DIROFILARIASIS AND OTHER ZOONOTIC FILARIASES:
AN EMERGING PUBLIC HEALTH PROBLEM IN DEVELOPED COUNTRIES

F. SIMON MARTIN¹ & C. GENCHI²

¹Laboratorio de Parasitología, Facultad de Farmacia, Universidad de Salamanca, Av. del Campo Charro s/n 37007 Salamanca, Spain
²Istituto di Patologia Generale Veterinaria, Università di Milano, Via Celoria 10, 20133 Milano, Italy

Received 23 November 1998; accepted 24 March 1999


SUMMARY: The tropical filariae which affect the human populations of different zones of the world constitute a parasitological and sanitary problem of the first order. As well as these species, there are other filariae of less importance, which infect, in the first place, wild and domestic animals, and which can be transmitted to man, causing zoonotic processes. Up to the present, zoonotic transmission has only been demonstrated in those species which infect mammals. Of these, the best known are species of the genus Dirofilaria, such as D. immitis, D. repens or D. conjunctivae. The relationships between some of these species and their hosts are described, presenting clinical aspects, immune response, evasion and geographical distribution. Diagnosis has undergone important advances with the application of the techniques of radiology, immunology and molecular biology. Specific identification is possible, both in animals and humans, using specific polypeptides and primers applied in enzyme immune assay and amplification of DNA (PCR), respectively. The application of these techniques in each particular case is discussed. Diagnosis constitutes the first step in the control of the zoonosis. Given that anti-Dirofilaria vaccines are still not available, control is exercised by chemotherapy. The therapeutic characteristics of some of the pharmaceutical products employed are described, especially those most recently introduced, such as melarsomine dihydrochloride, ivermectin and milbemycin, and the therapeutic guidelines recommended. Finally, a brief review of other species of zoonotic filariae, for which very limited data are available, is presented.

KEY WORDS: Zoonotic filariasis, dirofilariasis, Dirofilaria spp., life cycles, immune response, evasion, pulmonary dirofilariasis, subcutaneous/ocular dirofilariasis, diagnosis, treatment, epidemiology.

CONTENTS

INTRODUCTION

Five of the six most prevalent illnesses in human populations in tropical areas are caused by parasites: malaria, trypanosomiasis, leishmaniasis, schistosomiasis and filariasis. They have been included in the WHO’s Special Programme for Research and Training in Tropical Diseases (HYDE, 1990). It is, therefore, not surprising that the different species of tropical filariae, grouped principally in the genera Brugia, Wuchereria, Onchocerca and Loa, have been, and continue to be, studied extensively.

As well as these important filariae there are others with a wider geographical distribution which are also found in semitropical and temperate zones, where the majority of developed countries are situated. These species primarily infect animals, both domestic and wild. Up to the present, zoonotic transmission has only been demonstrated in those species which infect mammals (Table 1). In the Table, based on the data contained in McDougall, Magoon & Frischkl (1992) and Orihle & Eberhard (1998), some of the characteristics of the situation of the zoonotic filariae we have at present can be observed: A) The majority of human cases reported have been caused by species which infect domestic or peridomestic animals, but cases caused by species such as Loa loa and Meningonema peruzzi, which infect wild animals, are beginning to appear. This indicates that man is acquiring new species of filariae when they penetrate habitats...
from which they were formerly absent or penetrated sporadically. B) A great proportion of these cases have been reported in developed countries, in which the public health structures permit adequate medical and veterinary vigilance. Because of both the pathology caused by *Dirofilaria immitis* in its hosts and the consequences of the discovery of a pulmonary nodule in humans, and the number of cases produced by the group of species with subcutaneous/ocular location (*Dirofilaria repens, Dirofilaria tenuis/Dirofilaria conjunctivae*), these can be considered as the most important and best known of the zoonotic filariae. Thus, a review which includes biological, immunological, clinical and diagnostic aspects must be necessarily based on the cited species. Apart from their essentially parasitological interest, they have an added interest as a model to investigate human tropical filariases, which are not easily studied directly in those species.

The first description of *D. immitis* is owed to Joseph Leidy in 1850. Nevertheless, we should have to go back several centuries to find the first allusion to this parasite and the illness it produces in dogs. In the Hunting Treatise by Francesco Birago, published in Milan in 1626, of which there are only a few copies extant in the world, on page 58 rabies is described and «another incurable illness in the dog». The description given is as follows: «The dog suffers another disease, which is incurable, but not dangerous for other dogs nor for man; the dog generates two worms, which are half an arm's length long and thicker than a finger and red like fire. These worms moved and went to the dog's heart, making it vomit, but vomiting nothing, and two of my whippets died of this disease; and not knowing what illness this was, I had them opened and in the area of the heart I found one of these worms; and for this disease I found no remedy». It is evident that Birago is initially describing *Dioc-thophyme renale* and considers *D. immitis* an evolutionary stage of the former, but this demonstrates that dirofilariasis drew the attention of scholars several centuries ago. With regard to human dirofilariasis, the ocular case described by Amatus Lusitanicus in a girl resident in the south of France in the sixteenth century could have been due to *D. repens*, according to Pampiglione (1995).

### SYSTEMATICS AND PHYLOGENY

From the systematic point of view the genus *Dirofilaria* is situated within the Nematode Class and the Family Onchocercidae. At the level of subfamily, the situation is not so clear, since according to morphological studies *Loa loa* and *D. immitis* are very similar species which should be included in the Subfamily Dirofilariniae. Analysis of the 5S spacer region of the rDNA, however, seems to indicate that *Loa loa* is more related to *Mansonella perstans* and the genera *Brugia* and *Wuchereria*, while *D. immitis* is closer to *Onchocerca*, the constitution of the subfamily Dirofilariniae being questionable (Xie, Bain & Williams, 1994). *Dirofilaria* and *Onchocerca*, which also moult rapidly, have a similar biology and are opposed to *Loa loa* and the majority of Onchocercinae. This biological character supports the conclusions of the 5S spacer-DNA study (Xie, Bain & Williams, 1994; Bain et al., 1998). More recent studies, carried out at the University of Milan, based on the analysis of the sequences of the genes ftsZ and 16S rRNA of endosymbionts similar to rickettsiae of the genus *Wolbachia*, present in the filariae (Sironi et al., 1995), seem to confirm the phylogenetic proximity of *Dirofilaria* and *Onchocerca*. These endosymbionts, which have only been found in arthropods and very recently in filariae, have great host specificity and their relation with them se-

