

Retrospective study on the presence and pathogenicity of *Dirofilaria repens* in 5 Dogs and 1 Cat from Aosta Valley

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SUMMARY

Aosta Valley is the only region of Italy free of reported human cases of dirofilariosis due to *Dirofilaria repens*. Nonetheless, the retrospective analysis of five locally acquired *D. repens* infections in dogs, seems to indicate that an animal reservoir exist in the area. A feline case imported from Camargue, France, is also described. The most common dermatological signs were erythema, alopecia, crusting, papulae and nodules. All patients had lesional pruritus and dogs were affected by concurrent babesiosis. Eradication of the underlying condition, followed by a therapy with the specific adulticide and microfilaricide drugs led to a complete recovery of microfilaraemia. Increased pet travel and ecological changes are extending the ranges of vector borne, parasitic and zoonotic diseases. This fact pose a unique diagnostic challenge for the veterinarian, since the index of suspicion may be absent outside endemic areas.

Key words: *Dirofilaria repens* – dermatitis – zoonosis – dog – cat

Retrospektive Untersuchung zu Vorkommen und Pathogenität von *Dirofilaria repens* bei 5 Hunden und einer Katze im Aostatal

Das Aostatal ist die einzige Gegend in Italien, die frei ist von Dirofilariose verursacht durch *Dirofilaria repens*. Die retrospektiven Abklärungen bei 5 mit *D. repens* infizierten Hunden zeigen aber, dass in dieser Gegend doch ein Tier-Reservoir vorhanden sein muss. Ein Fall einer infizierten Katze aus der Camargue, Frankreich, wird ebenfalls beschrieben. Die häufigsten Hautveränderungen waren Erythema, Alopezie, Bildung von Krusten, Pappeln und Knötchen. Alle Patienten zeigten Pruritus und die Hunde hatten zusätzlich eine Babesiose. Nach Abklingen der vorhandenen Krankheitssymptome, führte eine spezifische Therapie gegen Adultstadien und Microfilarien zu einer vollständigen Genesung. Der zunehmende Reiseverkehr mit Kleintieren sowie klimatisch-ökologische Veränderungen erhöhen das Krankheitsrisiko vektorübertragener Parasitosen und Zoonosen. Diese Situation stellt für den Tierarzt eine wahre Herausforderung dar, da ausserhalb endemischer Gebiete der Verdacht auf solche Erkrankungen viel geringer ist.

Schlüsselwörter: *Dirofilaria repens* – Dermatitis – Zoonose – Hund – Katze

Introduction

Dirofilaria repens is a zoonotic filarial nematode parasite of dogs, cats and wild carnivores transmitted by mosquitoes (Pampiglione et al., 1995). Adult worms reside in the subcutaneous tissues of infected animals and release microfilariae that circulate in the blood (Tarello, 1999). Although dogs and cats are final hosts of *D. repens*, it also accidentally affects people, causing subcutaneous, conjunctival and pulmonary nodules often confused with neoplastic tumors (Pampiglione and Rivasi, 2000). Italy has the highest concentration of recorded cases of human dirofilariosis due to *D. repens*, in the world most of which are from Piedmont (Pampiglione et al., 2001), a region close to the work-

ing area of this study. To date, no human cases have been described in Aosta Valley and no data are available on the presence or absence of microfilaraemia in dogs and cats living in this region of North-Western Italy. It was thus worthy to report this retrospective study on 6 animal cases observed ten years ago in Aosta Valley, since it appears today evident that the geographic distribution of *D. repens* is expanding to higher sites from sea level (Rossi et al., 1996; Bucklar et al., 1998; Petruschke et al, 2001) and in formerly free areas of Central Europe (Doby et al., 1986; Raccurt, 2000).

