 2011; Kartashev et al., 2011; Lee et al., 2010; Simon et al., 2005). The distribution of dirofilariosis in humans does not coincide with the prevalence of dirofilariosis in dogs due to the lack of information on disease monitoring in humans and animals. Currently, cases of subcutaneous dirofilariosis in dogs are reported in regions where there have only been reports of cases of pulmonary disease in humans and vice versa. In the current distribution of dirofilariosis in humans, approximately 1782 cases were reported, of which 372 were patients with pulmonary dirofilariosis and 1410 were patients with subcutaneous / ocular dirofilariosis (Simon et al., 2010). Cardiopulmonary dirofilariosis predominates in the United States of America, where 116 cases have been reported, most of which in the South-East (Moore et al., 2005; Mumtaz et al., 2004; Skidmore et al., 2000). In North America, most cases of subcutaneous / ocular dirofilariosis were attributed to *Drofilaria ursi* and *D. tenuis*.

According to previous studies, the Mediterranean basin is endemic to human dirofilariosis. (Genchi et al., 2011). Although the incidence of cases in this region increased between 2000 and 2009, the distribution profile of dirofilariasis is not complete. Most of the pulmonary dirofilariosis cases have been reported in Spain, in the western part of the country, but subcutaneous dirofilariosis is reported more frequently on the Mediterranean Coast, based on serological studies (Simon et al., 2009). In France after 2000, 9 cases of dirofilariosis were reported: 7 of these due to *D. repens* and 2 to *D. immitis* infection. Between 1995-2000, dirofilariosis in humans was reported sporadically on the Atlantic Coast (Guillot et al., 1998; Weill et al., 1999), while during 2000-2009 the area became endemic for human dirofilariosis (Raccurt, 1996). Sub-cutaneous dirofilariosis caused by *D. repens* is the most common form of dirofilariosis in humans compared to *D. pneumitis* caused by *D. immitis* and other for subcutaneous tissue caused by other species of *Dirofilaria* (*D. tenuis, D.ursi*). Italy is the most affected country, where 200 cases of human subcutaneous dirofilariosis were recorded, followed by Sri Lanka and the Balkan area (Pampiglione, Rivasi, Angeli et al., 2001).

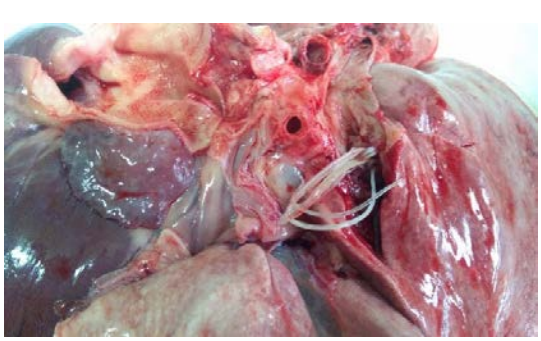
By 1999, most cases of dirofilariosis in humans were reported from the Mediterranean basin (Italy, France, Greece, Spain) (Pampiglione et al., 2000), all endemic to *Dirofilaria spp*., and very few cases of subcutaneous dirofilariosis reported in Germany, the Netherlands, the United States and Norway (Muro et al., 1999). According to studies from the last decade, the incidence of *Dirofilaria spp.* infestation has increased in the Mediterranean basin (France - 9 cases, Greece - 8 cases, Italy - 35 cases) and new cases of dirofilariosis came up in seven other regions (Bulgaria, Dubai, Georgia, Kazakhstan, Kenya, Iran, Israel), previously considered non-endemic (Simon et al., 2012).

In Europe, feline dirofilariosis was discovered in northern Italy, where Kramer and Genchi (2012) reported a prevalence rate of 7 to 27% in the hyperendemic region of the Po River valley. In the Canary Islands, two seroepidemiological studies have shown an increase in the prevalence of feline dirofilariosis from 18.3% to 33% between 2004 and 2011. In the United States, feline dirofilariosis was reported in 29 countries, with prevalence rates ranging from 3% to 19%, the highest being recorded in endemic areas for dirofilariosis in dogs (309). Various studies have shown that feline dirofilariosis is present in other countries, such as Australia, Sierra Leone, Armenia, China, Philippines, Malaysia, Tahiti and Papua New Guinea. Wild carnivores (*Canis lupus, C. latrans, C. aureus, Vulpes vulpes*) are frequently diagnosed with *D. immitis*: USA with a 21-42% prevalence in coyotes (Nagaki et al., 2000; Nelson et al., 2003) and California with a prevalence of 58-100% in foxes (Roemer et al., 2000).

In Europe, the prevalence of dirofilariosis in the red fox (*V. vulpes*) in Spain, Italy and Bulgaria ranges from 0.4% to 12% (C. Genchi, 2005, Gortazar et al., 1994; al., 2007), while in wolves (C. lupus) the prevalence is 2.1% (Segovia et al., 2001). On the territory of Bulgaria, dirofilariosis was detected in jackals (*Canis aureus*), with a prevalence of 8.9% (Kirkova, et al., 2007). Using the serological or even the post-mortem exam for detection, studies have generally revealed a modest microfilariemia in these hosts: the worm burdens in foxes are low, parasites are often of the same genus, therefore the risk of an infection reservoir is very low (McCall et al., 2008). In contrast, studies in California, USA, present coyotes as an active dirofilariosis reservoir due to the large number of microfilaria in the blood and of adults in the heart (Garcia and Voigt, 1989).

