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DERMATITIS ASSOCIATED WITH *Dirofilaria (Nochtiella) repens* MICROFILARIAE IN DOGS FROM CENTRAL ITALY

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Pruritic dermatitis associated with *Dirofilaria (Nochtiella) repens* microfilariae in the blood was diagnosed in 22 dogs from Fermo (Central Italy). According to the history, previous unsuccessful treatments with corticosteroids, antibiotics, restricted diet, flea control, levamisole and ivermectin were recorded in 17 dogs (77.3%). The combined filtration tests and antigen tests, performed during the study, were negative for *Dirofilaria immitis* and *Acanthocheilonema reconditum* in each case. Dermatological lesions included erythema, papules, single or multifocal alopecia, eczema, lichenification, crusting and nodules. All dogs had pruritus. Concurrent babesiosis was diagnosed in the blood smears of each case (100%), and 60% of the dogs were found to be carriers of canine granulocytic ehrlichiosis (CGE). Three dogs (13.6%) were positive for leishmaniosis. Eradication of the concurrent infections followed by specific macro- and microfilaricide treatment led to complete recovery from the dermatological syndrome. The main conclusion of the study is that *D. repens* infection can be more pathogenic than is currently considered, and it is apparently an opportunistic disease with serious dermatological consequences.

Key words: *Dirofilaria (Nochtiella) repens*, subcutaneous dirofilariosis, dermatitis, dog, microfilaria, babesiosis, ehrlichiosis

Dermatitis associated with the zoonotic filarial nematode *Dirofilaria (Nochtiella) repens* (Filarioidea: Onchocercidae) in dogs is seldom described (Tarello, 1999). Some authors, in occasional scientific papers since 1954 (Ajmerito, 1954; Restani et al., 1962; Kamalu, 1991; Scarzi, 1995), have come to recognise the infection by *D. repens* (otherwise mostly deemed as harmless and asymptomatic) as a possible cause of pruritic manifestations and dermatitis in dogs.

Nonetheless, the pathogenicity of the nematode is still poorly understood and disputed (Chauve, 1997), mainly for the following reasons: *(a)* skin lesions appear in only a small subset of infected dogs and are not predictable (Bredal et al., 1998); *(b)* the gastrointestinal symptoms (Mandelli and Mantovani, 1966)

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and poor physical performance (Marconcini et al., 1993; Papazahariadou et al., 1994) shown by symptomatic dogs are not strictly pathognomonic, or limitable to a presumptive filarial aetiology, and in fact, can dim the understanding of the overall clinical picture; *(c)* the traditional macro- and microfilaricide treatments seldom produce a complete recovery, recurrences are common, and no specific therapy has been claimed (Cazelles and Montagner, 1996; Pollono et al., 1998).

There has been increasing evidence, accumulated during the last 15 years, suggesting that the parasite may be conditioned by co-existing infections, such as babesiosis (Tarello, 1999), ehrlichiosis (Tarello, 2000*b*), leishmaniosis (Beaufils and Martin-Granel, 1987) and haemobartonellosis (Tarello, 2000*a*). It has been suggested that the apparent opportunistic behaviour of *D. repens* might well explain the presence of asymptomatic carriers, the concurrent observation of non-dermatological symptoms and the development of dermatitis associated with *D. repens* microfilariosis in only a small percentage of the parasitised dogs (Tarello, 1999).

It has also been noted that the development and sexual differentiation of adult nematode in humans is facilitated in patients affected by primary or secondary immunodeficiencies (Simmons et al., 1984; Nozais et al., 1994; Basset et al., 1996).

The present clinical documentation is aimed at enhancing the notion, not generally accepted at the present time, that *D. repens* can indeed have a pathogenic role in canine dermatology (Euzéby, 1961). For this purpose, history, clinical signs and the method of treatment were analysed retrospectively in 22 dogs with pruritic dermatitis associated with *D. repens* infection.

Materials and methods

Patients

Complete clinical records of 22 consecutive cases of dogs with pruritic dermatitis were reviewed. All dogs were coming from the province of Ascoli Piceno (Fermo) and were examined by the author in his private practice during an 8-month period (spring to autumn 1999). Each animal was laboratory-tested for *D. repens*, *D. immitis* and *Acanthocheilonema* (syn.: *Dipetalonema*) *reconditum*. Seventeen of these animals (77.3%) had already been treated elsewhere for their dermatological condition.

