

A Mini-Invasive Procedure for Removal of Adult *Dirofilaria Repens* From Subcutaneous Nodules in Dogs

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ABSTRACT

Dirofilaria repens is the causative agent of canine and feline subcutaneous Dirofilariasis, a mosquito-borne disease that has been increasingly reported in several countries in southern and central Europe, Africa and Asia. The natural hosts of *D. repens* are domestic and wild carnivores and the adults of these nematodes reside in the subcutaneous tissues of dogs and cats. Infection occasionally causes mild clinical signs such as subcutaneous nodules, itching, and dermal swelling, but most affected animals are asymptomatic. Here, the authors report a novel, mini-invasive procedure for removal of adult worms in four naturally-infected dogs presenting skin nodules. Ultrasonographic (US) and cytological diagnostic features are also presented. Worms were removed using a 19 Gauge needle, connected to a vacuum syringe. No adulticide therapy is currently available for *D. repens* and therefore surgical removal of worms could represent a non-invasive treatment leading to definitive cure of

symptoms.

INTRODUCTION

Dirofilaria repens is the causative agent of canine and feline subcutaneous dirofilariasis, a mosquito-borne disease that has become increasingly recognized in several countries in southern and central Europe, Africa and Asia. The *D. repens* life cycle consists of 5 developmental or larval stages in a vertebrate host and an arthropod (mosquito) intermediate host and vector. Adult female worms produce thousands of first-stage larvae or microfilariae that are ingested by a blood-feeding insect. Some microfilariae have a unique circadian periodicity in the peripheral circulation over a 24-hour period. Mosquitoes also have a circadian rhythm in which they obtain blood meals. The highest concentration of microfilariae usually occurs when the local vector is most actively feeding. Microfilariae then undergo 2 developmental moults in the insect. During subsequent feeding, the infected mosquito deposits third-stage larvae in a drop of hemolymph in the proximity of the bite wound. From here, larvae actively migrate to the subcutaneous tissue. Larvae

develop to the adult stage and prepatency lasts for six to nine months^{1,2,3,4,5}. The adults of *D. repens* reside in the subcutaneous tissues of dogs and cats and usually do not cause any symptoms. Occasionally, dermal swelling and subcutaneous nodules may be observed^{3,6,7}. Humans may act as accidental and dead-end hosts of *D. repens* and there is currently rising concern over the increase of reported zoonotic dirofilariasis in Europe. In most cases, the parasite is not able to develop to the adult, sexually-mature stage and infection is characterized by the presence of pre-adult stages located in subcutaneous nodules of different body areas, near the point of the mosquito vector bite. Ocular and pulmonary localization of the parasite has also been reported⁸. Subcutaneous or deep tissue localization of the parasitic nodule usually leads to a clinical suspicion of neoplasia and requires biopsy or more invasive surgery for differential diagnosis through histology and morphologic identification of the parasite, thus causing emotional distress and costly intervention⁸. In infected dogs and cats, diagnosis of infection is usually made by detection of circulating microfilaria by the modified Knott's test. Differentiation between *D. immitis* and *D. repens* larval stages is made on the basis of morphological features, by histochemical methods or by PCR. In the absence of circulating

microfilariae, ultrasonography and cytology may allow a diagnosis if nodules are present⁹.

To note that currently available tests to detect adult heartworm (*D. immitis*) antigens do not detect *D. repens* antigens and no cross reactivity has been described. Removal of the nodule and histo-pathological identification of the parasite may be time-consuming and costly. There is currently no registered adulticide treatment for *D. repens*. The off-label use of the arsenic adulticide melarsomine, combined with doramectin, a macrocyclic lactone with microfilaricidal activity, was recently described as effective in clearing *D. repens* infection in a dog⁷

For those dogs suffering from clinical signs of this disease, such as dermal swelling, sub-cutaneous nodules and pruritus, symptomatic therapy with steroids and/or antibiotics may be indicated, together with surgical removal of nodules³. Several macrocyclic lactones (ivermectin, moxidectin, selamectin) have been reported as effective for the prevention of *D. repens* infection in dogs^{10,11}. However, while there is no doubt that these drugs are able to clear microfilariaemia in infected dogs (important for breaking the transmission cycle and thus for zoonotic implications), most field studies were not based on necropsy confirmation of infection status and concern still remains about the ability of these compounds to completely prevent infection⁵. Here, the