PATHOGENESIS OF CARDIOVASCULAR DIROFILARIOSIS

The cardiovascular dirofilariosis in dogs and cats is characterized by acute and chronic inflammatory lesions in the lungs and other organs due to the presence of adults and microfilaria. *Dirofilaria immitis*, like most filarial worms, has its metabolism conditioned by the presence of an intracellular rickettsian symbiont which has been found in abundance in the Malpighi tubes of mosquitoes (Sironi et al., 1995). *Wolbachia* would appear to have a major role in filarial physiology because the literature reports a massive decrease in the number of larvae in peripheral blood when the definitive host is treated with tetracyclines, especially doxycycline, which is most active against these bacteria McCall et al., 2008). Kramer et al. (2014) show that by sequencing the Wolbachia symbiont genome and by comparing it with the *Dirofilaria* species, it has been proved that the two entities are closely linked, each of them being able to encode proteins, enzymes, vitamins, nucleotides that the other cannot encode. Darby (2012) suggested that for *Onchocerca ochengi, Wolbachia* plays the role of mitochondria, providing the energy required for organic processes and muscle contraction. The pathophysiological response in cardiovascular dirofilariosis is mainly due to the presence of *D. immitis* parasites in the pulmonary arteries. The first lesion occurs in the pulmonary artery (Figure 4) and in the pulmonary parenchyma due to intravascular adult localization; pulmonary hypertension occurs, which then leads to congestive heart failure. Another syndrome is the blood circulation disorder due to the location of the *Dirofilaria* in the right side of the heart (Figure 5), at the level of the tricuspid valve. These disorders lead to massive haemolysis and hemoglobinuria, being responsible for the cave vein syndrome (Ishihara et al., 1978; Kitagawa et al., 1987).


a

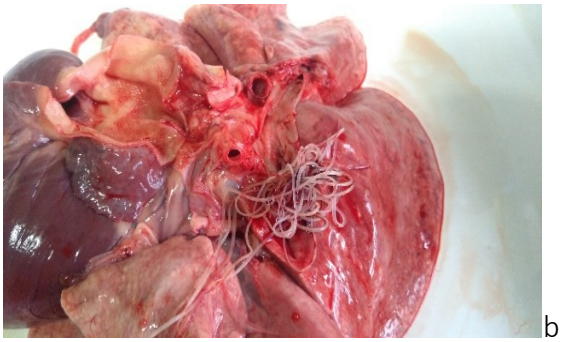

b

Figure 4. A bunch of adults of *Dirofilaria immitis* before (a) and after mechanical extraction (b) from a nodule from the traject of the right diaphragmatic lobar branch of the pulmonary artery in a male mongrel dog aged 12

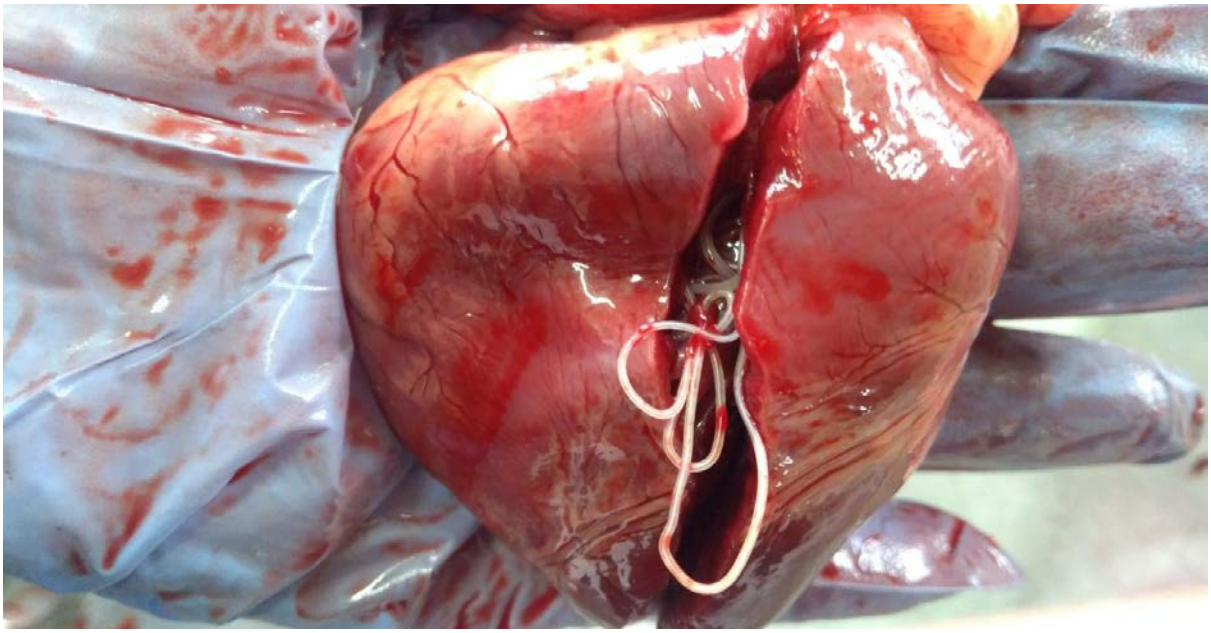
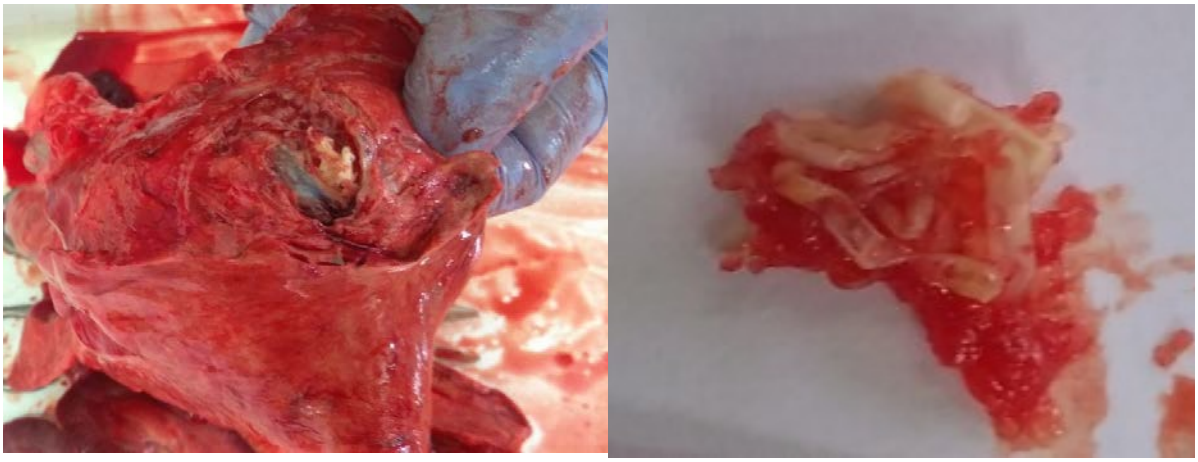