Table 1 provides a general overview on the signalment, history, clinical signs and the results of diagnostic tests of the 22 dogs. Pruritus was present in all subjects and therefore not listed individually in Table 1.

Table 1

Signalment, history, clinical findings and results of the laboratory tests in 22 dogs with *D. repens* infestation

Dog	Sex, age and breed	Duration of the disease	Previous tests and/or treatment (outcome)	Dermatological findings	Clinical signs	Knott results* and concurrent diseases
1	F, 10 years English Setter	1 month	None	Erythema and papules on LS region	Eye anaemia, muscular and joint pain	6 *DFR* µf. Babesiosis, CGE, TW
2	M, 13 years Poodle	1 year, constant	Corticosteroids and flea control (*transient relief*)	Erythema (LS region) and multifocal alopecia	Conjunctivitis, stomatitis, vomiting and lameness	11 *DFR* µf. Babesiosis
3	M, 3 years German Shepherd	3 years	Corticosteroids and antibiotics (*transient relief; recurrence*)	Erythema, crust, alopecia and haematoma on the neck	Anorexia, lethargy and lameness	3 *DFR* µf. Babesiosis + CGE
4	F, 13 years Pomeranian	5 years	Restricted diet and flea control (*transient relief; recurrence)*	Erythema and alopecia, (LS region and abdomen)	Vomiting	5 *DFR* µf. Babesiosis
5	F, 2 years Dalmatian	1 year, constant	Corticosteroids and restricted diet (*none*)	Erythema on abdomen and HL		4 *DFR* µf. Babesiosis + CGE
6	F, 4 years Yorkshire	4 years, constant	Flea control (*no benefit*)	Erythema, papules, lichenification and nodules (on abdomen and elbows)	Conjunctivitis, otitis, vomiting and diarrhoea	8 *DFR* µf. Babesiosis + CGE
7	M, 10 years German Shepherd	2 years, seasonal	Flea control (*no benefit*)	Erythema, crusts and papules on LS region	Tenesmus	3 *DFR* µf. Babesiosis + CGE
8	M, 3 years Dalmatian	4 months	Corticosteroids (*no benefit*)	Erythema and alopecia on neck, abdomen, HL and LS region	Conjunctivitis and vomiting	16 *DFR* µf. Babesiosis and leishmaniosis
9	F, 8 years Crossbred	3 years, seasonal	Corticosteroids and flea control (*no benefit*)	Erythema, crusts and papules on perineal and LS region	Conjunctivitis	2 *DFR* µf. Babesiosis

Legend: CGE = Canine Granulocytic Ehrlichiosis; *DFR* = *Dirofilaria repens;* FL = forelimbs; HL = hindlimbs; LS = lumbosacral; µf = microfilariae; TW = tapeworm. *All dogs were negative for *D. immitis* using both the Difil and the antigen tests. Pruritus was present in all cases.

Table 1 continued

Dog	Sex, age and breed	Duration of the disease	Previous tests and/or treatment (outcome)	Dermatological findings	Clinical signs	Knott results* and concurrent diseases
10	M, 9 years German Shepherd	3 years, seasonal	Corticosteroids (*no benefit*)	Erythema and eczema on flanks and LS region	Otitis Fever (39.5 °C)	3 *DFR* µf. Babesiosis + CGE
11	F, 9 years Maltese	2 years, constant	Corticosteroids (*no benefit*)	Erythema, papules and multifocal alopecia on entire body		5 *DFR* µf. Babesiosis
12	M, 2 years Yorkshire	1 month	Corticosteroids (*no benefit*)	Erythema and papules on head, thorax, abdomen, elbow and HL	Conjunctivitis and anorexia	11 *DFR* µf. Babesiosis
13	M, 1 year Dalmatian	2 weeks	none	Erythema on abdomen, acne of chin, pododermatitis and pyoderma	Conjunctivitis, asthma, anorexia, vomiting and fever (39.5 °C)	17 *DFR* µf. Babesiosis + CGE and leishmaniosis
14	M, 5 years Australian Shepherd dog	2 weeks	Levamisole (*no benefit*)	Erythema, papules, nodules and alopecia on elbows and thorax	Conjunctivitis, anorexia and fever (39.6 °C)	8 *DFR* µf. Babesiosis + CGE
15	F, 7 years Crossbred	4 years, seasonal	Corticosteroids (*transient relief; recurrence*)	Erythema on flanks, abdomen and perineal area	Conjunctivitis	14 *DFR* µf. Babesiosis
16	F, 1 year Doberman	2 months	Corticosteroids and antibiotics (*no benefit*)	Erythema, papules and alopecia on elbows, HL and LS region	Fever (39.3 °C)	3 *DFR* µf. Babesiosis
17	F, 5 months German Shepherd	2 months	Ivermectin (*no benefit*)	Erythema, papules and nodules on head, abdomen and HL	Conjunctivitis	2 *DFR* µf. Babesiosis + CGE
18	M, 9 months Maremma Shepherd dog	2 months	Flea control (*no benefit*)	Eczema and crusting on LS region	Lethargy and fever (39.6 °C)	3 *DFR* µf. Babesiosis + CGE

