# Canine filariosis caused by *Dirofilaria immitis* in Mozambique: a small survey based on the identification of microfilariae

E V Schwan[a*] and D T Durand[a]

**ABSTRACT**

*Dirofilaria immitis* was diagnosed in 4 of 13 indigenous dogs from the Province of Zambézia, Mozambique, by acid phosphatase staining of microfilariae. The finding reconfirms the occurrence of the parasite in Mozambique after 3 decades and emphasises the need for extensive surveys. Additionally, in 1 of the infected dogs, microfilariae of *Dipetalonema reconditum* were detected, which is the 1st record of this parasite in Mozambique.

**Key words**: acid phosphatase staining, *Dipetalonema reconditum, Dirofilaria immitis,* dog, membrane filtration, microfilariae, Mozambique.

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## INTRODUCTION

*Dirofilaria immitis* (Leidy, 1856) Railliet and Henry, 1911 (Filarioidea, Onchocercidae), colloquially known as 'canine heartworm', is a common parasite of the pulmonary arteries and right ventricle of mainly dogs and to a lesser extent cats as well as some wild Canidae, Felidae and other mammals[1]. With the exception of Antarctica, the parasite has an almost universal distribution[21]. Males are 12–20 cm long and 0.7–0.9 mm wide, and females are 25–31 cm long and 1.0–1.3 mm wide[20]. After a prepatent period of approximately 6–9 months, the parasite reaches maturity and females produce microfilariae that are unsheathed and measure 218–340 $\mu$m by 4–7 $\mu$m[3,7,19,24]. The appearance of microfilariae in the peripheral blood is nocturnal subperiodic, with maximum microfilaraemia observed during early evening and at night[9]. Typical for filarial parasites, adults and circulating microfilariae of *D. immitis* are long-lived. In dogs, the life expectancy of adults is about 5 years and that of circulating microfilariae 2.5 years[18,23]. Transplacental transmission may occur and microfilariae have been found in neonatal pups[13]. *D. immitis* follows an indirect life-cycle and about 60 anopheline and culicine mosquito species, belonging to the genera *Anopheles, Aedes, Culex, Psorophora* and *Mansonia,* are considered intermediate hosts which become infected by obtaining bloodmeals from microfilaraemic final hosts[12]. Microfilariae develop into 3rd-stage infective larvae in the mosquito. Under optimal conditions and depending on the vector species, this process may take only 15–17 days *e.g.* in *Aedes aegypti*[22]. At this stage of the life-cycle, mosquitoes are able to transmit the parasite during bloodfeeding. While feeding, infective larvae emerge from the mouthparts of the mosquito and migrate onto the skin of the host. After the mosquito has finished its blood meal and the fascicle is removed from the puncture wound, the larvae can then use this wound as their portal of entry into the host[15]. Following a complex migration, the worms finally reach their predilection site.

In South Africa, *D. immitis* has so far only been reported from imported dogs[25,26] and there are also no reports of endemic cases from the neighbouring countries Namibia, Botswana and Zimbabwe. In Mozambique, however, the parasite appears to be endemic and was first described by Dias in 1954[6] from a dog in Maputo. Unfortunately, the author did not mention on what particular finding (*e.g.* microfilariae in blood, macrofilariae encountered during necropsy) the diagnosis was based and hence the report should be interpreted with circumspection. Reliable records based on adult specimens recovered from dogs during necropsies, were reported from Beira in 1966 and Quelimane in 1969[4] but the number of animals affected was not mentioned. More recent reports, based on the examination of Giemsa-stained buffy coat films of 86 dogs, claimed 5 to be infected with microfilariae of *D. immitis*[10,11]. Considering, however, that 6 species of