Figure 5. Adults of *D. immitis* in the right chambers of the canine heart

The microfilariae appear to play a minor pathogenic role, but they can cause pneumonitis and glomerulonephritis. Some individuals may develop a hypersensitivity to microfilaria, so they disappear from the peripheral blood. Occasionally, parasites may have ectopic locations, such as the anterior chamber of the eye (Weiner et al., 1980). Massive infestations can result in obstruction of the right ventricle and pulmonary artery (figure 6), and fragments of dead parasites as well as microfilaria can cause emboli in the pulmonary capillaries and coronary arteries. Microfilaria can reach the encephalus, the spinal cord, the eye vessels, and even the anterior or posterior chamber of the eye. Toxic and antigenic action is caused by the substances produced by adult parasites in the arteries, the right side of the heart, and especially thromboxanes released by the blood platelets in contact with parasites (Uchida and Saida, 2005).



a b

Fig. 6. Nodule on the traject of the lobar branch of the pulmonary artery filled with organic debris of dead *D. immitis* worms (a). The aspect of the content removed from the nodule (b).

The heartworms act mechanically through their large body and tend to block, in particular, the right ventricle and the pulmonary artery, while the hematophagous regimen produces anemia and irritation. It forms emboli (the appearance and circulation in the bloodstream of foreign particles of the normal blood morphochemical composition) by pushing parasite fragments into the bloodstream and causing the sudden death of the animal due to breakage of cerebral vessels (Kitagawa et al., 2003).

The caval syndrome is a severe clinical form of dirofilariosis in a dog. The main mechanisms of this syndrome are: severe and acute tricuspid regurgitation, reduced cardiac output, and intravascular haemolysis. In this situation, a large number of *D. immitis* adults (over 60) migrate from the right side of the heart to the large vessels. Sudden shock, collapse and destruction of red blood cells, usually without early symptoms, occur. Death usually occurs within 1-2 days and the only effective treatment is to open the jugular vein and extract the worms with a special forceps. Survival of the dog depends on the surgical extraction of a sufficient number of adults so that blood circulation can be restored (Marck et al., 1998). Adults reaching the right ventricle are located in the tricuspid system and migrate to the right atrium. Their simple presence in the tricuspidian system produces severe damage of valves (Figure 7), followed by tricuspid regurgitation and aggravated by preexisting pulmonary hypertension. Very soon, there is right heart failure with right systolic murmur, hepatomegaly, splenomegaly, abdominal ascites (Wendy et al., 2007). Pulmonary hypertension as well as tricuspid regurgitation lead to reduced peripheral arterial circulation and reduced pulmonary venous circulation, and implicitly to decreased left heart volume with decreased cardiac output, decreased diastolic volume etc. (June et al., 1998).

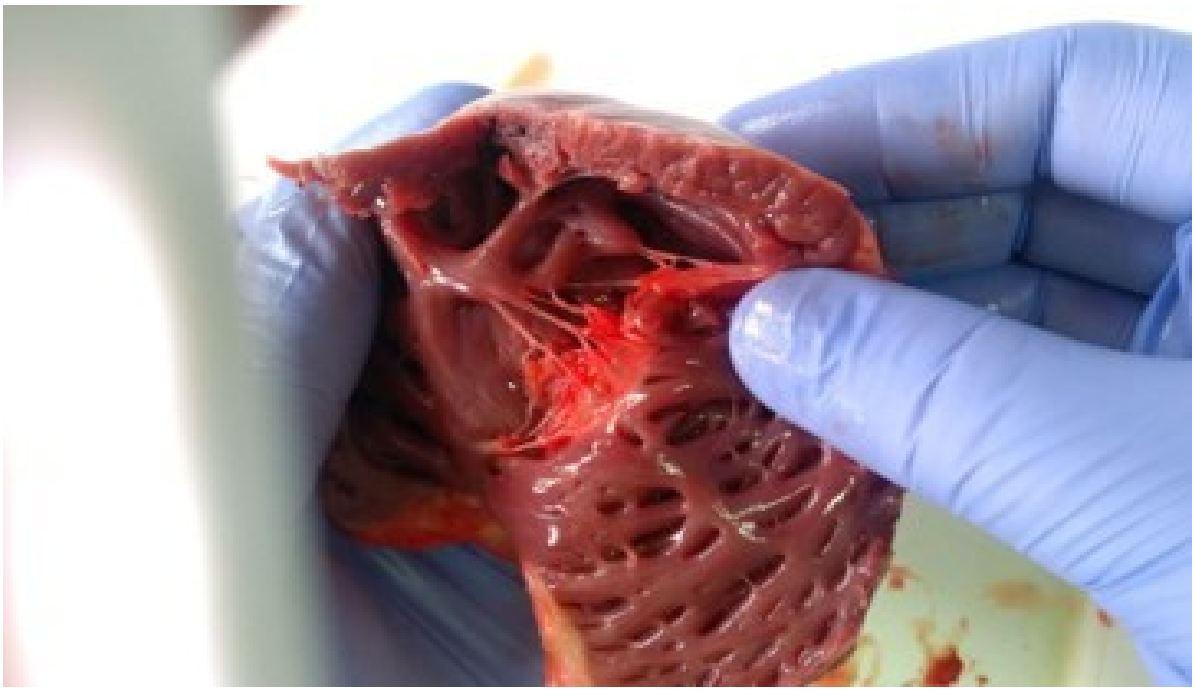


Figure 7. Dog endocharditis due to chronic heartworm disease

Intravascular haemolysis caused by canine heartworm remains a matter of speculation. Endothelial cell disruption and denudation of the intima are the first lesions that occur a few days after the parasites occupy the blood vessels. Evidence suggests that endothelial damage occurs as soon as the parasite is in place, too early for the host to develop an immune response.