Legend: CGE = Canine Granulocytic Ehrlichiosis; *DFR* = *Dirofilaria repens;* FL = forelimbs; HL = hindlimbs; LS = lumbosacral; µf = microfilariae; TW = tapeworm. *All dogs were negative for *D. immitis* using both the Difil and the antigen tests. Pruritus was present in all cases.

Table 1 continued

Dog	Sex, age and breed	Duration of the disease	Previous tests and/or treatment (outcome)	Dermatological findings	Clinical signs	Knott results* and concurrent diseases
19	F, 6 years Doberman	1 year seasonal	Antibiotics (*no benefit*)	Erythema on flanks and abdomen	Anorexia, sore throat, fever vomiting, muscular and joint pain, lymphadenopathy	7 *DFR* µf. Babesiosis
20	F, 3 years Pekinese	2 years, seasonal	None	Erythema and alopecia on LS region		4 *DFR* µf. Babesiosis + CGE
21	M, 7 years Maremma Shepherd dog	1 month	None	Erythema and papules on abdomen, elbows and hocks; alopecia on thorax, flanks and abdomen	Anorexia, lethargy, staggering, eye anaemia, lymphadenopathy	3 *DFR* µf. Babesiosis + CGE
22	F, 7 years Maremma Shepherd dog	1 month	None	Erythema and papules on elbows, hocks, head, neck and abdomen Eczema and alopecia on head, neck, thorax, flanks and abdomen	Anorexia, lethargy, and lymphadenopathy	2 *DFR* µf. Babesiosis + CGE

Legend: CGE = Canine Granulocytic Ehrlichiosis; *DFR* = *Dirofilaria repens;* FL = forelimbs; HL = hindlimbs; LS = lumbosacral; µf = microfilariae; TW = tapeworm. *All dogs were negative for *D. immitis* using both the Difil and the antigen tests. Pruritus was present in all cases.

Protocol

Once mixed infections and seronegative microfilaraemic heartworm disease cases (Tarello, 2001*a*) had been ruled out, at least three criteria were required for inclusion in the study group: *(a)* pruritus lasting more than 1 week, *(b)* presence of one or more skin lesions and *(c)* microfilaraemia due only to *D. repens,* i.e. not related to other dermatological causes. All the dogs described here met these requirements. No dog had received preventive medication for heartworm.

Examinations

Two samples of 2 ml each (one whole blood in EDTA, one serum in sterile vacuum) were taken from each dog, respectively, for a filtration (Difil) test and for a canine heartworm antigen test (Witness Dirofilaria, Merial). The samples for the differential identification of microfilariae were examined by light microscopy (× 4 and × 10). Differentiation between *D. immitis*, *D. repens* and *Acanthocheilonema reconditum* is mainly based upon the morphology of the microfilariae. The width of *D. repens* microfilariae is 7.1–8.3 µm, whereas that of *D. immitis* is 5.4–6.2 µm, and that of *A. reconditum* is less than 5 µm (Pollono, 1994). The measurement of length is a less reliable differential criterion (Schrey, 1996). The microfilariae observed in the present study (Fig. 1) had the typical wide and squat aspect of *D. repens* larvae. On the Knott and Difil tests, the tails of *A. reconditum* are hook-shaped and normally thin like those of *D. immitis* (Tarello, 2001*a*). The caudal end of *D. repens* microfilariae is much wider. The number of *D. repens* microfilariae (Fig. 1) reported in each dog is the sum of those observed on every Difil test performed on a 2-ml blood sample in EDTA. All samples were collected during the day, between 10 a.m. and 6 p.m., and this factor may account for the low number of microfilariae detected in each case. Fresh serum samples were simultaneously tested for the heartworm antigens without delay or refrigeration.