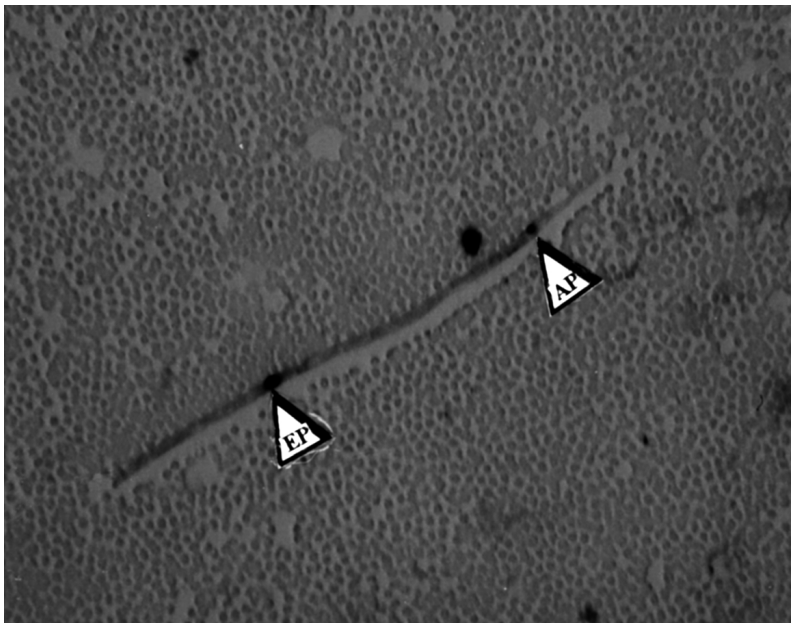


Fig. 1: ***Dirofilaria immitis* microfilaria. Acid phosphatase activity at excretory** (EP) **and anal** (AP) **pores.**

[a]Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa.

*Author for correspondence.
E-mail: vschwan@op.up.ac.za

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filariae have so far been described from dogs in the Afrotropical Region, species differentiation of microfilariae based on morphological characteristics has to be regarded as potentially unreliable and hence the latter report is not conclusive[17,24]. With the exception of the sheathed microfilariae of *Brugia* spp. that can easily be identified by morphological criteria, species identification of the unsheathed larvae of *Dipetalonema* and *Dirofilaria* requires acid phosphatase staining which is accepted as the most definitive differential technique[2,21,24].

Mozambique, with comparatively very little information available on its parasitological fauna, appears to be an endemic hotspot. According to unconfirmed reports, 10 % of the dog population of the capital Maputo is said to be infected with *D. immitis* (M E Mazibe, Instituto Nacional de Investigação Veterinária, pers. comm., 1993).

The objective of the present communication is to contribute to the extremely scarce information on canine filariosis in Africa, and Mozambique in particular. Furthermore, the intention is to create awareness of a parasite that, with increased traffic through the opening of borders, might eventually spread and become established in countries like South Africa, which so far appears to be unaffected.

## MATERIALS AND METHODS

During July 1996 a field trip was conducted to Mahimba ranch (18°16′S, 36°37′E), which is located 58 km south of Quelimane in the Province of Zambézia. The local inhabitants have many dogs that roam uncontrolled in the small gardens around the houses, on the roads and in most clearings on the ranch.

With the approval of their owners, blood samples were collected from 13 adult indigenous dogs. The blood was directly collected from the cephalic vein into 4.5 mℓ EDTA coated vacuum tubes (Vacutainer®, Becton Dickinson). The whole blood samples were stored at 6°C in a refrigerator and examined 7 days later in the laboratory. Of each sample, a 2 mℓ aliquot was screened for microfilariae by membrane filtration using 3.0 μm Isopore® membrane filters (Millipore)[5] stained with Giemsa[16].

## RESULTS

Examination of the stained filters revealed unsheathed microfilariae in 4 of the blood samples. Subsequent acid phosphatase staining identified microfilariae as those of *D. immitis* (all 4 samples), showing typical enzyme activity at the anal and excretory pores and *Dipetalonema reconditum* (1 sample), showing enzyme activity throughout the body[2,24]. Whereas microfilariae of *D. immitis* varied in width from 4 to 6 μm at the widest part of the anterior end and varied in length from 232 to 260 μm, those of *D. reconditum* had a width of 4 μm and varied in length from 200 to 204 μm.

## DISCUSSION

Since the only credible initial report of *D. immitis* in dogs in Mozambique based on the demonstration of macrofilariae during necropsy[4], the present report is the 1st one confirming the identity of the parasite by the reliable means of acid phosphatase staining of circulating microfilariae.

The fact that 4 of 13 dogs (31 %), in a sparsely populated rural area were found to be infected with canine heartworm emphasises the need to conduct extensive surveys to determine the prevalence in the various provinces of the country. Since heartworm disease is vector-borne, these studies would also be of major interest to neighbouring countries like South Africa and Zimbabwe in which *D. immitis* has not been reported other than from quarantined, imported dogs. *D. immitis* has, however, been reported from Tanzania, where the parasite is known to be endemic, as well as from Malawi[8,14]. Since no medication is registered at present for the prevention and treatment of heartworm infection in Mozambique and other southern African countries, a spread of the parasite, not only within Mozambique but also across its borders, could be expected in the long term. Dogs imported into South Africa from Mozambique, as well as other endemic countries, are subject to import regulations.


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