Furthermore, the disappearance of endothelial cells occurs without obvious degeneration and is followed by a build-up of cells and structural elements. This indicates that the cells were dislodged (broken). Macrophages, granulocytes and platelets are attracted to the site of endothelial lesion and adhere to the exposed sub-endothelial surface. Shortly after their arrival, the smooth muscle cells of the blood vessels migrate in the intimate and start an active miointimal process that produces a rapid increase in lesions. Although lesions produce a thickening of the wall of these elastic vessels and print a thick texture of the intima, this does not block the blood flow by narrowing the lumen. On the contrary, the distribution of large arteries produces dilation, so pulmonary hypertension becomes quite severe. Circulation of the pulmonary blood is prevented by the reduction of the vascular arterial bed caused by a peripheral vascular endoarteritis. Consequently, thrombosis and thromboembolism compromise pulmonary circulation. (Said and Saida, 2005; Hitoshi et al., 2003). Right ventricle hypertrophy appears as a compensatory response to the increased blood pressure load.

PATHOGENESIS OF SUBCUTANEOUS DIROFILARIOSIS

The pathogenicity of this nematode to the dog is very poorly known, as this infection is considered asymptomatic. Adults located in the subcutaneous tissue of the dog may cause dermatological clinical signs such as pruritus, erythema, papules, alopecia, hyperkeratosis, acanthosis, eczema or may well develop asymptomatically.

Serious infections with allergic reactions, possibly due to microfilaria, have also been reported. Generally, 85% of dogs with subcutaneous dirofilariosis exhibited at least one lesion of the subcutaneous tissue in the dorsal part of the body, in the lumbosacral region, the posterior limbs, or in the perianal region (Mandelli, Mantovani, 1966). Recent reports indicate the association of subcutaneous dirofilariosis with other diseases, such as babesiosis (100%), granulocytic erlichiasis (60%) leishmaniosis, most commonly in the Italian region (Tarello, 2010).

CLINICAL SIGNS

CARDIOPULMONARY DIROFILARIOSIS IN ANIMALS AND HUMANS

Normally, the expression of the cardiovascular dirofilariosis symptoms appears in the chronic form. The disease may develop asymptomatically over a period of several months or even years, the appearance of clinical signs being dependent on the number of adults in the heart or pulmonary artery, individual reactivity and physical activity of the dog (lesioning of the artery walls is directly proportional to the physical activity of the animal) (Dillon et al., 1995a). Ideally, the infection with *D. immitis* should be identified by serological testing prior to the appearance of clinical signs. However, at the earliest, antigenemia and microfilaemia do not occur until up to 5 and 6.5 months, respectively, after the infection. When dogs do not receive prophylactic treatment and are not properly tested, the infection is not detected and it progresses as the number of adults of *D. immitis* increases. Clinical signs such as cough, exercise intolerance, apathy, dyspnea, cyanosis, hemoptysis, syncope, epistaxis and ascites (right congestive heart failure) may occur. The frequency and severity of clinical signs correlate with pulmonary pathology and the physical activity level of animals. In sedentary dogs, signs are often not observed even though the number of adults of *D. immitis* in the heart may be relatively large. Infected dogs experiencing a dramatic increase in physical activity, such as during the hunting season, may show obvious clinical signs. Also, parasite death and thromboembolies precipitate expression and worsening of clinical signs (McCall, et al., 2008).

In congestive heart failure, the following are usually noted: abdominal distension, edema of the limbs, anorexia, weight loss and dehydration. At this stage of the disease, there are sounds of heart murmur on the right side of the chest due to tricuspid valve insufficiency, and abnormal heart rhythm due to atrial fibrillation. Sudden death happens very rarely and dogs usually die due to respiratory emergency or cachexia. Occasionally, acute episodes can also be observed during the chronic period of disease progression, so after severe adult death severe thromboembolism may occur, and dogs may display acute dyspnea and hemoptysis with a fatal outcome. Based on the assessment of the number of adults in the right side of the heart, animal health, and age and lifestyle, a dog may be classified as having a low or high risk for the development of clinical signs of infection with *D. immitis* (Furlanello et al., 1998; Calvert et al., 1985; Venco et al., 2001).

There is also a more complex classification system in which dogs are classified from I to IV based on the severity of clinical signs: Class I dogs with mild infection; dogs in Class II have coughing; Class III dogs are severely affected and show cough, haemoptysis, weight loss, lethargy, exercise intolerance, dyspnoea, heart failure (ascites), and radiographic findings suggestive of cardiovascular infection (large primary pulmonary arteries and lobar pulmonary arteries are truncated, arteries pulmonary sinuous lung and infiltrated lymphadenopathy). Class IV dogs are those with caval syndrome characterized in principle by haemodynamic changes (AHA, 2014). The main signs are: dyspnoea, tricuspid heart murmur, acute intravascular haemolysis, and the sign considered pathognomonic for caval syndrome is hemoglobinuria. In this situation, in the absence of surgery to eliminate the heart parasites, the animal will not survive (Atwell and Buoro, 1988; Kitagawa et al., 1986, 1987; Venco, 1993).

Dogs aged 5-7 are at a higher risk of infection with a high number of *D. immitis* adults, probably due to increased exposure time and the development of the disease. There are also other factors that affect the evaluation of the risk of *D. immitis* infection, such as cardiopulmonary disease or other systemic diseases and pathologies of other organs. Another important aspect is the extent to which the physical activity of the animal can be restricted during the treatment period (Venco et al., 2001).

Typically, cat dirofilariosis develops with the pulmonary localization of filaria. From the clinical point of view, it can develop acutely, chronically or asymptomatically. Infected cats may be asymptomatic carriers of the parasite, or may have suggestive clinical signs of respiratory or digestive origin. Clinical signs are nonspecific, but most often there is vomiting and cough, signs that are associated with the moment when immature stages of *D. immitis* arrive in the lungs or when adult death occurs. At this stage, it is infiltrated into distal pulmonary arteries and often associated with eosinophilic pneumonia (Dillon et al., 2000).