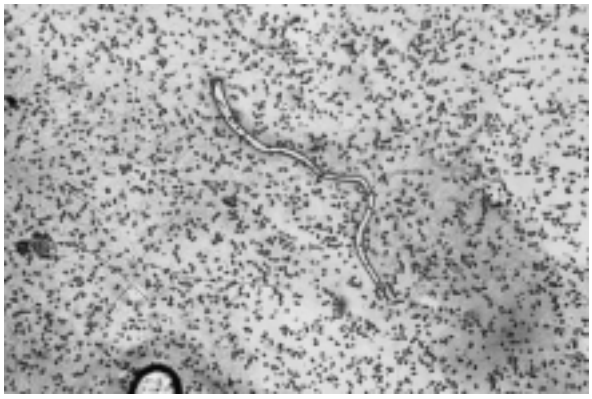

Fig. 1. Dirofilaria (Nochtiella) repens microfilaria (Difil test, × 10)

A fresh Wright-stained blood smear was also done in each case and checked for other canine haemoparasites. The test for leishmaniosis (Leishcan 16) was performed in all suspected cases.

Difil and antigen tests were also carried out on two control groups, i.e. on 7 dogs with patent *D. immitis* infestation (Group A) and on 11 healthy dogs without pruritus and skin lesions (Group B) during routine clinical checks for heartworm disease performed in the same area in 1999.

Diagnosis of D. repens *infection*

The diagnosis of subcutaneous dirofilariosis was based upon the microscopic finding of *D. repens* microfilariae (Fig. 1) and a negative test for circulating *D. immitis* antigens (Tarello, 1999; Tarello, 2001*a*).

Therapy

The protocol of the therapy was based on the eradication of the concurrent condition(s) detected in each case, followed by a treatment with the classic macrofilaricide (melarsomine: Immiticide, Rhône-Merieux, 2.5 mg/kg; 1 ml/10 kg, im., twice at an interval of 24 h) and microfilaricide drugs (ivermectin: Ivomec, MSD, 0.05 mg/kg; 1 ml/25 kg, sc., single injection 10 days after completion of macrofilaricide therapy). Against the associated infections with multiple tick-borne pathogens (*Babesia* and *Ehrlichia* spp.) the treatment of choice was programmed with imidocarb dipropionate (Carbesia, Mallinckrodt Veterinary, 1 ml/17 kg, sc., once a week, 4 times) associated with doxycycline (Ronaxan, Merial, 10 mg/kg/day, for 21 days). Leishmaniosis was treated with aminosidine (Aminofarma, 10 mg/kg/day, im., for 10 days).

Controls

The direct follow-up of the dogs with *D. repens* infection and of the control animals included a clinical examination and a repeated Difil test, one month after the end of treatment. Then, phone calls to the owners and repeated physical examinations, whenever possible, were made in the period August-September 2000, approximately 1 year after the diagnosis and treatment of the condition.

Results

Previous treatment/s and outcomes

Seventeen (77.3%) of the 22 dogs with *D. repens* infection had already been treated previously for their dermatological symptoms with various combinations of therapies (Table 1), including corticosteroids (n = 10; 45.5%), flea

control (n = 6; 27.2%), antibiotics (n = 3; 13.6%), restricted diet (n = 2; 9.1%), levamisole (n = 1; 4.5%) and ivermectin (n = 1; 4.5%), with constantly poor or absent responses. Transient relief followed by recurrence was reported by the owners of dogs no. 2, 3, 4 and 15.

These animals had been treated with corticosteroids (no. 2, 3 and 15) and restricted diet (no. 4) in association with flea control (no. 2 and 4) and antibiotics (no. 3).