Case report Cat

A 5-year old female Persian cat from Aosta, living in apartment without outdoor access, and relapsed after previous treatments with flea control and corticosteroids, was presented in January 1992 with a 3-year history of pruritic dermatitis, showing erythema on abdomen, hind limbs, back and flanks, multifocal alopecia on whole body and hyper-keratosis on the abdomen and lumbo-sacral region. Initially, the condition was seasonal (spring-summer) and became constant during the last year. Before the onset of the condition the cat spent a 6-months period in Camargue (France). The modified Knott's test showed the presence of a high number of *Dirofilaria repens* microfilariae in the circulating blood and a diagnosis of feline subcutaneous dirofilariosis (Tarello, 2000a; 2000b) imported from an endemic area of Southern France (Bain, 1978) was consequently made. The cat was treated with the microfilaricide levamisole (Ascarilen, Teknofarma, 11 mg/kg/day, sc., for 6 days) showing 1-month partial improvement followed by clinic relapse. A new Knott test revealed the presence of microfilariae and a therapy with ivermectin (Ivomec, Merial, 50 µg/kg, sc.) administered once a month was strongly recommended. The cat was not seen again by this author.

Case report Dogs

Between January 1992 and December 1993, five dogs were diagnosed with subcutaneous dirofilariosis in Aosta Valley, based on the following criteria (Tarello, 1999; 2002a-c; 2003a-b): a) pruritus lasting for more than one week, b) presence of cutaneous lesions, c) microfilariosis caused only by *Dirofilaria (Nochtiella) repens*. Adult nematodes of *D. repens* are localized in the subcutaneous tissues and these were not found, nor



Figure 1: Nodule on a hind limb of a dog with subcutaneous dirofilariosis.

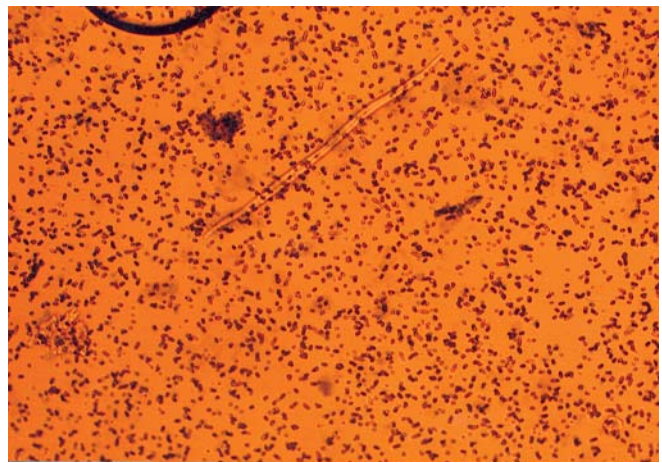


Figure 2: *Dirofilaria (Nochtiella) repens* microfilaria in the blood of a dog with cutaneous lesions ($\times 10$).

were they searched for, during this study. No animal had received preventive medication for heartworm disease. At the time of inspection the condition lasted between 3 months and 3 years and all patients had already been treated previously for their dermatological symptoms with various combinations of therapies, including flea control, corticosteroids, antibiotics and liver-protectors with poor or negative responses (Tab. 1). Laboratory testing for *Dirofilaria repens*, *Dirofilaria immitis* and *Acanthocheilonema reconditum* was carried out using a canine heartworm antigen test (DiroChek™, Synbiotics Corp.) and a modified Knott's test performed on 1 milliliter whole blood samples collected in EDTA-tubes. Six preparations were made from each sample and examined at light microscopy ($\times 4$ and $\times 10$, Leitz).

A Wright-stained fresh blood smear was also done in each case to check for other canine haemoparasites. All dogs had pruritus, manifested by localised scratching, licking and biting. The gross appearance of skin lesions is described in Table 1 and an example shows in Figure 1. Microscopic examination of six slides from every Knott concentration test was carried out and *Dirofilaria (Nochtiella) repens* microfilariae were found in the blood of all dogs (Fig. 2), their count ranging from 8 to 19 per sample. *Acanthocheilonema reconditum* and *Dirofilaria immitis* microfilariae were not found and the test (DiroChek) for heartworm antigen was negative. Concurrent babesiosis due to *Babesia canis* was also diagnosed in all dogs and the tick borne infection was treated first with the anti-babesial imidocarb dipropionate (Carbesia, Schering-Plough Animal Health, 7.13 mg/kg, s.c., weekly for 2 weeks). Within a few days this treatment resolved the systemic clinical signs, such as anorexia, vomiting, fever, lethargy, conjunctivitis and lameness (Tab. 1). A partial improvement of the cutaneous lesions and itching was observed in all dogs treated. Therapy with the adulticide drug thiac-