Figure 1. Subcutaneous nodules caused by *Dirofilaria repens* infection in the treated dogs (white circles)



Figure 2 Scrotal nodule and swelling in one of the treated dogs



Table 1. Four dogs affected by subcutaneous dirofilariosis (*D. repens*)

Breed	Age	Gender	# Nodules
Boxer	7 years	Female	4
Italian Hunting Dog	5 years	Male	3
Mixed breed	5 years	Male	6
Petite Bleu da Gascogne	7 years	Male	1

Table 2. Parasitological analyses of affected dogs.

Dog	Worms	Microfilaremia
Boxer	7 (2 male, 5 female)	<i>D.repens</i> + / <i>D.immitis</i> +
Italian Hunting Dog	5 (1 male, 4 female)	<i>D.repens</i> + / <i>D.immitis</i> +
Mixed breed	6 (3 male, 3 female)	<i>D.repens</i> - / <i>D.immitis</i> +
Petite Bleu de Gascogne	1 (male)	<i>D.repens</i> - / <i>D.immitis</i> -

authors report a novel, mini-invasive procedure for removal of adult worms in four naturally-infected dogs presenting skin nodules.

MATERIALS AND METHODS

Four dogs from the province of Pavia (Po River valley, Northern Italy) presented with multiple subcutaneous nodules ranging in diameter from 1 to 2 cm. The number of skin nodules ranged from 1-6 (Table 1, Fig. 1a and 1b). Only one on them was currently on chemoprophylaxis with ivermectin (dosage 9 µg, oral route, once a month).

Fine needle aspirates were carried out on all nodules in all dogs and smears were stained with a rapid, modified Romanowski (Diff-Quick®). Ultrasonographic examination of skin nodules was performed by using a linear 12 mHz probe.

A modified Knotts' test to detect circulating microfilaria and discriminate morphological features was performed in each dog, as previously described¹⁵.

Following diagnosis of subcutaneous dirofilariosis by *D. repens*, a mini-invasive procedure was carried out to remove adult

parasites. A 5 ml syringe connected to a 19 Gauge Butterfly needle was used (Figure 2). The needle was firmly inserted within the nodule and gently moved while negative pressure was applied to the syringe until a very small amount of white or pink fluid could be noted (Figure 3). The negative pressure was maintained during needle withdrawal allowing removal of the intact adult worm (Figure 4, 5, 6). Ultrasound control was performed in order to confirm that all the worms in the nodule were removed.

Worms underwent identification based on morphological features (Figure 7a-7b), which was subsequently confirmed by PCR analysis, as previously described^{13,14,15}. No sedation or local anaesthesia treatment was performed.

RESULTS

Results of parasitological analyses are summarized in Table 2. In two of the dogs, a mixed infection with both *D. immitis* and *D. repens* microfilariae was found, only *D. immitis* larvae were present in the peripheral blood in one case while in the dog

Figure 3. A 5 ml syringe connected to a 19 Gauge Butterfly needle used for adult *Dirofilaria repens* removal



Figure 4. The needle is firmly inserted within the nodule and gently moved while negative pressure is applied to the syringe until a very small amount of white or pink fluid can be noted



Figure 5. The negative pressure is maintained during needle withdrawal allowing to start the intact adult worm removal



Figure 6. The adult worm is removed intact



Figure 7. Adult male worm of *D.repens* removed from a scrotal nodule



Figure 8. Female adult mature worm of *Dirofilaria repens* after removal.



Figure 9 Male adult worm (magnification 40 x). Transverse striations are observed on the cuticles of *D.repens* together with longitudinal ridges that are lacking on *Dirofilaria immitis* adult worms

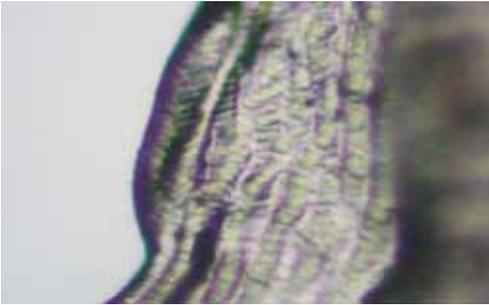


Figure 10 Multinucleated morulae (arrows), which represent the intrauterine pre-larval stage of development of the parasite