<table>
<thead>
<tr>
<th>Gen. and sp.</th>
<th>Animal reservoir</th>
<th>Location in humans</th>
<th>Geographic distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. immitis</em></td>
<td>Dog, cat, fox...</td>
<td>Pulmonary. At times subcutaneous</td>
<td>Cosmopolitan</td>
</tr>
<tr>
<td><em>D. lousianensis</em></td>
<td>?</td>
<td>Heart</td>
<td>Southern USA</td>
</tr>
<tr>
<td><em>D. spectans</em></td>
<td>Otter</td>
<td>Digital artery</td>
<td>Brazil</td>
</tr>
<tr>
<td><em>D. magalhaesi</em></td>
<td>?</td>
<td>Heart</td>
<td>Brazil</td>
</tr>
<tr>
<td><em>D. repens</em></td>
<td>Dog, cat, fox...</td>
<td>Subcutaneous/ocular.</td>
<td>Europe, Asia, Africa</td>
</tr>
<tr>
<td><em>D. tenuis = D. conjunctivae</em></td>
<td>Racon</td>
<td>At times pulmonary</td>
<td>Southeastern USA</td>
</tr>
<tr>
<td><em>D. ursi</em></td>
<td>Bear</td>
<td>Subcutaneous/ocular</td>
<td>Japan, Russia, Northern SA, Canada, Alaska</td>
</tr>
<tr>
<td><em>D. subdermata</em></td>
<td>Porcupine</td>
<td>Not reported</td>
<td>Northern USA, Canada, Southeastern USA, South America</td>
</tr>
<tr>
<td><em>D. striata</em></td>
<td>Silvatic felids</td>
<td>Subcutaneous/ocular</td>
<td>Southeastern USA</td>
</tr>
<tr>
<td><em>D. lutrae</em></td>
<td>Otter</td>
<td>Not reported</td>
<td>USA, Canada, Russia, Switzerland, Japan</td>
</tr>
<tr>
<td><em>Onchocerca sp.</em></td>
<td>?</td>
<td>Subcutaneous</td>
<td>USA, Colombia</td>
</tr>
<tr>
<td><em>Dip. arbuta</em></td>
<td>Porcupine</td>
<td>Ocular</td>
<td>Oregon (USA)</td>
</tr>
<tr>
<td><em>Dip. sprenti</em></td>
<td>Beaver</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Brugia sp.</em></td>
<td>?</td>
<td>Lymphatic nodules, conjunctiva...</td>
<td>USA, Colombia</td>
</tr>
<tr>
<td><em>Loatina spp.</em></td>
<td>?</td>
<td>Ocular</td>
<td>Colombia</td>
</tr>
<tr>
<td><em>Meningonema peruzzi</em></td>
<td>African monkeyes</td>
<td>Cerebral</td>
<td>Central Africa</td>
</tr>
</tbody>
</table>

Table 1.—Mammalian filariae species in which zoonotic transmission has been demonstrated. * = *Dirofilaria spectans* and *D. lutrae* are very similar to *D. immitis*, and *D. subdermata* is similar to *D. ursi*. ** = some cases have been refered as *D. conjunctivae*. 
Dirofilariasis and other zoonotic filariases

ens very stable. Both these characteristics have been taken as a basis to simultaneously construct the phylogenetic trees of the selfsame endosymbionts and the filariae (BANDI et al., 1998) (Fig. 1). What is more, the studies carried out with ribosomal DNA appear to confirm that the filariae of mammals have appeared recently, possibly in the Cretaceous period, approximately 60 million years ago (MAGGENTI, 1983). Furthermore, the origin and the speciation of Onchocerca in Africa could have occurred even more recently, only 2.5 million years ago, during the Pleistocene (BAIN, 1981).

THE LIFE CYCLES OF DIROFILARIA SPECIES

*D. immitis* and *D. repens* develop in two hosts: the definitive hosts, which are canines and felines, both wild and domestic, and the vectors which belong to diverse species of culicid mosquitoes (BARRIGA, 1982).

The adult worms of *D. immitis* are located in the right ventricle and first part of the pulmonary artery. They are very long and thin. The females measure around 25 cm and the males around 14 cm. The females are ovoviviparous and lay larvae of the first stage denominated microfilariae (mf) (naked) in the bloodstream of the hosts. These are ingested by the vectors when they feed on infected animals. The microfilariae remain in the stomach of the vector for the first 24 hours. They then migrate to the Malpighian tubules, where they moult to L2 and to L3. The evolution from L1 to L3 takes place in 12 days at 24°C and takes considerably longer (16-20 days) at 22°C. During the following 3-4 days the L3 grow and perforate the basal membrane of the tubule penetrating into the general body cavity of the mosquito. They mature completely during the migration towards the cephalic space and the proboscis, reaching a length of 800-900 µm, and even 1300. These larvae are transmitted to other hosts when the vector feeds on them later. It has been demonstrated that the L3 and L4 of *D. immitis* have positive thermotaxis when located on a thermal gradient (MOK et al., 1986). The ca-

Fig. 1.— Phylogenetic tree showing the relationships of *Wolbachia* endosymbionts found in arthropods (A and B) and filarial nematodes (C and D). The names at the terminal nodes are those of the host species. The scale bar indicates the distances in substitutions per nucleotide. *Anaplasma marginale* was used as an outgroup. The tree (neighbour joining method) is based on the *ftsZ* gene sequences available in the data bases. The *ftsZ* gene sequences of filarial wolbachiae have been published by BANDI et al. (1998).
pacity of the L3 to detect minimal changes of temperature could be crucial for success in penetrating the warm-blooded host. Moreover, the increase in temperature towards the interior of the vertebrate hosts could constitute a stimulus such that the L3 and L4 move towards a specifically located site. When L3 are inoculated into subcutaneous tissue, they moult to L4 between 3 and 8 days post-infection. Approximately 50 days after the inoculation the L4 begin to migrate towards the heart, where, after one final moult, they mature into adult worms between 170 and 190 days post-infection. Once fecundated, the females begin to produce microfilariae, which circulate freely in the blood. The microfilariemia increase during the following 10 months and afterwards they remain constant for more than 5 years, disappearing in the following 7 to 9 years. The adult worms can live for around five years and the microfilariae more than two. In cats, which are resistant hosts, the maturing process is lengthened, there is not usually microfilariaemia, and when it does appear, it is usually of low intensity and short duration.

D. repens is located in the subcutaneous tissue of its definitive hosts. The males are also smaller than the females. The female produces unsheathed microfilariae quite similar to those of D. immitis, which appear in the peripheral bloodstream and subcutaneous interstitial lymph; from there they are ingested by the vectors. When the vector takes blood again, it inoculates the larvae into the subcutaneous tissue of the host, where they evolve to the adult stage in 175-238 days, with two successive molts.