Table 1: Signalment, History, Clinical findings and results of Laboratory Tests in 5 Dogs with *Dirofilaria (noctiella) repens* infestation from Aosta Valley

Dog No.	Sex, age & breed	Duration of the disease	Previous tests and/or treatment (outcome)	Dermatological findings	Clinical signs	Knott's test result* & concurrent diseases
1	M, 5 years Crossbreed	1 year	Flea control (no benefit)	Erythema, crusts, alopecia & nodules on lumbo-sacral region and hind limbs	Anorexia, fever (39.4°C) & vomiting	10 DFR μ f. Babesiosis
2	F, 5 years German shepherd dog	3 years	Flea control & corticosteroids (partial benefit followed by relapse)	Erythema, eczema & multifocal alopecia on abdomen, neck and lumbo-sacral region	Lethargy, conjunctivitis, vomiting	19 DFR μ f. Babesiosis
3	M, 3 years Siberian Husky	3 months	Corticosteroids & liver-protector (no benefit)	Papulae and nodules on abdomen, thorax, head and fore limbs	Anorexia, lethargy	16 DFR μ f. Babesiosis
4	M, 2 years Yorkshire terrier	5 months	Flea-control, corticosteroids & antibiotics (no benefit)	Erythema, crusts, alopecia & papulae on lumbo-sacral region and hind limbs	Anorexia, lameness, fever (40°C)	9 DFR μ f. Babesiosis
5	F, 8 years Crossbreed	1 year	Antibiotics, anti-acid, vitamins & liver-protector (no benefit)	Erythema, alopecia & lichenification on perianal area & hind limbs	Vomiting	8 DFR μ f. Babesiosis

DFR = *Dirofilaria repens*; μ f = microfilariae; *All dogs were *Dirofilaria immitis* negative using both the Knott and the antigen test

etarsamide sodium (Caparsolate, Abbott Laboratories, 2.2 mg/kg, i.v., four times q. 12 hours) began 2–3 days after completing the treatment for babesiosis and this led to a further improvement of cutaneous lesions including pruritus. The microfilaricidal ivermectin (Ivomec, Merial, once 50 μ g/Kg, s.c.) was administered 10 days after the adulticide treatment. The speed of recovery depended on the duration of the disease and on severity of the lesions. Clinical re-examination and a Knott test carried out 1 month after the completion of treatment showed the resolution of the cutaneous lesions and the absence of *Dirofilaria repens* microfilariae in the blood.

Discussion

Five dogs and 1 cat from Aosta Valley affected by chronic pruritic dermatitis unresponsive to previous therapies were found to carry *Dirofilaria repens* microfilariae in the blood, confirming that dogs and cats are valuable reservoirs of the nematode in this region of North-western Italy. Human cases have never been reported in that area (Pampiglione and Rivasi, 2000). Concomitantly, in the author's knowledge, this is also the first report of babesiosis due to *Babesia canis* infection in dogs from Aosta Valley. Onset of the condition in the cat was apparently linked to a previous period of living in Camargue, an endemic area for *D. repens* in Southern France (Bain, 1978; Doby et al., 1986;

Pampiglione et al., 1995). Differently, all five dogs examined in this study were born in the Aosta Valley and never travelled outside the region, indicating that the infection was acquired locally.

The importance of such findings resides in the fact that no dog, cat or wild carnivore from this area has ever been found to carry *D. repens* microfilariae in the past, although suitable vectors and animal reservoirs exist in strictly close areas of Piedmont (Pampiglione et al., 1995; Rossi et al., 1996; Tarello, 1999; 2000a–b; 2002b), where human dirofilariosis is frequently by seen (Pampiglione et al., 2001). These observations may provide a better epidemiological indication and will permit a risk assessment in view of a possible transmission to humans, since there is clear evidence that *Dirofilaria spp.* infections are spreading within the human population in Europe (Muro et al., 1999). Canids are recognized reservoirs and definitive hosts of the zoonotic nematode *D. repens* (Pampiglione et al., 1995), the endemic area of which is expanding northwards (Bucklar et al., 1998), to newly colonized areas of France (Raccurt 2000) and Switzerland (Petruschke et al., 2001) and to higher sites on sea level in the neighboring endemic areas of Northern Italy (Rossi et al., 1996). Dogs in this study lived in rural areas located in the lower Aosta Valley, from Fenis to Pont-S-Martin, 400–500 meters on sea level and within 1 km distance from the Dora Baltea River. They were allowed access to outdoor environments or

resided perpetually outdoor. This fact enhances the risk of mosquito bite and consequently the risk to acquire *Dirofilaria immitis* and/or *repens* (Rossi et al., 1996).