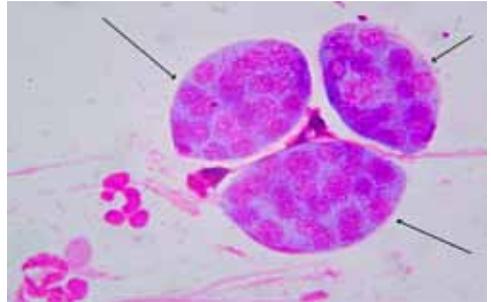


Figure 11. At ultrasound examination (12 MHz linear probes) the adult parasite appears as double, parallel and hyperechoic lines within the subcutaneous nodules (white arrows)

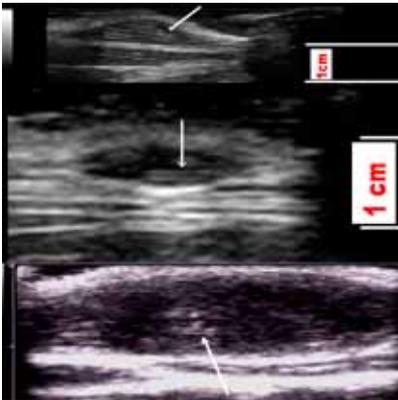


Figure 12 Ultrasound examination (12 MHz linear probes) of the scrotal nodule. The white arrow shows the nodule with inside double line filarid worm echoes. (T : testicle)

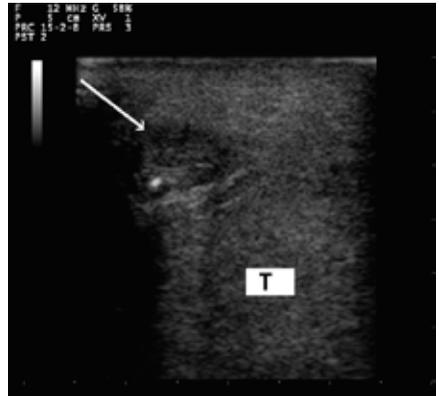


Figure 13 Ultrasound examination (12 MHz linear probes) 7 days after worm removal (previous case). Resolution of the lesion.



on chemoprophylaxis no microfilariae were detected.

Cytology was consistently characterized by a mixed, predominantly eosinophilic inflammatory infiltrate, the presence of several microfilariae and, in one case, by the presence of multinucleated morulae, which represent the intrauterine pre-larval stage of development of the parasite (Figure 7).

At US examination, the parasite appeared as double, parallel and hyperechoic lines within the subcutaneous nodules (Fig. 8a and 8 b), similar to that described for *Dirofilaria immitis* in pulmonary arteries¹².

Most nodules⁹ contained one intact worm, while several others⁵ contained two intact worms. The procedure resulted in the removal of a total of 12 female and 7 male adult worms (Table 2).

Morphological and PCR examination confirmed that the parasite was *D.repens* in all of the cases.

All nodular lesions disappeared within a few days following the removal of the parasite with the mini-invasive technique, without any further treatment.

DISCUSSION

The mini-invasive procedure described here is easy to perform and is followed by complete resolution of symptoms related to infection by *Dirofilaria repens* when nodules are present. It requires no anaesthesia or sedation. However, it cannot be performed in asymptomatic dogs (no nodules) and therefore cannot be considered as curative, from a parasitological point of view, for clearing the infection.

At this time, however, no adulticide drug is currently registered for treatment against *Dirofilaria repens* and some concerns remain about its prevention, because of the results of experimental infections⁵ and the findings of the dog of this study on chemoprophylaxis. Therefore, in the authors opinion, the described surgical method appears to be the best option when nodules are present. Furthermore, as shown here, it is important to remember that clinical infection may be present without microfilaremia (as

in one case) and that mixed infections by *Dirofilaria immitis* and *Dirofilaria repens* are not uncommon. To conclude, a mini-invasive surgical procedure for removing adult *Dirofilaria repens* nodule in naturally infected dogs is a safe, easy and effective procedure for relief of symptoms related to infection.

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