Man can be infected by both species in the same way as the habitual hosts, by the bite of a mosquito carrying L3, but as the host is inadequate the parasites do not complete their habitual development. The larvae can be destroyed by the immune response in the subcutaneous tissue, or cause subcutaneous nodules in the case of D. repens, or begin to migrate towards the heart in the case of D. immitis. However, in this case, they are habitually detained in a branch of the pulmonary artery where they produce an embolism and later a pulmonary nodule. Worms of this species have also been found accidentally in different zones of the human anatomy. Sexually mature but isolated adults (which makes reproduction impossible) have also been observed occasionally both in pulmonary and extra-pulmonary locations. NOZAIS, BAIN & GENTILINI (1994) have described a human case of subcutaneous dirofilariasis with microfilariae in blood, in Corsica.

Regarding the remaining species, it is worth mentioning that D. ursi has black flies as vectors and that the data for other species are so scarce that even the reservoir animals are unknown (see Table 1).

RELATIONSHIPS BETWEEN PARASITES AND RESERVOIR ANIMALS

From the clinical point of view, although the proper name of the disease (cardio-pulmonary dirofilariasis) suggests an initial cardiac pathology, dirofilariasis must be understood as a very complex vascular and pulmonary illness, which in its last stages affects the right chambers of the heart. The presence of worms in the pulmonary artery and right ventricle produces progressively more serious damage (FURLANELLO et al., 1998):

- Vascular disease: this is produced in the caudal arteries. The causes are varied: traumatic processes, the action of immune cells (neutrophils and platelets) and the sudden death of some worms. The traumatic processes appear because of the arrival of the worms and have, as a consequence, the increase of the intercellular spaces and the disorganisation of the longitudinal axes of the endothelial cells of the vessels. The active substances released by the platelets produce an increase in division of the smooth muscle cells, intravascular villi appearing. All these processes diminish the calibre of the vessels and cause hypertension.
- Pulmonary disease: the egress of blood fluids to the perivascular spaces causes pulmonary oedema.
- Cardiac disease: As a consequence of the processes in the former phases an increase in the activity of the right ventricle is produced, which leads to congestion and heart failure. For some variable period of time there are no symptoms. The more physical exercise performed, the greater is the damage to the arteries. The symptoms, which appear gradually, are chronic coughing, difficulty in breathing, lipohymiae after exercise, weakness, anorexia, weight loss and dehydration. Pulmonary noises appear in the caudal lobules, arrhythmia and cardiac noises caused by tricuspid insufficiency. The abdomen and legs swell because of the accumulation of liquids when there is congestive failure. After serious embolisms, acute respiratory difficulty and hemoptysis appear. Sudden death does not usually occur.

In cats, the development of the disease is somewhat different and difficult to predict: in some cases no symptoms appear and spontaneous healing takes place. In others, apparently healthy animals suddenly begin to show acute symptoms: dyspnoea, hemoptysis and vomiting. Sudden death is not unusual in these cases.
- Caval syndrome: the vena cava syndrome can appear in both hosts. This is a clinical variant with acute development during cardiopulmonary filariasis. It appears suddenly with intra-vascular haemolysis, anaemia, hemoglobinuria and cardiac-circulatory shock (VENCO, 1993). The prognosis is generally unfavourable. This syndrome is reported in the literature in highly endemic zones, during the spring and summer period and most of all in young dogs of 3-5 years. There are studies that suggest the existence of factors of predisposition in males or of protection in females (ATKINS, 1988) and incidence is also very high in some zones of the north of Italy (GENCHI et al., 1992).

Although the pathogenic mechanisms are still not perfectly defined, it seems clear that the cause is the unforeseen location of adult worms in the right ventricular
The most profound study of the haemodynamic modifications in the course of the illness has not confirmed this hypothesis. A similar picture to that of caval syndrome is rapidly induced by inserting synthetic fibres, similar to adult worms, into the right ventricular atrium. Their withdrawal causes the disappearance of the symptoms. The insertion of the same filaments into the vena cava does not produce the symptoms. The presence of a critical mass of parasites in the right ventricular atrium is the cause of serious disturbances in the laminar flow. The turbulence induces a serious mechanical stress on the walls of the erythrocytes, which haemolyse rapidly. In affected dogs, a reduction has also been observed in the activity of the enzyme cholesterol-lecithin-acetyltransferase. The consequent increase in the proportion of free cholesterol/esterified cholesterol at serum level creates a favourable gradient for the diffusion of free cholesterol across the cytoplasm membrane of the erythrocytes, altering the characteristics of deformability and resistance. Finally, the mass of parasites opposing the venous return to the heart has a strong effect on the systemic circuit, leading to a state of cardiocirculatory shock in the affected subjects. Therefore, the tendency is to use the nosological term «hemoglobinuria caused by dirofilariasis».

Apart from the cases in which caval syndrome appears, the greater part of canine infections are chronic. This implies a survival capacity of the filariae for long periods of time in their habitual definitive hosts, which seem to be due, in part, to the type of immune reaction of the host and also to the capacity of the parasite to evade the immune response. Bearing in mind the data on incidence and longevity of the parasite, the rate of accumulation parasite/year/individual must be much higher than empirical data reveal (GENCHI et al., 1988). These facts appear to reveal scarce efficiency of the protective immune response of the dog and/or the parasite's great capacity for avoiding the immune response of its host. The immune effective mechanisms unleashed during canine cardiopulmonary dirofilariasis are a puzzle in which not all of the pieces are yet known. A recent review of the present state of knowledge in this field has been carried out by SIMON, GENCHI & PRIETO (1998). The known data appear to indicate that the immune response is directed to the control of the successive re-infections and to retain the production and activity of the microfilariae present in the bloodstream during the first infection, in some cases suppressing the microfilaraemia (amicrofilaremic cases).

The mechanisms of evading the immune response are not yet well known, as has been indicated previously. Nonetheless, certain facts suggest that each evolutionary stage of the parasite adopts a different evasive strategy. L3, which is a phase of short duration, seems to evade the immune response by eliminating a great quantity of antigenic molecules before and after the moult. This permits it to present a low antigenic profile to the immune system of the host, which makes it very difficult to detect. It has been demonstrated, both in vivo and in vitro, that L3 eliminate part of their antigenic content (principally constituted by two molecules of 35 and 6 kDa), and these components are not replaced on the surface of the larva (IBRAHIM et al., 1989). This type of evasion would be sufficient for a phase of short duration such as is the case of L3, which later gives way to another larva with different antigenic properties. The evolutionary phases, such as the L4, adult and the microfilaria, which must survive in the host for long periods of time, seem to evade the immune response by masking processes. It has been demonstrated that the surface of adult worms retains very few platelets and is capable of adsorbing albumin, IgG and the fraction C3 of the complement from dogs (BILGE et al., 1989; KADISPAOGUL & BILGE, 1989). In microfilaria, proteases directed against specific antibodies have been found (TAMASHIRO, RAO & SCOTT, 1987). The proteolytic activity of the microfilaria was not detected in supernatants, suggesting that these proteases could be associated to the surface of the parasite and that lysis occurs when IgG links with the epicuticle of the larvae. Much more extensive studies are necessary for an adequate comprehension of the evasion processes.