Clinical findings

The gross appearance of skin lesions (Table 1) was as follows: erythema (n = 21; 95%) (Fig. 2), papule (n = 11; 50%) (Fig. 3), single or multifocal alopecia (n = 10; 45.5%) (Fig. 4), crusting (n = 4; 18.2%), nodule (n = 3; 13.6), eczema (n = 2; 9.1%), haematoma (n = 1; 4.5%), acne (n = 1; 4.5%), pododermatitis (n = 1; 4.5%), pyoderma intertrigo (n = 1; 4.5%), lichenification (n = 1; 4.5%).

All dogs were reported to have lesional pruritus, which manifested itself by scratching, licking and biting of the altered regions (Fig. 2). One dog (no. 11) showed lesions on its whole body. The localisation of skin lesions was as follows: abdomen (n = 11; 50%) (Fig. 4), lumbosacral region (n = 10; 45.5%), hindlimbs (n = 6; 25.3%), elbows (n = 6; 25.3%), flanks (n = 5; 22.7%), head (n = 4; 18.2%) (Fig. 3), thorax (n = 4; 18.2%), hocks (n = 4; 18.2%), neck (n = 3; 13.6%) and perineal region (n = 2; 9.1).

Fig. 2. Dog no. 21 with pruritus and *Dirofilaria repens* microfilariae in the blood. Erythema and alopecia are present on head, neck, elbows, hocks, thorax, flanks and abdomen

Fig. 3. Dog no. 13. Papule on the chin in a Dalmatian with many *D. repens* microfilariae (17) in the blood, and concurrently affected by leishmaniosis, babesiosis and canine granulocytic ehrlichiosis

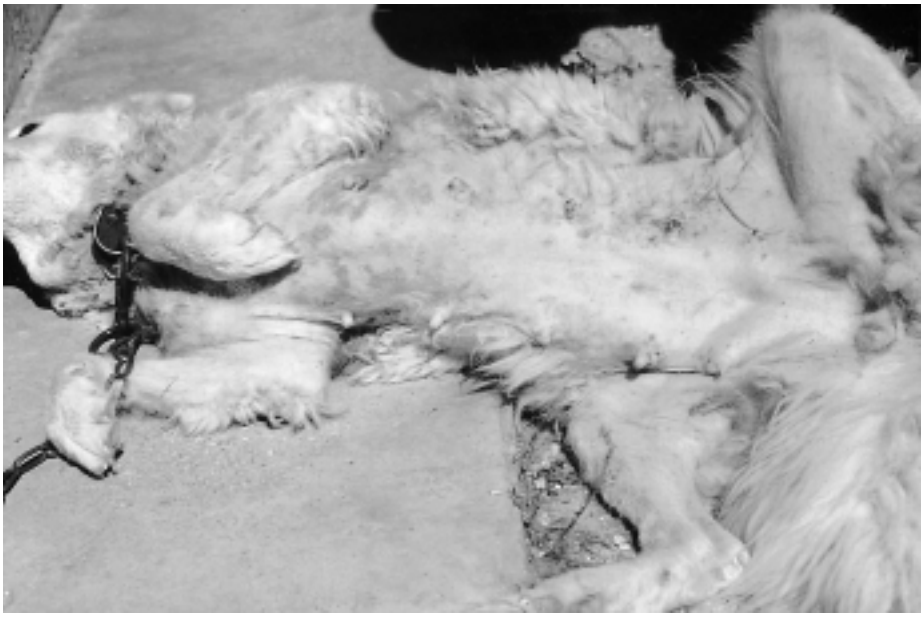

Fig. 4. Dog no. 22. Papule, erythema and alopecia on head, neck, thorax, flanks and abdomen in a patient with *D. repens* microfilariae in the blood

Other symptoms

Symptoms and signs, other than dermatological (Table 1), were as follows: conjunctivitis (n = 9; 41%), anorexia (n = 7; 31.8%), vomiting (n = 6; 27.3%), fever (n = 6; 27.3%), lethargy (n = 4; 18.2%), submandibular lymphadenopathy (n = 3; 13.6%), lameness (n = 2; 9%), muscular and joint pain (n = 2; 9%), eye pallor (n = 2; 9%), otitis (n = 2; 9%), tenesmus (n = 1; 4.5%), diarrhoea (n = 1; 4.5%), asthma (n = 1; 4.5%), pharyngitis (n = 1; 4.5%) and stomatitis (n = 1; 4.5%).

