Dirofilaria repens in a cat with acute liver failure

E V Schwan[a], D B Miller[b], D de Kock[c] and A van Heerden[d]

ABSTRACT

Acute liver failure was diagnosed in a 12-year-old cat. Fine needle aspirate cytology revealed high numbers of unsheathed microfilariae and a hepatocellular reaction with no evidence of bacterial infection. The microfilariae were identified as those of *Dirofilaria repens* by acid phosphatase staining. The high number of microfilariae seen in both the blood and the liver aspirate samples as well as the favourable response to ivermectin amongst other drugs administered, is suggestive that *D. repens* was the cause of the liver insult. A positive result obtained with an antigen-capture ELISA (Dirochek®) for *Dirofilaria immitis* antigen was interpreted as false. This is the 1st report of *Dirofilaria repens* for South Africa.

Key words: acid phosphatase staining, acute liver failure, antigen capture ELISA, cat, *Dirofilaria repens,* ivermectin, microfilariae, South Africa.

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INTRODUCTION

Dirofilaria repens Railliet & Henry, 1911, is a nematode (Filarioidea: Onchocercidae) which in some geographical areas is a common parasite of mainly dogs and cats as well as some wild carnivores (*Felis viverrina, Felis sylvestris, Felis chaus, Panthera leo, Genetta tigrina, Vulpes vulpes, Canis aureus*)[11,29]. The parasite is endemic to countries in Europe, Asia and Africa[28] Cases reported from the Americas either relate to animals imported from Europe or are regarded as incorrect identifications[29]. In Africa, *D. repens* has been reported from Kenya[16,27,34], the Central African Republic[14], Egypt[26], Nigeria[17,18,35,36,40], Zimbabwe[20], Uganda[7,8], Sudan[13,19], Tunisia[3] and Morocco[5]. The parasite has not previously been reported from South Africa. *D. repens* has an indirect life-cycle. In the carnivores that act as final hosts, the predilection site of the male and female adult parasite is the subcutaneous tissue[16].

Males are 4–7 cm, females 9–17 cm long[9,33]. In the final host, after a prepatent period of approximately 6 months, the parasite reaches maturity and the females produce microfilariae, which eventually appear in the blood[42]. Microfilariae are unsheathed and 283–377 µm long[9,42]. There is periodicity in the appearance of microfilariae in the peripheral blood, with minimum microfilaraemia observed during the day and a peak around midnight (nocturnal periodic)[17]. Anopheline and culicine mosquitoes of the genera *Aedes, Anopheles, Culex, Armigeres* and *Mansonia* are considered to be intermediate hosts and become infected by obtaining bloodmeals from microfilaraemic final hosts[39]. The microfilariae develop to 3rd-stage infective larvae in the mosquito. Under optimal conditions, and depending on the vector species, this process may take only 10–11 days[42]. At this stage of the life-cycle, mosquitoes are able to transmit the parasite during blood-feeding. The presence of *D. repens* in the subcutaneous tissues of dogs and cats is generally considered insignificant for the host, although it has been suggested that the pathology may be significant in cases of massive infection[18,21,23,32]. Pruritus, with or without non-specific skin lesions, is the most common clinical sign associated with *D. repens* infection (cutaneous dirofilariosis)[17,21]. In those few cases found to be massively infected with adult worms and with simultaneously high microfilaraemia, gross and histopathological changes in the spleen, liver, gastrointestinal tract, kidneys, lungs, heart and brain have been reported[18,21,32]. The nature of these lesions suggest combined mechanical and immunopathological effects elicited by both micro- and macrofilariae[23]. Humans can become infected accidentally with *D. repens,* acting as a final host[22]. Carnivores, as preferential hosts, constitute the reservoirs of infection for humans and suitable culicid vectors. In humans the parasite is mainly localised superficially in granulomas of subcutaneous and submucosal tissues as well as in the eyelids and under the conjunctiva, and constitutes the most important aetiological agent of the various human dirofilarioses with regard to incidence, variety of localisations and geographical spread[29].

This paper represents the 1st report of *D. repens* for South Africa. The parasite was encountered in a cat.