The complex relationships that are established between the parasite and its vector contain three fundamental facts with regard to transmission: A) there is a tendency towards a stable population of parasites in infected animals; B) only some of these infections are microfilaremic, which signifies that in a given population there are parasites (mf) available for vectors; C) in the majority of cases, the infections are chronic, which implies that the parasites are available for vectors during long periods of time.

### RELATIONSHIPS BETWEEN PARASITES AND VECTORS

The relationships between the parasites and vectors (susceptibility/resistance, rhythm of blood sucking, etc.) and other factors, such as the dimension of the mosquito population, longevity, seasonality, the introduction of new species which adapt well to the new habitats and the existence of genetic factors, have a decisive influence on vector capacity. With regard to zoonotic transmission, an essential element is that at least one of the potential vector species, present in a given area, feeds, without distinguishing, on carnivores (dogs) and man.

The immune reaction of the vector in the form of melanisation capacity is stronger in the first 14 days of life (CHRISTENSEN, LAFOND & CHRISTENSEN, 1986; CHRISTENSEN & FORTON, 1986), which determines that in a given population, the older individuals are more susceptible than the young and therefore better vectors. In experimental and field studies, it has been demonstrated that Culex pipiens molestus infected with D. immitis microfilariae present significantly higher rates of mortality.
than *Culex pipiens pallens* (ZAITSU, 1988). Biochemical differences in the enzymatic activity of the monophenol oxidase of haemocytes of *Aedes trivittatus* and *Aedes aegypti* have been related to the differences that both species present in their capacities to destroy microfilariae of *D. immitis* (LI, TRACY & CHRISTENSEN, 1989). Certain anatomical characteristics, such as the presence of a bucco-pharyngeal armature, in relation to the diameter of the microfilariae of the distinct species, can result in more or less extense damage in the cuticle of the worms, or in their complete anatomic structure (CANCRI, 1998). The ingestion of larvae and their consequent development modifies some behaviour patterns of the vector. It has been observed that mosquitoes infected with less than 4 larvae do not alter their flying activity, while those infected with more than 4 show a considerable increase in their activity. Moreover, infected mosquitoes are much more active during the normal daily period of quiescence (BERRY, ROWLEY & CHRISTENSEN, 1987).

**RELATIONSHIPS BETWEEN PARASITES AND HUMANS**

With regard to pathology, the characteristic lesion is a solitary pulmonary nodule (Fig. 2), which is revealed by radiological exploration, though in some cases multiple nodular lesions have been observed. The immense majority of the nodules are found in a sub-pleural location, which corresponds to the relationship between the diameter of the worm and the calibre of the peripheral pulmonary arteries. Their radiological characteristics suggest benignity: the edges are well defined and smooth; they are spherical or oval in shape with homogeneous density. Apart from the classical appearance of a stable solitary pulmonary nodule, it is suspected that many of them could be transitory (FARBER & LAGUARDA, 1987; CORDERO et al., 1990, 1992) and in some cases, as has recently been demonstrated, residual lesions appear in the form of small calcified nodules (CORDERO et al., 1992).

From a clinical point of view, pulmonary dirofilariosis appears with a variety of clinical pictures. The majority of the cases are without symptoms and the solitary pulmonary nodules are detected by chance on carrying out a thoracic radiography because of another illness of a very different nature. Sometimes symptoms appear. They are usually non-specific and are constituted of different combinations of cough, pleural pain, non-pleural pain, purulent expectoration, hemoptoic sputa, fever and other symptoms.

Subcutaneous dirofilariosis appears as a small subcutaneous nodule which gradually grows during weeks or months. Its consistency is hard and elastic, and it presents a marked erythema. Palpation may or may not be painful. The location is varied but with a clear tendency to situations in zones on the upper part of the body (PAMPIGLONE, CANESTRITI & RIVASI, 1995). When the

---

**Fig. 2.** Human dirofilariosis: a) stable pulmonary nodule caused by *D. immitis*; b) small calcified granuloma caused by *Dirofilaria immitis*. 
Diagnosis of canine and feline dirofilariasis

The techniques employed in the diagnosis of cardio-pulmonary dirofilariasis in the reservoirs are varied:
- direct detection of parasites: the detection of microfilariae in peripheral blood can be carried out directly, or by applying concentration methods; the most usually employed are those of Knott and filtration;
- diagnosis by image: echocardiography alone or associated with Doppler impulses permits the visualization of the worms in the right chambers of the heart (atrium and ventricle) (Venco, Furlanello & Vezzoni, 1998) (Fig. 3); other types of diagnosis by image, such as radiology, can reveal alterations in arteries, the heart and lungs (Vezzoni & Venco, 1998);
- molecular biology techniques: the amplification of the DNA of the microfilariae with specific primers using polymerase chain reaction (PCR) has recently been introduced into the diagnosis of these parasites (Favia et al., 1996);
- serological techniques: at present, the tests most employed in the diagnosis of canine dirofilariasis are those which detect circulating antigens of adult females; those which detect antibodies are of great value in the detection of feline dirofilariasis (Manfredi, 1998).

The possibilities of each of these techniques in each of the possible hosts should be valued. Detection by echocardiography depends principally on the location of the worms at the moment of carrying it out. When the worms are located in the right atrium or ventricle they are easily identifiable, but if they are located inside the pulmonary artery or its ramifications, they cannot be observed. The radiographic alterations can cause suspicion, but confirmation by other diagnostic methods is always required.

In an affected canine population, there is always a percentage of amicrofilaremic infected individuals; the techniques of detection and identification of microfilariae only reveal the presence of the parasite when there are circulating microfilariae in sufficient quantities to be detected in small volumes of blood; thus a negative result does not discount the existence of infection. Furthermore, in the case of positive detection, the specific identification by morphology alone may be difficult at the level of microfilaria, specially when the examiner is not used to a such a kind of identification, and sometimes it is necessary to use histochemical techniques, such as the identification of the points of activity of the acid phosphatases.