Microscopy

Microscopic examination of the filter retaining the microfilariae from every Difil test performed, showed *D. repens* microfilariae (Fig. 1) in the blood of all of the 22 dogs, and their count ranged from 2 to 17 per sample (average 6.3). The search for *D. immitis* and *A. reconditum* and the tests for heartworm antigen were negative.

The associated conditions were diagnosed by fresh blood smears stained using the Wright technique, microscopic evaluation of the fresh stools, and the serological tests for *Leishmania* (Leishcan 16). The concurrent infections were: babesiosis (22 dogs: 100%), canine granulocytic ehrlichiosis or CGE (13 dogs: 60%), leishmaniosis (3 dogs: 13.6%), and tapeworm infection (1 dog: 4.5%).

Control groups

In the control group A of 7 dogs with patent heartworm disease, all animals had *D. immitis* microfilariae in the blood and absence of *D. repens* microfilariae. In the control group B of 11 healthy dogs without dermatological problems, three animals (27.2%) were found to be carriers of *D. repens*, and two of these subsequently developed pruritic dermatitis during the summer of 2000.

Therapy

During the preliminary therapy against babesiosis and ehrlichiosis, the collateral signs (lethargy, conjunctivitis, anorexia, vomiting and fever) progressively decreased and disappeared. Simultaneous improvement of the superficial lesions was also observed, along with evidence – though admittedly hard to evaluate – of marked reduction of the itching. The same observation was done in cases no. 5, 8 and 13 already while under anti-leishmania treatment alone (aminosidine; Aminofarma, 10 mg/kg/day, im., for 10 days), which preceded the one with imidocarb-doxycycline.

In general, in all of the 22 dogs, the macrofilaricide (melarsomine; Immiticide) treatment began 2–3 days after completion of the antibabesial-ehrlichial therapy – apparently producing marked healing and a fast, progressive disappearance of all visible signs of dermatitis (Fig. 5), along with cessation of the

dogs' scratching, licking and biting, symptoms of itching. Obviously, this also depended on the duration of their disease, and on the severity of the lesions seen at the first visit. Microfilaricide treatment (ivermectin) was always carried out in all dogs 10 days after the end of the adulticide therapy. A clinical control and a Difil test, made one month after the completion of treatment, confirmed the resolution of the dermatological symptoms and the disappearance of *D. repens* microfilariae from the blood.

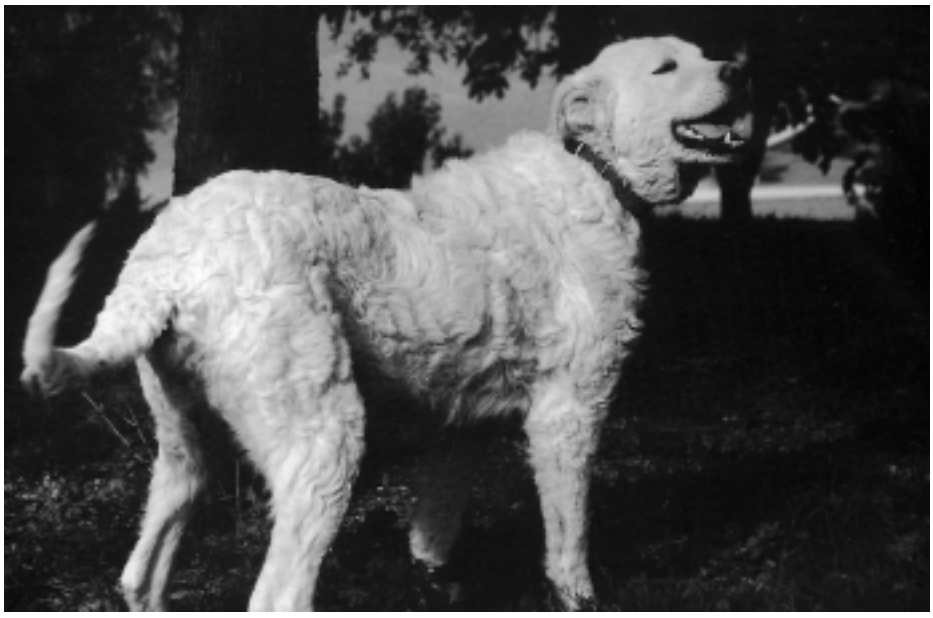

Fig. 5. Dog no. 22. Complete dermatological recovery 1 year after the end of therapy for *D. repens* infection