CASE HISTORY

A 12-year-old intact male cat was presented to the Animal Farm Veterinary Clinic in Pretoria, South Africa. The cat had been kennelled 7 days previously. After its return, the cat was reported to have a poor appetite, being depressed and having vomited once. The animal originally lived in Phalaborwa in the Northern Province, but had not left the Pretoria area during the last 3 years.

Physical examination on Day 1 revealed a rectal temperature of 39.2 °C and a pulse rate of 160/min. Respiration was shallow and rapid. The cat was slightly dehydrated. Abdominal palpation did not reveal any abnormalities and there was no abdominal pain. The mucous membranes were severely icteric. Routine biochemistry revealed moderately elevated creatinine (265 µmol/ℓ), highly elevated alkaline phosphatase (281 U/ℓ) and extremely elevated alanine aminotransferase (894 U/ℓ) and gamma glutamyltransferase (20 U/ℓ) (Table 1). The haematology results were all within physiological range (Table 2). Treatment was initiated for an acute hepatic insult. Intravenous physiological saline (Intramed) spiked with dextrose to 2.5 % was administered as a constant rate infusion with an infusion pump. Amoxycillin (Clamoxyl RTU, Pfizer Animal Health) at 20 mg/kg subcutaneously twice daily, enrofloxacin (Baytril, Bayer Animal Health Division) at 5 mg/kg subcutane-

[a]Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa.
E-mail: vschwan@op.up.ac.za

[b]Department of Companion Animal Medicine, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa.
E-mail: dmiller@op.up.ac.za

[c]Animal Farm Veterinary Clinic, 827 Old Farm Road, Faerie Glen, 0043 South Africa.
E-mail: ddekock@cybernet.co.za

[d]Pretoria Cytology Service, PO Box 369, Reyton, 1001 South Africa.

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Table 1: **Serum biochemistry results.**

Parameter	Results				Reference range
	Day 1	Day 2	Day 3	Day 4	
Urea (mmol/ℓ)	8.89	–	–	–	5.7–12
Creatinine (µmol/ℓ)	265	–	–	–	71–212
ALT (U/ℓ)	894	712	418	222	12–130
ALP (U/ℓ)	281	182	97	58	14–111
Amylase (U/ℓ)	820	–	–	–	500–1800
Lipase (U/ℓ)	1749	–	–	–	100–1400
GGT (U/ℓ)	20	6	12	0	0–1
Total protein (g/ℓ)	82	–	–	–	57–89
Albumin (g/ℓ)	35	–	–	–	26–39
Globulin (g/ℓ)	52	–	–	–	28–51
Total bilirubin (µmol/ℓ)	–	126	–	–	0–9

ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gamma glutamyltransferase.

ously once daily, vitamin K (Kyrovite K Injection, Kyron Laboratories) at 2 mg/kg intramuscularly once daily, as well as ketoprofen (Oruject, Rhône-Poulenc Rorer SA) at 2 mg/kg subcutaneously once daily completed the treatment protocol. The fluid deficit was replaced over 6 hours and then a maintenance fluid rate of 70 mℓ/kg/24 h was infused. A naso-oesophageal feeding tube was placed (a radiograph taken to ensure correct placement). Hill's Prescription Diet Canine/Feline a/d was fed according to caloric requirements.

Physical examination on Day 2 revealed that the condition of the cat was unchanged. The rectal temperature was 39.6 °C and the mucous membranes were still icteric. Repeat serum biochemistry revealed extremely elevated total bilirubin of 126 µmol/ℓ and, although still elevated, ALT, ALP and GGT had decreased to 712 U/ℓ, 182 U/ℓ and 6 U/ℓ respectively (Table 1). The partial thromboplastin time was slightly prolonged (32.1 s) (Table 2). A fine needle aspirate was obtained from the liver. On microscopical examination, the sample was found to be hypercellular and extremely platelet-rich. There was no evidence of bacterial infection even though the white blood cells, predominantly neutrophils with a strong left shift, were markedly increased. Eosinophils were prominent with few macrophages and lymphocytes. Very high numbers of microfilariae were noted. A fairly high number of hepatocytes revealed markedly swollen and prominent nuclei. Marked but not massive lipidosis was noted. The bile duct epithelium appeared normal. Examination of an EDTA blood sample revealed microfilariae on Giemsa-stained thin blood films (Fig. 1). The microfilariae were unsheathed. They varied in width from 6.25 to 7.5 µm at the widest part of the anterior end and varied in length from 320 to 350 µm. The microfilariae were identified by acid phosphatase staining as those of *Dirofilaria repens,* showing typical enzyme activity at the anal pore and innerbody[2,41,43]. Examination of a serum sample for antigen of *Dirofilaria immitis* by means of a commercial ELISA (Dirochek®, Symbiotics) was positive. Faecal flotation was negative, thus there was no evidence of gastrointestinal helminth infection. Intravenous saline supplemented with KCl was continued, together with twice daily amoxycillin, enrofloxacin, vitamin K and, additionally, vitamin B_1, B_2, nicotinamide and d-panthenol (Vitamin B CO, Centaur) subcutaneously once daily as well as Na clanobutin (Bykahepar, Centaur) at 200 mg/kg intramuscularly. Ivermectin (Ivomec, 1 % m/v, Injection for Cattle, Sheep and Pigs, Merial) 0.02 mℓ was given once subcutaneously for the microfilaraemia. The product was used extra-label with the owner's permission. Naso-oesophageal feeding was continued.