The design of specific primers (Favia et al., 1996) based on the nucleotide sequence of a cuticle antigen of D. immitis, which contains multiple tandem repeats (Poole et al., 1992), and on a highly repetitive sequence of D. repens, which constitutes approximately 3% of the genome of this species (Chandrasekharan et al., 1994), which is why differential diagnosis should be imposed as a first measure.

THE DIAGNOSIS OF DIROFILARIASIS

Diagnosis is a fundamental aspect of dirofilariasis, both in veterinary and human clinical practice, and in epidemiological studies. In each of the hosts, diagnosis presents specific problems, which determine, to a great extent, the techniques to be applied in each case. In animals, the identification of the species is necessary, since the line to follow is imposed by the consideration of whether it is a case of D. immitis or some other species, given the pathogenic nature of the former. Furthermore, it constitutes the first step in the control of the zoonosis. In the cat, given that acute development of the disease often appears, early diagnosis is also fundamental. In man, the detection of pulmonary or subcutaneous nodules always produces the suspicion of a malign cause,
permits diagnosis by the technique of PCR, with a specificity of 100% and a sensitivity of 1 ml/sample, but can only be applied in microfilaremic cases. These same primers can be applied for the detection of infecting larvae in mosquito vectors. The random amplified polymorphic DNA technique (RAPD) has also been employed (Genchi et al., 1993a).

The serological tests for the detection of antigens, of which several exist on the market, are at this time extremely sensitive and specific and do not depend on the location of the worms or the presence or absence of microfilariae. A recombinant molecule, DiT33, of *D. immitis* (Hong et al., 1996) of 26.4 kDa, homologous to Ov33 of *O. volvulus* and Bm33 of *Brugia malayi*, which have demonstrated their diagnostic capacity in human filarioses, has recently been proposed for the early detection of canine dirofilariasis. Bearing all these considerations in mind, at present the simultaneous application of tests for the detection of microfilariae and circulating antigens is recommended in laboratory diagnosis. These techniques can be complemented with others described, should it be necessary.

In cats, given the variable evolution of the disease and the possibility of sudden death of the infected animal, early diagnosis is of the first importance, to avoid the consequences of the worms reaching the heart. The advantages and disadvantages of diagnosis by image are similar to those present in dogs. The detection of microfilariae has little value, since, as has been mentioned earlier, the majority of the cases are microfilaremic and when microfilariaemia are present, the presence is transitory and of low intensity. As natural infections are normally produced by 1-3 worms, at times immature or of one sex, the detection of the antigens released by the females is difficult. For this reason techniques for the detection of specific antibodies are applied. With the exception of some studies, not directed specifically towards dirofilariasis, there are very few existing investigations at present of the immune response that felines develop against these parasites. A strong response of IgG antibodies against the somatic (SA) and excretory/secretory (E/S) antigens of adults of *D. immitis* has been observed in cats with natural infection confirmed by echocardiography (Prieto et al., 1997). In experimentally infected cats, antibodies against antigens of adults were detected 2 months after infection, which seems to demonstrate the great sensitivity of ELISA with these antigens in the early detection of the infection. This technique also demonstrated its capacity in the evaluation of the pre-adul ticide action of a semi-synthetic macrolid in experimental infections. Significant differences have been observed in the levels of specific antibodies in two groups of cats, one composed of individuals that had been infected and treated and the other of infected but untreated cats, 3
months after the application of the medication (PRIETO et al., 1999). The diagnostic capacity of the specific antibody detection tests is similar or superior to those of other types of diagnosis employed in dirofilariasis (GENCHI et al., 1999). Moreover, specific markers have been identified for feline D. immitis in the SA complex of adults (polypeptides of 22, 26, 30 and 40 kDa) and in the E/S antigens (22 and 25 kDa) (PRIETO et al., 1997), which, applied in ELISA, contribute considerably to the decrease of cross-reactivity.

**Diagnosis of human dirofilariasis**

The diagnosis of *D. immitis* and *D. repens* in humans poses different problems from the moment of detection (Fig. 4). In the case of subcutaneous nodules, it is the patients themselves who discover them and who seek medical attention, while the majority of pulmonary nodules go unnoticed and only a part are detected accidentally by thoracic radiography, applied for other reasons unrelated to dirofilariasis. Given that both the subcutaneous and pulmonary nodules cause suspicions of a malignant tumour or other pathological conditions (tuberculosis, fungal infections, hamartomae, etc.), the most important thing is to carry out a differential diagnosis. When there has been an absence of parasite forms in the corporal fluids, diagnosis has been carried out by the application of invasive techniques (thoracotomy, puncture/aspiration) to obtain biopsies that permit the identification of the parasites by histology. In this situation, it should be borne in mind that, while the excision of a subcutaneous nodule is a simple surgical process, pulmonary biopsy necessarily involves a much more aggressive, and potentially iatrogenic, action. Afterwards, the possibility of identifying tissues from worms depends on a certain number of considerations, which must be taken into account. According to McDONALD et al. (1992), specific identification in situ can be problematic due to the similar morphological characteristics of the cuticles of the diverse species and the destruction caused by the histological reaction of the host. The key characteristics that have been borne in mind for specific identification (size of the cuticle ridges, their number and the distance between them) are, in the opinion of ORIHEL & EBERHARD (1998), very variable at different levels of the body of a single worm, and even in the same transverse section, which is why they do not constitute an adequate criterion. According to these authors, the discovery in the subcutaneous tissue of a worm with a smooth cuticle poses problems of identification, since all the species of the genus *Dirofilaria* described in humans have cuticle ridges, with the exceptions of *D. immitis* and *D. luteae*. Since this latter species has not been reported in man, the discovery of a smooth worm in this tissue is usually attributed to *D. immitis*.

Very recently the technique of PCR has begun to be applied. FAVIA et al. (1996) have demonstrated that the primers they designed amplify the DNA extracted from adult worms of *D. immitis* and *D. repens*, preserved in different media, with the exception of formalin, which inhibits the activity of the Taq polymerase. Nevertheless, this technique does not avoid surgical intervention previous to obtaining the samples of tissue or the worms themselves.