Recurrences were reported, during the following year (2000) only in three dogs (no. 7, 18 and 20 = 13.6%), the owners of which neglected to administer the preventive treatment with oral ivermectin (Cardotek, 1 amp/month for 8 months).

Discussion

To the author's knowledge, this is the first clinical study reporting on a relatively large group of dogs (22 cases) affected by pruritic dermatitis associated with *D. repens* microfilariae in the blood (Fig. 1). The differential diagnostic work-up should include, and was performed in this work to exclude other causes of dermatitis, infestation by skin parasites (fleas, *Demodex, Cheyletiella*), dermatophytes, *Malassezia pachydermatis*, hypersensitivity (food, atopy, ectopara-

sites), nutritional (zinc deficiency) and immune-mediated dermatosis, bacterial (*Staphylococcus)* and viral infections, as well as endocrine (hypothyroidism, hyperadrenocorticism) and psychogenic conditions (Tarello, 2000*a*).

Previous unsuccessful treatments with other medicaments were recorded in 17 (77.3%) out of 22 patients included in the present study. According to Bredal et al. (1998), who reported a single case, clinical signs of *D. repens* infestation are claimed to be present in only a small percentage of the parasitised dogs. However, this is in contrast with the results obtained here, where all animals in the study group had dermatological symptoms and two out of 3 asymptomatic *D. repens* carriers in control group B developed pruritic dermatitis during the following year.

Dogs infected with *D. repens* may show seasonal variations in the number of microfilariae in the blood, with the highest concentrations in August and September (Worms, 1972; Cancrini et al., 1975). The high number of microfilariae may be associated with clinical signs (Restani et al., 1962; Braca et al., 1977) caused by mechanical, toxic and immune-mediated actions (Mantovani, 1965). The pathogenicity of *D. repens* in humans is attributed to the movements of the adult nematodes in the subcutaneous tissue, associated with itching and pain (Pampiglione et al., 1995; Cancrini et al., 1998). The same mechanism has been suspected in dogs (Mandelli and Mantovani, 1966). In fact the symptomatic evidence of early cessation of the pruritus (the animals stopped scratching, licking and biting) was the most striking feature of the benefits from macrofilaricide treatment in all animals. A similar result has been described in an earlier Italian report (Restani et al., 1962) on 6 dogs submitted to the same treatment and previously unsuccessfully treated with several antibiotics, topical and general chemotherapeutics.

Adult nematodes of *D. repens* were not found nor searched for during the present study. Nevertheless, the detection of microfilariae in the blood seems to be of diagnostic importance for the presence of adults in *Dirofilaria* spp. infestations, as indicated by recent scientific committee guidelines (BSAVA's Scientific Committee, 1998). The importance of other unknown or hidden factors has been previously suspected (Tarello, 1999) in the development of skin lesions in dogs carrying *D. repens*. Earlier reports (Bonvicini, 1910; Sadignian, 1969; Beaufils and Martin-Granel, 1987; Cazelles and Montagner, 1996; Pintore et al., 1997) indicate co-infection with other pathogens in dogs with *D. repens* infestation. This hypothesis seems to be confirmed by the present work which found concurrent babesiosis (22 dogs; 100%), canine granulocytic ehrlichiosis or CGE (13 dogs; 60%), leishmaniosis (3 dogs; 13.6%), and tapeworm infection (1 dog; 4.5%). The association between babesiosis and CGE occurred most commonly (60%), and all animals were found to be infected with at least one of these agents. Concurrent infections with different pathogens may complicate illnesses, and consequently, the clinical presentation may become unusual (Kordick et al., 1999).

Babesiosis was diagnosed by detecting small bodies between 1 and 2.5 μm in the erythrocytes, which were identified as *Babesia gibsoni*.