Table 2: **Haematology results.**

Parameter	Results		Reference range
	Day 1	Day 2	
Haemoglobin (g/dℓ)	12.4	–	8–15
RBC (×10^{12}/ℓ)	8.02	–	5–10
Haematocrit (%)	38.4	–	30–45
MCHC (g/dℓ)	32.2	–	32–36
MCV (fℓ)	47.8	–	39–59
MCH (pg)	15.4	–	13–17
RDW (%)	13.8	–	11.8–14.8
Reticulocytes (%)	0.3	–	0.0–1.5
Platelets (×10^9/ℓ)	387	–	300–700
Wbc (×10^9/ℓ)	11.76	–	5.5–19.5
Neutrophils (abs) (×10^9/ℓ)	10.11	–	2.5–12.5
Eosinophils (abs) (×10^9/ℓ)	0.12	–	0.0–0.9
Lymphocytes (abs) (×10^9/ℓ)	0.94	–	1.5–7
Monocytes (abs) (×10^9/ℓ)	0.94	–	0.0–0.85
PT (s)	–	10.7	7.1–10.9
PTT (s)	–	32.1	11.5–19.9

RBC, Red blood cell count; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; RDW, red cell distribution width; WBC white blood cell count; PT, prothrombin time; PTT, partial thromboplastin time.

Physical examination on Day 3 revealed a slight improvement in habitus. The rectal temperature was 39.8 °C. Intravenous fluid therapy was continued with Ringer's lactate solution, together with amoxycillin, enrofloxacin and ketoprofen. Naso-oesophageal feeding was continued. Serum biochemistry revealed a further decrease of the still highly elevated ALT (418 U/ℓ). ALP was again within physiological limits (Table 2).

Physical examination on Day 4 revealed a remarkably improved habitus. The naso-oesophageal tube was removed owing to blockage. The owner was encouraged to hand-feed the animal 3–4 times a day. Intravenous fluid therapy was continued with Ringer's lactate solution, together with amoxycillin and enrofloxacin. Serum biochemistry revealed only moderately elevated ALT (222 U/ℓ), with ALP and GGT within physiological limits.

On Day 5 the habitus of the cat was normal and the animal was discharged on enrofloxacin (Baytril Tablets, Bayer Animal Health) at 5 mg/kg/day and amoxycillin with clavulanic acid (Synulox 50, Pfizer Animal Health) at 12.5 mg/kg twice daily orally. An EDTA blood sample