Dogs may be affected by microfilariae due to *Dirofilaria immitis*, *Dirofilaria repens* and *Achantocheilonema reconditum* and differentiation is mainly based upon their morphology (Schrey, 1996; Tarello, 2001). *D. repens* microfilariae are 325–375 µm in length and 6–8 µm in width, showing a cephalic space roundish, short and empty (Pampiglione et al., 1995). Microscopically, they are easily differentiated from other microfilariae because they are larger and longer, showing a tail not so thin like those of *D. immitis* and of *A. reconditum* (Schrey, 1996).

D. immitis microfilariae are shorter (220–330 µm) and thinner (5–6 µm) whereas those of *A. reconditum* are very thin (4–5 µm) and often bented as a hook in their caudal end (Schrey, 1996).

Exclusion of *D. immitis*, the causative agent of heartworm disease, through a negative antigen test was an important confirmation that microfilariae observed were only due to *D. repens*. Detection of microfilariae in the blood is diagnostic for the presence of adults in *Dirofilaria* infections (Anon., 1998). Canine dirofilariosis due to *D. repens* has received little attention in the past, if compared with studies carried out on *D. immitis* (Rossi et al., 1996; Bredal et al., 1998). Microfilariemia has been observed in dogs from Sri Lanka, France, Greece, Russia, Ukraine and Italy (Pampiglione et al., 1995) and most of human cases are recorded in these countries (Pampiglione and Rivasi, 2000). The observation of affected animals showing itching dermatitis responsive to specific medicaments (Tarello, 2002a–b, 2003a–c) seem to confirm a pathogenic role for *D. repens* in dogs and cats, previously disputed (Bredal et al., 1998). Erythema and pruritus are described also in humans, where microfilariemia is absent and usually a single adult parasite is recovered (Pampiglione and Rivasi, 2000;

Pampiglione et al., 2001). On the other hand, the concurrent babesiosis observed in all dogs in this study is not usually associated with cutaneous lesions and accounts for the collateral signs such as anorexia, vomiting, lethargy and fever (Zahler et al., 2000), which promptly recovered following specific therapy. Treatment of subcutaneous dirofilariosis has been rarely reported in dogs (Tarello 2002a–b) although it is suggested for animals suffering from clinical signs of this disease (Baneth et al., 2002). A proper therapy is also important in order to decrease the risk of infection to humans in the vicinity of the affected animal when suitable mosquito vectors are present (Baneth et al., 2002). Recently, melarsomine (Immiticide, Merial, 2.5 mg/kg, IM, twice every 24 h) has been successfully used as adulticide (Tarello, 2002a–c) and novel microfilaricidal drugs, such as doramectin (Baneth et al., 2002), selamectin, moxidectin and milbemycin have proved effective in the eradication of microfilariae.

Lack of previous evidence of the blood borne parasites detected in these cases may be due to the fact that during the last years the ranges of certain vector-borne diseases such as dirofilariosis and babesiosis are extending due to ecological and climatic changes (Irwin, 2002). In many regions, the emergence of parasitic diseases is also a consequence of increased pet travel, as indicated by the feline case.

Recommendation has been recently made that each and every case of human dirofilariosis observed in Italy should be recorded, in order to determine the true extent of the condition (Pampiglione et al., 2001). In companion animal practice veterinarians have the responsibility of providing accurate information about the zoonotic transmission of parasite infections from pets (Irwin, 2002). This also includes that canine and feline subcutaneous dirofilariosis cases should be reported in order to assess the prevalence and pathogenicity of *Dirofilaria repens* in the animal reservoirs and final hosts.

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