In the very rare cases in which *D. immitis* adults are in the right side of the heart, an abnormalsound may be heard due to tricuspid valve insufficiency and galloping heart rate (Atkins et al., 1998a). Neurological signs such as ataxia, syncope, blindness can be observed when the ectopic localization of the filaria occurs (Atkins et al., 1998a, Dillon et al., 1996, 1997a, b, 1998, McCall et al., 1994). Although rarely observed clinically, pulmonary edema, pneumothorax, or chylothorax were reported in cat dirofilariosis (Atkins et al., 1998b, Dillon et al., 1997b, Glaus et al., 1995, Treadwell et al., 1998). In principle, there are two phases of clinical expression in the evolution of dirofilariosis in the cat: the first stage in which D. immitis larvae L5 reach the pulmonary arteries and die, and the second stage is marked by the death of *D. immitis* adults (Atkins et al., 1998a Dillon et al., 1995b).

Generally, most cases of cat dirofilariosis are undiagnosed. Mostly immature stages of nematode *D. immitis* do not get mature and die as they reach the pulmonary arteries. Thus, the absence of adults makes it impossible to diagnose the infection in the absence of the cuticular antigen. The death of the larvae in the pulmonary arteries induces severe changes in the respiratory system, which is why the disease is now recognized as a pulmonary syndrome called HARD-Heartworm Associated Respiratory Disease (AHA, 2014). Clinical signs describing this associated respiratory syndrome are: anorexia, rapid heart rate, difficulty in breathing, lethargy, vomiting, coughing, collapse, convulsions, diarrhea, weight loss, sudden death, blindness (McCall et al). The results of some studies conducted by Dillon (2007), Levy (2007) demonstrated the presence of respiratory lesions caused by the death of *D. immitis* L5 larvae in pulmonary arteries, in non-adult cats. These lesions are due to a vascular and parenchymal inflammatory response. Cats have specialized macrophages (pulmonary intravascular macrophages) in the capillary beds of the lung, and their activation is largely responsible for the exacerbated pulmonary reaction. Thus, the consequences of these reactions are: the lung does not work in the normal parameters and the occurrence of acute respiratory syndrome, which is often misdiagnosed as asthma or allergic bronchitis. In cases of acute infection where which cats survive, they may go into chronic form or become completely asymptomatic. Chronic dirofilariosis generally prevails in respiratory and / or gastrointestinal symptoms, leading to severe internal degradation of internal organs, and eventually cachexia. Although ferrets (*Mustela putorius furo*) are very susceptible to infection with *D. immitis*, unlike dogs, even a small number of adults can cause their death (Kemmerer, 1998). *D. immitis* adults are frequently located in the cranial veins and the cava vein as well as in the pulmonary arteries and heart cavities. The symptomatology is similar to what occurs in the dog but the course of the disease is faster. Also, ferrets frequently develop the caval syndrome, as exemplified in the study by Supakorndej et al., in which 1 in 7 infected ferrets developed this syndrome. Clinical signs include: lethargy, exercise intolerance, pleurisy, cyanosis and dyspnoea. When sudden death occurs, this is caused by pulmonary embolism.

SUBCUTANEOUS DIROFILARIOSIS IN ANIMALS AND HUMANS

Subcutaneous dirofilariosis in dogs is usually asymptomatic. Clinical manifestations are classified in two clinical syndromes: multifocal nodular dermatitis, which is generally located on the face and prurigo papularis dermatitis. Numerous dermatological manifestations such as: pruritus in 100% of animals, erythema (79%), papules (62%), focal or multifocal alopecia (55%), hyperkeratosis (14%), nodules (12%), acanthosis (5%), eczema (3%), pyoderma (3%) and edema (1%) have been noted. Extradermal localisations of *D. repens* adults include: conjunctivitis (46%), anorexia (35%), vomiting (26%), fever (25%), lethargy (20%), enlarged lymph nodes (10%) (417, 418). A recent study conducted by Mircean (2017) reveals the implications of *D. repens* microfilaria in kidney and liver imbalances and the presence of adults in abdominal and ocular cavities. These changes and injuries have been attributed to both mechanical and immunopathological processes. Consequently, experimental investigations on pathogenic mechanisms of subcutaneous dirofilariosis are required.

HUMAN DIROFILARIOSIS

Dirofilaria immitis is located in the lung, causing nodules most often confused with pulmonary neoplasms (benign or malignant - carcinomas, metastases), tuberculosis or fungal infections (Ro et al., 1989; Awe et al., 1974). Parasitic granuloma surrounds the fourth stage larva (L4), blocking the pulmonary artery and causing local embolism and inflammation. From the histological point of view, the pulmonary nodules are most commonly formed by a cellular infiltration made up of eosinophils, neutrophils, lymphocytes and plasma cells, and by a histiocytic reaction in the tissues surrounding the capillary vessels. Nodules originating from an infarct due to the pulmonary embolism process are met more rarely. The most common are cases where the lung node presents a necrotic region with lysis of the wall of the pulmonary artery due to larvae that have left the nodule (Araya et al., 2007). Usually, the nodules are found in the right pulmonary tract, frequently in the sub-pleural regions (Muro et al., 2001). In many cases, the lung nodule cannot be detected because the larva has already been destroyed with the passage of time and only a cellular response can be observed (Simon et al., 2005). Pulmonary dirofilariosis develops asymptomatically, and if clinical manifestations occur, these are nonspecific: cough with chest pain, fever, dyspnoea. In general, pulmonary dirofilariosis develops through the appearance of a solitary, well defined node, of spherical or ovoid shape, homogeneous density and benign profile (Muro et al., 2001). There have also been reports of pulmonary dirofilariosis with the presence of several pulmonary nodules, with up to five parasitic

nodules in the same individual (Kochar et al., 1985). Previous studies have reported different time intervals for the formation of the pulmonary node, the interval ranging between 2/3 up to 8 months (Kahn et al., 1983; Navarrete et al., 1972).