Small babesiae have been reported in the countries most affected by *D. repens*, such as Spain, France and Italy, rarely associated with skin autoimmune diseases (Tarello, 2001*b*), and mostly showing anorexia, vomiting, fever and lethargy (Zahler et al., 2000). These four clinical signs were observed respectively in 7 (31.8%), 6 (27.3%), 6 (27.3%) and 4 (18.2%) of the reported cases in this study. These signs, with the ophthalmological ones (11 dogs; 50%) were recorded with greater frequency. A diagnosis of CGE (currently attributed to *E. ewingii* and/or *E. equi*) in dogs consists of direct identification of ehrlichial morulae in peripheral neutrophils (Goldman et al., 1998). Naturally occurring infections produce nonspecific signs of illness overlapping those induced by *Babesia* spp., including fever, lethargy, conjunctivitis, vomiting, diarrhoea, muscular/joint pain and lameness. It might be of interest to note that 10 of 13 dogs (77%) diagnosed with CGE in the present report showed at least one and up to three, of these symptoms (Table 1). Imidocarb dipropionate is a specific antibabesial medicament and also appears beneficial in the treatment of *E. ewingii* (Kordick et al., 1999). Doxycycline (10 mg/kg/day) is presently recommended as the proper therapy for all ehrlichial organisms, and has also had a prophylactic action against highly pathogenic strains of *Babesia canis* (Vercammen et al., 1996). For this reason, these drugs were both administered to this study group, even to the dogs only affected by babesiosis. The results obtained with these preliminary treatments appeared to help the recovery of affected dogs with the elimination of collateral symptoms such as conjunctivitis, lethargy, anorexia, vomiting and fever. Reduction of itching occurred while an improvement in the dermatological signs was noticed by both the owners and the author as early as the first stages of treatment against the co-infections.

These results are in agreement with the observation that infections with *Babesia* and *Ehrlichia* spp. induce immunosuppression and favour secondary opportunistic infections (Kordick et al., 1999).

Not in contrast with an earlier report (Ajmerito, 1954), similar outcomes were obtained also in dogs no. 5, 8 and 13 during treatment with anti-leishmania drugs. It is not possible to prove that the dermatological lesions in the dogs of the present study were directly caused by the microfilariae or by the concurrent babesiosis, but it does seem worthy of notice that (a) seventeen (77.3%) out of 22 dogs had previously been unsuccessfully treated with several therapeutic regimens, not aimed directly against *D. repens*. All patients recovered after specific treatment for the concurrent etiologic agents observed and for all stages of *D. repens;* (b) the Difil tests of control made 1 month after the end of therapy were negative for *D. repens* microfilariae; (c) relapses during the following year occurred only in three dogs (no. 7, 18 and 20; 13.6%), not submitted by their owners to the suggested 8-month microfilaricide prevention protocol.

The incidence of *D. repens* in the control group B (27.2%) is similar to the infection rate (20.5–25%) observed in some epidemiological surveys recently carried out on untreated dogs (Pollono, 1994; Rossi et al., 1996; Pollono et al., 1998) and cats (Tarello, 2000*a*) in Italy.

Taken together, these data are compatible with the fact that Italy has the highest concentration of recorded cases of human dirofilariosis in the world (Pampiglione et al., 1999; Pampiglione et al., 2001). In fact, the geographical distribution of the human cases follows that of the canine (Széll et al., 1999) and feline population carrying *D. repens* microfilariae (Pampiglione et al., 1995). The present report seems also to confirm the high *D. repens* infestation rate already observed in dogs from the study area (Ascoli Piceno province) in an earlier epidemiological survey (Mantovani et al., 1965).

In summary, in 22 dogs with pruritic dermatitis, living in Central Italy, negative antigen and filtration tests ruled out *D. immitis* and *A. reconditum*, whereas Difil tests proved positive for *D. repens*. The association between cutaneous lesions, microfilariae of *D. repens* variety and concurrent pathologic conditions was extremely close, constant through all patients and confirmed by their uniform response to specific therapy. The main conclusion is that *D. repens* infection in dogs is not as harmless as is currently considered, and apparently it is an opportunistic disease, often manifesting together with other infections.

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