SeroLOGY is an alternative to the invasive methods. As we have mentioned earlier, in spite of the small number of worms that cause human infections there is a considerable antibody response, which can be used for diagnosis. The main problem to solve is that of specificity. The SA of adults or larval stages, which can be obtained in appreciable quantities with relative ease, produces strong cross-reactions with other species of helminths, principally *Toxocara canis*, causing the syndrome of visceral larva emigrant in man. The E/S products of the adults permit a greater specificity in epidemiological studies, but are less sensitive than the somatic products for the detection of cases of pulmonary dirofilariasis (SANTAMARIA et al., 1995). This has given rise to a series of studies which has led to the identification of specific polypeptides. At least two have been employed experimentally in the diagnosis of human pulmonary dirofilariasis: a Japanese group cloned the gene that encodes for a protein of 35 kDa (Di35) (SUN & SUGANE, 1992), previously identified and characterised by PHILLIP & DAVIS (1985) in its native state. The recombinant protein encoded by this gene was employed in ELISA and was found to give very high specificity and sensitivity, even against sera from patients with tropical filariases. We have identified a molecule (really a group of molecules), in the region of 22 kDa (denominated Di22), which is specific for pulmonary dirofilariasis (PERERA et al., 1994), and which we have employed with success in Western blot in the confirmation of the diagnosis of clinical cases. The later evaluation for its use in ELISA (PERERA et al., 1998) has demonstrated that this technique has a sensitivity of 100%, a specificity of 90% and positive and negative predictive values of 75% and 100%, respectively.

Nevertheless, given the relatively low probability pretest (the probability of encountering a case of pulmonary dirofilariasis in a case of pulmonary nodule), the positivity of a serological test must be complemented by radiology, the antecedents, the zone of residence, etc., before the application of any type of measure.

Regarding *D. repens*, various specific polypeptides have been identified very recently in the Mw range of 26 to 40 kDa. These molecules permit not only the discrimination between subcutaneous dirofilariasis and other parasitic and non-parasitic illnesses, but also between clinical cases and infections without subcutaneous or ocular alterations (SIMON et al., 1997).

Although, as has been mentioned earlier, differential diagnosis is the most important, once it is demonstrated that the cause of the nodule is a dirofilaria, the specific identification is, from the clinical point of view, a secondary matter. Nonetheless, it can be of interest from the epidemiological point of view. As has been mentioned previously, the subcutaneous nodules are produced by *D. repens* and the pulmonary ones by *D. immitis*. There are,
however, a small number of published cases in which pulmo-

nary nodules were due to *D. repens* (PAMPILIONE,
CANESTRI TROTTI & RIVASI, 1995) and subcutaneous no-
dules due to *D. immitis* (SANTAMARIA et al., 1995; GUTIE-
RREZ et al., 1996). Nevertheless, with certain frequency,
publications are found in which the attribution to one or
the other species is based on epidemiological motives and
the location of the nodule, since the worms are completely
destroyed. In some of these cases serology has been the
key to the identification of the species (SANTAMARIA et
al., 1995). It is also possible that molecular biology tech-
niques could be a great help in this aspect.

**CONSEQUENCES OF THE DIAGNOSIS
OF A DIROFILARIASIS**

At present, there is no vaccine against canine and fe-
line dirofilariasis, although considerable efforts are
being made in this field. Up to now, irradiated L3
(WONG, GUEST & LAVOIPIERRE, 1974; MEJIA & CAR-
LOW, 1994; YOSHIDA et al., 1997) and chemically abbrevi-
ated infections (BLAIR & CAMPBELL, 1981, GRIEVE et
al., 1988; YOSHIDA et al., 1997) have been employed to
stimulate the immune system of dogs, obtaining percent-
gages of protection between 42 and 98%. The protective
capacity of a recombinant molecule (Di20/22) from di-
verse expression vectors has recently begun to be tested
in feline dirofilariasis The best results have been obtai-
ned with Di20/22 expressed in feline herpes virus, which
reduces the percentage of individuals infected to 12.5%
(FRANK, SABIN & CHANDRASHEKAR, 1998). Although,
in general, the results are hopeful, it appears that we are
still a long way from achieving an efficient and fully
operative vaccine. The experiments are costly and are
carried out with a very small number of infected ani-
mals, which limits the value of the results obtained, For
this reason control is carried out by chemotherapy.

The guidelines to follow, once the presence of these
parasites has been discovered, vary in each of the hosts.
In endemic areas in which dirofilariasis is taken into account, both from the veterinary and zoonotic points of view, diagnosis in reservoirs is carried out routinely for all the animals brought to surgery. This attitude permits preventive treatment and, when necessary, curative treatment of the animals, which has important positive repercussions on the control of the zoonosis.

When the result of diagnosis is negative, preventive treatment can be begun, which is one of the most important aspects of the chemotherapy of dirofilariasis. Initially diethylcarbamazine citrate was applied, derived from piperazine, a pharmaceutical product developed in the 1940’s for the therapy of human filariasis. Nevertheless, its side effects and its low efficiency, which necessitate its daily administration from very shortly before the commencement of mosquito activity until 2 months after the end of the season, have caused it to fall into disuse. It has been substituted by semi-synthetic macro-lids, such as ivermectin and milbemicin. Their monthly administration at doses of 6-12 mg/kg and 500-900 mg/kg, respectively, is completely effective. Administration must begin a month before the beginning of the vector activity and be prolonged until one month after its possible end (Giertlerro, Paiaro & Soll, 1993; Genchi et al., 1993b).

If the diagnosis is positive macrofilaricide treatments are applied. These can be of two types: by chemotherapy and surgery. The guidelines for chemotherapy in relation to the level of gravity of the dirofilariasis have been established. Macrofilaricide treatment can be performed using thiacetarsamide. This product damages tissues and its effectiveness is variable in relation to the age of the parasites. Levamisol also has side effects such as cardiac arrhythmia, haemolytic anaemia, granulomatosis encephalomyelitis and histopathological alterations at the level of the brain and meninx. Its efficiency varies from 0-100%. The arsenical mersalosmine dihydrochloride is the most recent pharmaceutical product approved. At the prescribed dosage of 2.5 mg/kg (twice with an interval of 24 hours), it is non-toxic. Injection into the lumbar musculature is recommended, between the 3rd and 5th lumbar vertebrae. It has a demonstrated efficiency of more than 95% against adults and immature parasites of 4 months. It also has a microfilaricide action (45%) (Vezzoni, Di Sacco & Genchi, 1993). When the infections are severe, macrofilaricide therapy must be accompanied by symptomatic therapy to reduce the allergic reactions caused by the death of the worms. Microfilaricide therapy represents the last phase of causal therapy and must be carried out at least from 3-4 to 4-6 weeks after the macrofilaricide treatment. The recommended dosage for ivermectin is 50 mg/kg; milbemicin has a microfilaricide action even at doses 5 times lower than those prescribed for prophylaxis. The extraction of the worms by surgery is applied in individuals with serious infections to avoid the risk of death as a consequence of chemotherapy. Because of the appearance of large clots (for more information see Venco, Furlane-

Llo & Vezzoni, 1998). This can be performed using flexible probes with alligator forceps at the end, which are introduced through the right jugular vein, and monitoring the progress to the heart by radioscopy. Once there the worms are trapped and extracted.