Dirofilaria repens causes subcutaneous dirofilariosis in humans, with the presence of nodules in the subcutaneous tissue, deep in the dermis or submucosa. Subcutaneous nodules have a firm, elastic consistency and are associated with erythema (Pampiglione et al., 2007). The adult or pre-adult form of *Dirofilaria repens* grows within the subcutaneous nodule within a few weeks or months (Kramer et al., 2007). Very few cases have been reported of localization of the pathogen in the muscle tissue, lymph nodes or viscera (Pampiglione et al., 1996c; Gros et al., 1996). In contrast, most reports of subcutaneous dirofilariosis were localized in the ocular region (74%) (eyelids, subconjunctival, orbital region) and in the upper limb region (11%) (Pampiglione et al., 2000). Clinical evolution may be severe in patients with ocular dirofilariosis, and symptoms may include visual impairing or even loss of vision (Genchi et al., 2011).

Epidemiological studies have shown that 10% of patients with ocular dirofilariosis exhibit a number of secondary complications such as retinal detachment, glaucoma, and visual acuity disorders (Avdiukhina et al., 1996); secondary complications due to surgical extraction in the optic nerve region (Korkhov et al., 2009); secondary complications as a result of localization of the pathogen in the orbital area: palpebral ptosis (Stringfellow et al., 2002).

In Sri Lanka, a number of cases with unusual localizations of *Dirofilaria repens*, such as in the male genital region (scrotum, epididymis, penis) have been reported in children aged 5 (Dissanaike et al., 1997); other early localizations of *D. repens* were reported by Hoop et al. (1997) in a patient with a granuloma in the parotid gland; in the submucosa of the oral cavity and root of a tooth by Avdiukhina et al., (1997); in the mammary gland of a woman by (Genchi et al., 2011).

DIAGNOSIS OF DIROFILARIOSIS IN ANIMALS AND HUMANS

PARACLINIC DIAGNOSIS

In optimal conditions, the lifecycle lasts 184-270 days, so that the dog can become microfilaremic within ca. 7-9 months after the infection. Not all infected dogs become microfilaremic (in unisex infestations, when administering drugs that induce sterility of *Dirofilaria immitis* females, in individual situations of occurrence of immune-mediated reactions leading to the death of microfilaria) (McCall JW, et al., 2008).

The diagnosis of dirofilariosis is based on the presence of circulating microfilaria and / or circulating antigens from adult females. Not all microfilaria found in the blood of dogs are *Dirofilaria immitis* (Acantocheilonema reconditum, *Dirofilaria repens*, *Dipetalonema* dracunculoides and, very rarely, *Dipetalonema grassi*). Adults of *Dirofilaria immitis* live between 5-7 years. Transplacentary trasmitted microfilaria or those transmitted by haematransfusion are incapable of developing in adults (Castillo JC, et al., 2011).

The antigen detection test was first described in the early 1980s. Weil et al. (1984) showed the detection of adults of *D. immitis* by counterimmune electrophoresis (CIE). Subsequently, the authors described the ELISA based on monoclonal antibodies (Weil et al., 1985). Both techniques are characterized by high specificity and sensitivity to the detection of circulating microfilaria. Also, the antigen screening test was able to assess the degree of infestation. Indeed, Brunner et al. (1988) showed that the sensitivity of the tests was not affected by the presence of circulating microfilaria of *D. immitis*, but was largely influenced by the large number of *D. immitis* adults.

Tests with false negative results may be due to the presence of male or female worms (unisex infections are extremely rare in dogs, Rishniw et al., 2012), elimination by means of immune system mechanisms or the use of macrocyclic lactones (LM, Rawlings et al., 1982). Antigen screening tests, which can be performed on whole blood, plasma or serum, can also produce false negative results because of antigen-antibody complexes that inhibit immunoassay tests to identify antigens and develop subsequent colorimetric reaction (Tonelli et al., 1989). Recently, it has been found that long-term use of monthly macrocyclic lactone in infected dogs (so-called "slow killing") can also cause false negative test results for antigen detection, probably due to an intense antibody response to antigens released from adults of *Dirofilaria immitis* that die (Drake et al., 2015).

Interestingly, most of the diro's screening tests used an antigen recovery method to

minimize the effects of immune complex formation on the performance of the test (Little et al., 2014). Also, the use of chemicals (e.g., pepsin and acid treatment) has been reported to remove antigen-detectable inhibitors (Rodríguez-Iglesias et al., 1992).

It has been argued that pretreatment of heat serum samples before testing for antigens is able to reverse false negative results due to antigen-antibody complexes in hosts infected with *D. immitis*, both experimentally and naturally (Little et al. , 2014a; Little et al., 2014b; Velasquez et al., 2014; Ciuca et al., 2016). Thus, heat treatment disrupts antigen-antibody complexes and releases antigen which is subsequently made available for detection. This may have important consequences for the diagnosis of clinical disease but also for epidemiological studies, especially in areas where the prevalence of infection is not well known.

Diagnosis of *D. repens* infection is based on the presence of circulating microfilaria or on parasite observation in the subcutaneous nodules, as there are currently no screening tests available for antigens for serological diagnosis.

D. immitis and *D. repens* can also be identified by histochemical staining of the anatomical regions of microfilaria with phosphatase activity and amplification of microfilaria DNA by the PCR method. *D. immitis* microfilaria shows two areas of phosphatase activity near anal and excretory pores, while mff. *D. repens* have only one area of phosphatase activity near the anal pores. Recently, a duplex real-time PCR method capable of detecting and differentiating the two filaria as well as the multiplex PCR method for simultaneous detection of filaria in the dog have been described.

There have been several studies published on the prevalence of *D. immitis* and *D. repens* infection in dogs living in endemic areas for both parasites (Pantchev et al., 2009, 2011; Demiaszkiewicz et al., 2014; Ionică et al., 2015). The Knott method, along with the antigen detection test and the PCR technique were used to determine the state of monoinfection or co-infection of the final hosts. However, many dogs in which the antigen detection test had a negative result, whether or not microfilaremic, were considered either uninfected or infected only with *D. repens* on confirmation by the PCR method. None of these studies subjected the serum samples to heat treatment, probably underestimating the actual prevalence.