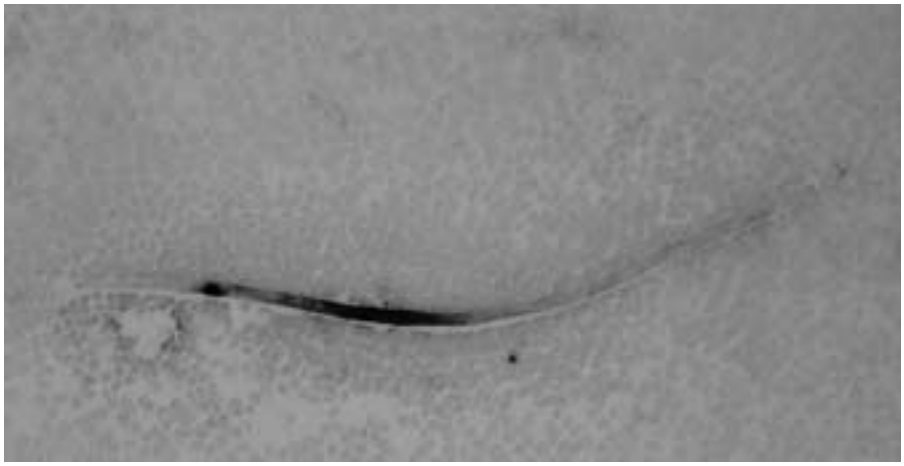

Fig. 1: ***Dirofilaria repens* microfilaria. Acid phosphatase activity at innerbody and anal pore.**

from the owners other cat was negative for microfilariae.

DISCUSSION

Dirofilaria repens has never before been recorded from any domestic or wild animal in South Africa. However, *D. repens* has been reported from a 10-year-old dog in Norway that had been imported from Durban, South Africa, 14 months previously[6]. *Dirofilaria* spp. are not endemic in Norway and the 3 confirmed cases reported over the past 20 years were all from imported dogs[15]. Whether the dog was infected with *D. repens* in South Africa is uncertain, since infection could also have occurred in transit. *D. repens* has gained significant attention in recent years as the most important aetiological agent of the over 400 reported cases of human dirofilariosis[29]. Although regarded as largely non-pathogenic in its principal final hosts, dog and cat, there is evidence suggesting that under certain circumstances the parasite may not be as innocuous as generally supposed.

In this case, a cat was diagnosed with acute liver failure and fine needle aspirate cytology revealed high numbers of microfilariae and a hepatocellular reaction with no evidence of bacterial infection. The microfilariae were identified as those of *D. repens.* Although there is no proof that *D. repens* was the cause of the acute liver insult in this case, the high number of microfilariae seen in both the blood and the liver aspirate samples as well as the cat's favourable response to treatment, which included ivermectin amongst other drugs, is suggestive. The aetiology of acute hepatic failure comprises hepatotoxins, infectious agents, systemic disorders as well as hepatic injury[38]. In order to shed more light on the aetiology of the present case, an ultrasound-guided true-cut liver biopsy was recommended. This, however, did not meet with the owner's approval. The microfilaraemia responded well to ivermectin; on Day 5, 3 days after treatment, no microfilariae were detected on thin blood films and the cat's habitus and serum biochemistry results had improved. The microfilaricidal properties of ivermectin are well documented for a broad range of filarial helminth species occurring in both human and animal hosts[4,12]. However, to the best of our knowledge there are no reports on the efficacy of ivermectin against microfilariae of *D. repens.* The recommended dose for clearing microfilaraemia in *Dirofilaria immitis* infection is 50 µg/kg[31]. In the present case, no systemic side-effects, which are a common sequel in the treatment of microfilariaemia caused by *D. immitis*[1], were noted. However, the practise in heartworm-endemic areas of using ivermectin chemoprophylactically at 6 µg/kg monthly has been shown to be an effective means to prevent infection with *D. repens*[10,24,30]. The positive result obtained with the Dirochek® ELISA for *Dirofilaria immitis* antigen is interpreted as false.

In South Africa, *D. immitis* has so far only been reported in imported dogs. Considering the dramatic improvement of the cat, the absence of any typical clinical signs usually associated with cardiovascular dirofilariosis as well as the fact that the animal never left South African territory, precludes acceptance of the test result. Dirochek®, like several other commercially available test kits, is an antigen-capture ELISA based on monoclonal antibodies manufactured in the USA. Both the manufacturer of Dirochek® as well as independent surveys conducted in the USA claim a 100 % specificity[25]. Cross-reactions with *Dipetalonema reconditum,* the only other filarial species found in dogs, but not in cats, in the USA can be excluded. However, this is not the case in other geographical areas, as has been demonstrated in Europe, where cross-reactions, particularly with *D. repens,* as well as other canine and feline filarial worms that are not endemic to the USA, have been reported[37,41]. Consequently, the interpretation of positive antigen-capture ELISA test results for *D. immitis* should be undertaken with great circumspection in Africa.

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