In humans, given the benign characteristics of the infection, the problem is not the treatment, but how the practitioner acts once a nodule with certain radiological characteristics and a positive serology to Dirofilaria is discovered. With increasing frequency it is recommended to wait and see the evolution of the nodule for a prudent period.

**EPIDEMIOLOGY**

The definition of the areas where *D. immitis* and *D. re-
pens* are endemic in canine populations, the degree to which these populations are affected and the species of vectors present are primordial factors for the determination of the risk of infection for the human population (Muro et al., 1999). Epidemiological studies have demonstrated that canine dirofilariasis is cosmopolitan. Nevertheless, the maps of distribution also reflect the interest in this parasite and the existing possibilities for investigation in different geographical areas. Prevalences are very variable (0.9% in zones of Canada - 95% in zones of the Po valley close to Milan). In Europe, the dirofilariae are widely distributed throughout the southern countries and are much less frequent or completely absent in the others. However, cases are being reported with ever-increasing frequency, both in animals and in humans, in the central and northern countries (Hinayd, Bacowsky & Hinterdorfer, 1987; Leuthier & Gothe, 1993; Auer et al., 1997), and it is suspected that they could have been acquired during stays in countries of the south during the summer period. In Spain, canine cardiopulmonary dirofilariasis has been found in almost all the mainland except in the Cantabrian Cornice (Rojo et al., 1988). The highest average prevalence is found in the Canary Islands, and on the mainland it diminishes towards the north. Canine subcutaneous dirofilariasis is suspected on the Mediterranean coast and in the Balearic Islands, since human cases of subcutaneous/ocular dirofilariasis have been reported in these zones (Ruiz Moreno et al., 1998). The distribution within the endemic areas is not homogeneous, as the highest prevalences are found in river valleys and in humid zones, for example irrigated zones, where the conditions for the breeding of mosquitoes are more favourable.

The distribution of human dirofilariasis is more difficult to define. A map of distribution can only be constructed attending to the clinical cases previously published, or also taking into account the epidemiological studies which measure the actual situation at a given time, which, unfortunately, are very scarce. The retrospective studies of published clinical cases show that there are far fewer points where human dirofilariasis has
have been reported than those where dirofilariasis has been found in animals. Furthermore, at each of these points only a few cases have been identified. Agreement has not been reached regarding the first human case published. FAUST, THOMAS & JONEA (1941) found an adult worm 12 cm long in the inferior vena cava of a woman of 73 from New Orleans. Although initially they gave it the name of *D. louisianensis*, FAUST himself (1957) suggested that the true identity of the worm was *D. immitis*. Generally, the work of FAUST, THOMAS & JONEA (1941) is considered the first report of human dirofilariasis. MAGALHAES (1887) reported a case in Rio de Janeiro, attributed to *D. magalhaesi*, but the documentation was scarce and this latter species is somewhat different from *D. louisianensis*, which is identifiable with *D. immitis*. The first case of human pulmonary dirofilariasis is that described by DASHIEL (1961) in the United States. Since then the number has increased in parallel to the interest in these parasites and the process of their becoming more widely known, as is shown by the fact that between 1960 and 1990 165 cases were published, while in only the last 8 years at least another 100 cases have been published. Retrospective analysis shows that the infection predominates in males (2:1) with respect to females and that the highest incidence is produced between 40 and 59 years (CLFERRI, 1982). The cause of the greater number of cases (possibly more than 500) is the subcutaneous nodules-ocular location brought about by *D. repens, D. tenuis, D. ursi* and other species of the genus *Dirofilaria* (see Table 1).

Epidemiological studies based on the use of serology present a quite different picture. In endemic areas, human populations present seroprevalences close to those corresponding to canine populations. Moreover, in areas in which *D. immitis* and *D. repens* co-exist, the seroprevalences for each of them in humans correlates significantly with those presented by the canine population (personal unpublished data). Taking all the aforementioned into account and bearing in mind the transitory nature of many pulmonary cases and their asymptomatic character, we can state that human dirofilariasis is under-diagnosed simply because it is not taken into consideration by practitioners and that the retrospective review of published cases only reveals a part of the problem. Finally, we must point out that the use of serology has permitted the determination of the existence of human dirofilariasis in areas where the detection of the disease by the initial discovery of pulmonary nodules is practically impossible. We have demonstrated the existence of specific anti-*D. immitis* antibodies in a community of Tikuna Indians isolated in the Colombian Amazon, and the existence of microfilaremic infections by *D. immitis* and a *Dipetalonema* species in their dogs (VIEIRA et al., 1997). This study has allowed the inclusion of this remote zone in the area of distribution of dirofilariasis and also poses the question of how the parasite reached this zone, whether by the colonists’ hunting dogs, or by some animal reservoir still to be identified.

### OTHER ZOOONOTIC FILARIAE

Besides the diverse species of the genus *Dirofilaria*, the causes of most of the human cases so far reported, other genera and species have been identified sporadically (though with increasing frequency) as agents of zoonotic infections (Table 1). An extensive review of them has recently been published by ORIHEL & EBERHARD (1998). Infections caused by *Brugia spp* and *Onchocerca spp* have special importance because of the number of human cases reported. The zoonotic infections they produce have been described outside the endemic zones of human filariasis and in individuals who have not travelled in tropical areas. The worms found have morphological characteristics that permit their identification to the level of genus, but present differences regarding the species of parasites of humans of tropical endemic zones:

- *Brugia spp.*: human infections by *Brugia* spp have been reported in the United States (29 cases), in individuals who had never travelled to endemic zones of *Brugia malayi*. Furthermore, cases have been discovered in Colombia, Brazil, Peru and Ethiopia. The morphological characteristics of worms found in the north were different from those of the south, which indicates that there are various species implicated. In two cases gravid females were found, from which it can be deduced that there must also have been a male which had fecundated them. Initially one of the cases was diagnosed as lymphoma, but the posterior histological study demonstrated the presence of the worms in the lymphatic nodules.

- *Onchocerca spp.*: in all the cases described (6), the worms were similar to *O. gutturosa*, a world-wide parasite of bovine cattle.

- *Molinema spp.*: three cases attributed to *Molinema arbuta* (= *Dipetalonema arbuta*) and *M. sprenti* (= *Dipetalonema sprenti*) (parasites of the porcupine and the beaver respectively), have been reported in Oregon (U.S.A.). These two species have been previously included in the genus *Dipetalonema*.