CLINICAL DIAGNOSIS OF DIROFILARIOSIS IN ANIMALS

The evaluation by thoracic radiography, echocardiography and electrocardiography provides a perspective on the clinical condition of each patient with cardiopulmonary dirofilariosis. Chest radiographs identify pulmonary artery enlargement, lung parenchymal changes, and right cardiomegaly in the advanced stages of the disease. This technique

cannot be used to evaluate parasitic burden. Echocardiography is an examination by means of which the adults can be visualized in the right heart chambers, the caudal vena cava, the main pulmonary artery, and the proximal tract of both caudal lung arteries. Adults can be identified as short, double linear, floating in the right heart chamber or in the lumen of the vessels (Moise, 1988; 276). Cardiac ultrasound also provides information about heart parasite load and disease status, important factors in establishing appropriate therapy. An important aspect is that cardiac ultrasound should be considered in cases where clinical and imaging characteristics suggest a severe infection. The Doppler echocardiography can accurately determine the presence and severity of pulmonary hypertension. The electrocardiogram is a useful exam through which abnormalities of the electrical action of the heart (right electrical axis deviation, atrial fibrillation) can be identified, but these changes are usually found in the last severe stage of the disease (McCall et al., 2008).

In feline cardiopulmonary dirofilariosis, the radiological profile may be normal despite the presence of the infection. Also, single-adult infections can cause changes that are not visible by radiography, leading to a misdiagnosis. Experimental animal studies have shown that after 6 months of inoculation with infective larvae, the radiological pulmonary changes suggestive of cardiopulmonary dirofilariosis in the cat involve the presence of a large area of radiopacity in the pulmonary parenchyma, the torsion of caudal pulmonary arteries and the occurrence of interstitial pneumonitis.

DIAGNOSIS IN HUMAN DIROFILARIOSIS

In case of subcutaneous or conjunctival nodules, the patient is the first to discover infestation with *Dirofilaria* and to request a medical examination. Indeed, the pulmonary nodules are deeply localized, most commonly without clinical expression, and the radiological examination can only occasionally detect fragments of the lung nodules. Typically, the radiological examination recommended by a physician does not aim to exclude or confirm dirofilariosis from the pathology of the patient, but rather a suspicion of malignant lesion (Simon F., et al., 2007).

In order to perform a correct diagnosis, two essential procedures for the confirmation of dirofilariosis should be followed: collection of appropriate samples and correct identification of the pathogen (McDaugall, et al., 1992). In the absence of blood microfilaria, the identification of the pathogen is done by biopsy to help confirm the presence of the parasite in the node (Cancrini et al., 1991). It is an invasive procedure with a high potential for iatrogenic complications, especially in the case of pulmonary nodules. Identification of the pathogen from the biopsy of a lung node may be difficult due to the gradual

decomposition of the parasite. Its identification becomes difficult due to the morphological similarities of the cuticle of several species of parasites. For example, filarial identification features include: the number and dimensions of the cuticle ridges, their spacing, and their placement on the cuticle. All these characteristics, similar to many species of filaria, prevent accurate identification of the species (Orihel et al., 1998). According to studies conducted by this author, all species of the Dirofilaria genus, especially those that infest humans, show ridges in the cuticle except for the species *Dirofilaria immitis* and *D. lutrae*, where the cuticle is smooth. So far, there is no information on infesting humans with this species, but the initial stage of *D. immitis* is located in the subcutaneous tissue (Moorhouse et al., 1978)

Molecular and immunological techniques are used as complementary or alternative methods in the diagnosis of dirofilariosis. In cases where the parasites show modifications due to the host's immune response and cannot be identified based on morphology, PCR is used to identify the *Dirofilaria* species, a highly sensitive and specific test even for small amounts of DNA (Eccher, et al., 2008). Immunohistochemistry is another diagnosis method which singles out the Wolbachia symbiont in nodules (Simon et al., 2007). Another complement to the invasive method is serology-ELISA. Even if infestation occurs with a small number of parasites, the host develops a strong immune response. Thus, various antigenic complexes are used to identify anti-*Dirofilaria spp*. antibodies (Montoya et al., 2010).

Serology is a complementary technique to non-invasive methods in the diagnosis of lung dirofilariosis in humans. Various antigen complexes have been used to detect human dirofilariosis-specific antibodies (Santamaria et al., 1995; Simon et al., 1991). The sensitivity of serological testing of patients with dirofilariosis is increased due to the use of various epitopes resulting from the sequencing of polypeptides in antigenic complexes specific to *Dirofilaria immitis* and *D. repens*.

Thus, molecular weight proteins (Di 35, 35-kD) have been identified and characterized by various authors (Philipp et al., 1986). Those proteins have been cloned and used as recombinant proteins by means of the ELISA technique, thus demonstrating an increased sensitivity and specificity in the diagnosis of dirofilariosis in humans (Sun et al., 1992). Using the Western Blot method, protein markers have been successfully used in the diagnosis of lung dirofilariosis. On the ELISA plate, these proteins exhibit 100% sensitivity and 90% specificity with positive values 75% and 100% positive for negative ones (Perera et al., 1998). Thus, serological testing should be supplemented with other paraclinical examinations such as: radiological examination, data from medical history and area of residence prior to the use of invasive methods in the diagnosis of lung dirofilariosis in humans (Muro et al., 1999). Polypeptides with molecular weights ranging from 26-40 kDa specific for *Dirofilaria repens* (Simon et al., 1997) have also been identified.

In conclusion, taking into account the results obtained in the literature, serological correlation using methods such as ELISA and Western Blot with other invasive and non-invasive methods is the main action in the diagnosis of dirofilariosis in humans (Simon et al., 2012). Difficulties in serological testing of serum samples may be due to the interference of Dirofilaria species with other helminthes, such as *Toxocara canis (larva migrans), Ascaris suum, Echinoccocus granulosus*, but these obstacles can be overcome by choosing protein markers with specificity and treatment of serum with helminth-specific antigens. Diagnosis of dirofilariosis in humans is still a large subject of research given the poor knowledge of the disease by most people in urban and rural areas. The apparently small number of cases identified in the literature by serological studies should be reassessed due to ocular disease infections and diagnostic difficulties (Genchi et al., 2005).