- *Loa* spp.: this genus was proposed by ORIHEL & EBERHARD (1984) to group some species of the genus *Dirofilaria*, since the biological and morphological characteristics of the microfilariae and adult worms are clearly different from those of the genus *Dirofilaria*. *Loa* has many characteristics similar to *Loa, except in the cuticular protuberances*. In spite of the fact that its geographical distribution is not completely known, it is known that *L. ondatrae* and *L. scapiceps* are parasites of the rabbit in the United States and that *L. roemeri* is a natural parasite of the kangaroo in Australia. BARTLETT & GREINER (1986) place *L. scapiceps* and *L. roemeri* but not *L. ondatrae* in the genus *Pellecitus*. One human case with ocular location occurred in Colombia and has been attributed to a *Loa* sp. (BOTERO et al., 1984), whose reservoir remains unknown.

- *Meningonema peruzzi*: this species is a parasite of cercopithecid monkeys of Central Africa, located in the
CONCLUSIONS

In conclusion, we emphasise the following facts: at present canine and feline pulmonary dirofilariasis is considered as a veterinary problem of the first order, since it affects animals which are highly appreciated by man. This has determined great advances in the fields of diagnosis, therapy and surgery and the same is beginning to occur in that of vaccines. Moreover, the possibility of employing these species as a model to learn aspects of the biology of the filariae, which could be applied to the control of human tropical filariases, has contributed even more to this development.

Man is an inadequate host for dirofilariæ, thus the development is seen to be substantially altered. Although the processes it originates are benign, there are two reasons for taking human dirofilariasis into consideration:

A) the accidental discovery of subcutaneous and especially pulmonary nodules due to dirofilariasis always produces the suspicion of a malign cause; thus surgical intervention is usually employed, producing unnecessary damage in patients;

B) until recently, dirofilariasis consisted of a few hundred published cases. At present, thanks to serological diagnosis techniques, it has been demonstrated that human populations resident in endemic areas present seroprevalences close to those which appear in the canine populations of these areas and, in principle, any individual who is infected could develop subcutaneous and/or pulmonary nodules. It is very probable that, within a few years, dirofilariasis will be included in the differential diagnosis of pulmonary and subcutaneous nodules, as numerous specialists of the countries most advanced in its study are insisting (Yano et al., 1989; Nicholson et al., 1992), with the consequent demand for trustworthy methods of diagnosis, as far as possible of a non-invasive nature. When we achieve this situation we will be able to avoid, at least in part, the iatrogeny derived from discovery and the costs caused by surgical interventions.

In 1969, when barely 30 human cases had been reported, pathologists and clinics in the United States published a review (Schloattaer, Harrison & Thomson, 1969) in which they stated: «In conclusion, it seems clear that in the United States dirofilariasis must be considered as an emergent zoonotic problem. Although the number of human cases is small, practitioners must consider the possibility of this diagnosis when the symptoms and lesions suggest a parasitic infection». In 1998, with close to 900 cases of zoonotic filariases published, ORIHEL & EBERHARD (1998) indicate the following: «The infections by zoonotic filariae will continue to be diagnosed with increasing frequency in new geographical areas, finding new locations in the hosts and as a result of the invasion by new species. Clinics, pathologists and parasitologists must continue on the alert faced by the possibility of these infections, both in their present appearance and in other new ones».

ACKNOWLEDGEMENTS

We thank Mr. G. Jenkins for the translation of the manuscript. Dr. C. Bandi for providing the phylogenetic tree of filariae, and Dr. L. Venco for the photograph of Figure 3.

REFERENCES


Auer (H.), Weinkamer (M.), Bstech (A.), Schnyder (C.), Dietze (O.), Kunz (G.) & Aspock (H.), 1997.—Ein seltener Fall einer Dirofilariä repens-Infektion des Nebenhodens. Tropenmedizin und Parasitologie, 19: 53-58


Bartlett (C.M.) & Greiner (E.C.), 1986.—A revision of Pellici-
Dirofilariasis and other zoonotic filariases


Isaiah (M.S.), Tamashiro (W.R.), Moraga (D.A.) & Scott (A.L.), 1989.– Antigen shedding from the surface of the infective stage larval of Dirofilaria immitis. Parasitology, 99: 89-97.


Li (J.), Tracy (J.W.) & Christensen (B.M.), 1989.– Hemocytene monophenol oxidase activity in mosquitoes exposed to microfiliariae of Dirofilaria immitis. Journal of Parasitology, 75: 1-5.

Magalhaes (P.S.), 1887.– Description de uma espécie de filarias encontradas no coração humano, precedida de uma contribuição para o estudo de filarioses de Wucherer e do respective parasito adulto. Revista de medicina d'observação dos corpos e do coração. São Paulo: Imprensa. 67: 646-650.

Mok (M.), Abrahm (D.), Grieve (R.B.) & Thomas (C.B.), 1986.– Thermotaxis in third and fourth-stage Dirofilaria immitis larvae. Journal of Helminthology, 60: 61-64.

Muro Alvarez (A.), Cordero Sanchez (M.), Ramos (A.) & Simon Martin (F.), 1991.– Seasonal changes in the levels of anti-Dirofilaria immitis antibodies in an exposed human population. Tropical Medicine and Parasitology, 42: 371-374.


Perera Madezio (L.), Muro Alvarez (A.), Cordero Sanchez (M.), Villar Ledesma (E.) & Simon Martin (F.), 1994.– Identification, purification and evaluation of a 22 kDa Dirofilaria immitis antigen for the immunodiagnosis of human pulmonary dirofilariosis. Tropical Medicine and Parasitology, 45: 249-252.


Phillip (M.) & Davis (T.B.), 1986.– Biochemical and immunologic characterization of a major surface antigen of Dirofilaria immitis. Journal of Immunology, 136: 2621-2627.

Prieto (G.), Venco (L.), Simon (F.) & Genchi (C.), 1997.– Feine heartworm (Dirofilaria immitis) infection: detection of specific IgG for the diagnosis of occult infections. Veterinary Parasitology, 70: 209-217.

Prieto (G.), Simon (F.), Genchi (C.), McCall (J.W.) & Venco (L.), 1999.– Utility of adult antigens of Dirofilaria immitis for the early detection of dirofilariosis and for the evaluation of chemoprophylactic treatment in experimentally infected cats. Veterinary Parasitology, 86: 5-13.


VENCO (L.), 1993. – Approccio diagnostico e terapeutico alla sindrome della vena cava. *Veterinaria*, 2 (Suppl.): 57-68.