TREATMENT AND PROPHYLAXIS

Treatment in cardiopulmonary dirofilariosis is complex and difficult to establish in conditions where adulticides can cause thromboembolism and death of the patient. In conclusion, the therapy schedule should be used depending on the animal's health status and burden with adults of *D. immitis*, and the association with other competing diseases. In principle, the treatment is aimed at eliminating microfilaria from the blood and disrupting the development of larval stages in adults and the elimination of preexisting adults. Assessment for the treatment of adulticides and the risk of thromboembolism should be performed individually for each infected animal. If so far the disease was considered to have a 4-stage evolution (Di Sacco and Vezzoni, 1992), researchers have now reduced the disease to two categories of evolution: mild (low risk of thromboembolism) and severe (high risk of thromboembolism).

In order to place the patient into the appropriate class, the doctor should consider the following: the parasitic load (the number of adults present in the pulmonary arteries and the right side of the heart), the size and age of the animal [dogs aged 5-7 are at risk of having a large numbers of adults (Venco et al., 2004)], lung changes and the degree of restriction of physical exercise. Dogs with low risk of thromboembolism include: a small number of adults without clinical signs, normal radiological profile, cardiac ultrasound does not reveal the presence of adults in the right side of the heart, low titre of circulating antigens or negative antigen test, but the presence of microfilaria in blood, the absence of concurrent disease association, andthe availability of physical activity restriction (owner involvement).

Dogs with high risk of thromboembolism include: high adult loads, clinical signs specific to the disease (cough, abdominal distension), adult observation in the right side of the heart to cardiac ultrasound, severe pulmonary changes, high circulating antigens, absence of restriction of physical activity (absence of owner involvement) (Venco et al., 2011, Maccal et al., 2008).
The supportive therapy has the role of reducing and controlling pulmonary inflammation, pulmonary edema and reducing complications resulting from adulticide therapy (Dillon et al., 1995). Corticosteroid use (prednisolone 1-2 mg / kg for 4-5 days), diuretics (furosemide 1mg / kg) and digoxin can only be used when atrial fibrillation is present.

ADULTICIDE THERAPY

The only substance approved and recommended by AHA (American Heartworm Society) is melarsomina, used in a 2.5 mg / kg dose, two doses at 24-hour intervals. Recently, the AHA recommendation (2005) proposes two-phase melarsomine therapy to reduce the risk of pulmonary thromboembolism consisting of intramuscular injection of two doses at 24 hours followed by a third dose at 30 days. This treatment scheme involves the initial elimination of 90% of adult males and 10% of adult females, reaching a 50% reduction in the total number of adults. The third dose eliminates the remaining adults, so reducing the risk of thromboembolism and shock when adult death is achieved gradually (AHA, 2012). Generally, adulticidal therapy causes pulmonary thromboembolism, especially if parasitic load is high. Pulmonary thromboembolism can be controlled by movement restriction at least one month after adulticide therapy, administration of heparin and corticosteroids to reduce pulmonary inflammation and to avoid severe respiratory shock due to adult elimination (Venco et al., 1998).

Numerous studies suggest that macrocyclic lactone therapy (ivermectin), which has been shown to be partially adulticidal when used in doses of 6-12 mcg / kg every month for 16 months or even 30 months, has an efficacy of 100% (McCall et al., 2001). In contrast, other studies demonstrate a worsening of the animal's health when adult elimination is achieved slowly and over a long period of time (Venco et al., 2004).

Surgical extraction is recommended in dogs with a large parasitic charge as the only safe method of eliminating adults without the risk of pulmonary thromboembolism (Morini et al., 1998). The surgical extraction of *D. immitis* adults is performed with a Flexible Forceps Alligator (Fuji Photo Optical LTD, Japan) which is inserted along the jugular vein with guidance provided by fluoroscopy (Ishihara et al., 1990).

In cats with cardiopulmonary dirofilariosis, therapy consists of administering the corticosteroid support medication to control pulmonary changes. According to some studies, cats can often be cured spontaneously, with evidence of regression of lung imaging signs and negative results in antigen tests.

A daily dose of prednisolone is recommended starting from 1 to 2 mg / kg body weight every 12 to 24 hours, gradually decreasing to 0.5 mg / kg every 2 days for 2 weeks, followed by observation without treatment for another 2 weeks.

The risks of adulticide therapy and the severity of infection, especially in dogs, makes prophylaxis play a very important role. Studies have demonstrated the efficacy and safety of macrocyclic lactone administration, such as ivermectin, milbemicinoxima, moxidectin or selamectin in the prevention of dirofilariosis in dogs. The American Heartworm Society recommends testing animals to identify the *D. immitis* antigen before administering

prophylactic substances to avoid the risk of pulmonary thromboembolism when dosage is inadequate to the stage of infection. Tests for the *D. immitis* antigen are also recommended every year, and chemoprophylaxis in endemic areas can be initiated from the age of 8 weeks, one month before the start of vector activity and one month after the end of their activity (Rubin et al., 2010).

Doxycycline therapy (10mg/ kg) for a period of 4-6 weeks, followed by macrocyclic lactone administration in the usual doses for microfilaricide therapy, leads to female sterilization and amicrofilaremia, prevention of reinfestation and slow killing of adults. Adult death, not susceptible to efflux of endosimbionts, is characterized by low risk of thromboembolism and inflammation (Kramer et al., 2014).
Prevention of *D. repens* with macrocyclic lactones is questionable and, to date, drugs containing continuous release moxidectin appear to be effective, according to experimental studies. Monthly prophylaxis with macrocyclic lactones is the only effective option for cats living in endemic areas of dirofilariosis in dogs. The monthly doses of prophylactic substances are as follows: 24 µg / kg body weight of ivermectin, 2 mg / kg milbemycin oxima, 1 mg / kg moxidectin and 6 to 12 mg / kg selamectin, starting at 8 weeks of age (Genchi et al. 2007).

Prophylactic medication for dirofilariosis is not recommended for humans. The most important aspects are: the development of a differential diagnosis to eliminate other causes that might have led to the appearance of the nodules that have to be removed surgically, the avoidance of surgical intervention in the pulmonary nodules until an etiologic diagnosis has been completed.

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