HISTOCHEMICAL DIFFERENTIATION OF MICROFILARIAE OF *BRUGIA*, *DIROFILARIA* AND *ACANTHOCHEILONEMA SP.* FROM CANINE BLOOD USING A COMMERCIAL, ACID PHOSPHATASE LEUCOCYTE KIT

Ambily, V.R[1*]., Usha.N.Pillai[2]., Pramod,S[3]., Kanaran,P.P [4]

[1]Assistant Professsor, [2]Associate Professor, Department of Clinical Veterinary Medicine

[3] *PhD Scholar, Centre of Excellence in Pathology College of Veterinary and Animal Sciences, Mannuthy, Thrissur*

Kerala Veterinary and Animal Sciences University, Kerala

[4]Assistant Director, AHD, Kerala

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ABSTRACT

The present paper deals with histochemical differentiation of microfilariae from canine blood using a commercial kit, acid phosphatase leukocyte. Out of hundread dogs presented with clinical signs suggestive of microfilariosis, eighty dogs screened positive for microfilariae. Blood samples obtained from these positive cases were processed as per standard procedure and smears were prepared and stained for the demonstration of acid phosphatase activity using Acid Phosphatase Leucocyte kit. The differential acid phosphatase staining pattern enables quick speciation of microfilariae.

Keywords: Dog, Acid phosphatase, *Brugia*, Microfilaria

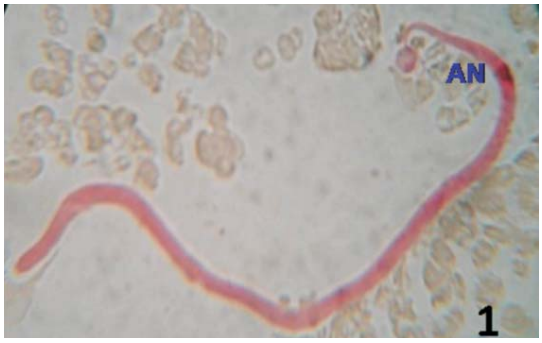
INTRODUCTION

Canine filariosis is caused mainly by filarial nematodes belonging to *Dirofilaria*, *Acanthocheilonema* and *Brugia* species. Techniques like observation of microfilarial activity in fresh drops of blood and differentiation by size and morphologic characters in stained smears are often difficult to differentiate each species. Histochemical technique using Naphthol AS-TR Phosphate is the most reliable method for their differentiation. This technique is based on demonstration of acid phosphatase enzyme activity in various body parts of each species of microfilaria. The standard technique of acid phosphatase activity as per the Barka method (Balbo and Abate, 1972)) which utilizes Naphthol AS-TR phosphate as the substrate and pararosanilin as the chromagen. In the present paper we propose the use of a test kit, Acid Phosphatase Leucocyte kit (Far diagnostics, Italy), commonly employed in the detection of acid phosphatase reaction in leucocyte for the diagnosis of leukemia. This kit utilizes similar reagents as in Barka's standard technique.

MATERIALS AND METHODS

The present study was carried out by screening dogs of both sexes above six months of age presented to College Veterinary Hospital, Mannuthy from different parts of Kerala with clinical signs suggestive of microfilariosis *viz* anorexia, vomiting, fever, conjunctivitis, limb and scrotal oedema. Wet

film examinations were carried out to detect microfilaria. Three milliliters of blood was collected from the cephalic vein of microfilaremic dogs and allowed to clot. The serum obtained is centrifuged at 3000 rpm for 5 minutes. The supernatant was discarded and the sediment was examined for microfilaria. Smears were prepared from the sediment , air dried and fixed in chilled absolute acetone for one minute, air dried and stained for the demonstration of acid phosphatase activity using Acid Phosphatase Leucocyte kit (Far diagnostics, Italy). This kit utilizes Naphthol AS-BI phosphate instead of Naphthol AS-TR phosphate. Smears were stained following the procedure described by the manufacturer of the kit, except counter staining with Giemsa solution. Smears prepared from these cases were also fixed in methanol for Giemsa staining.



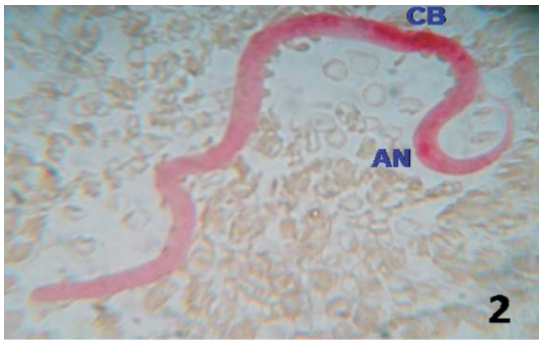


Fig.1 & 2: *Dirofilaria repens* microfilariae

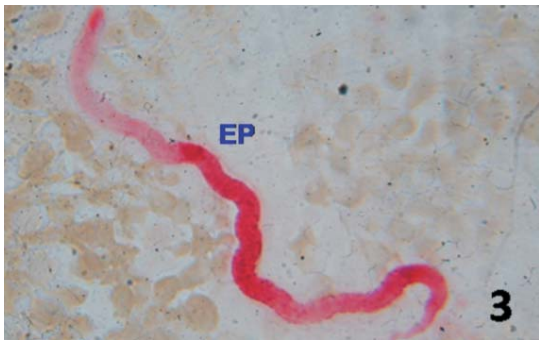


Fig.3: *Dipetalonema reconditum* microfilariae

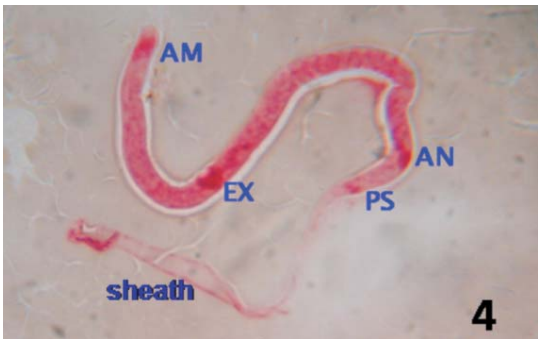


Fig.4: *Brugia malayi* microfilariae

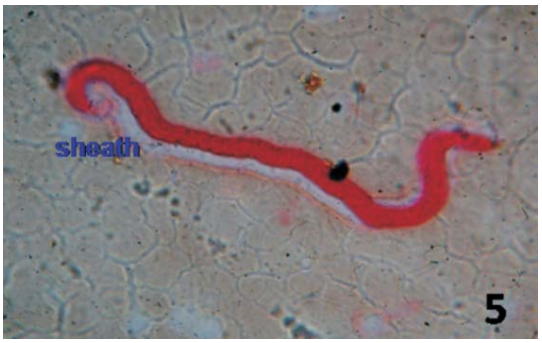


Fig.5: *Brugia pahangi* microfilariae

RESULTS AND DISCUSSION

Giemsa staining of blood smears revealed both sheathed and non sheathed microfilariae. Histochemical differentiation of nonsheathed microfilariae revealed three distinct patterns. Nonsheathed microfilaria that had acid phosphatase activity at the anal pore only is *Dirofilaria repens* (Fig.1) in accordance with the findings of Balbo and Abate (1972) and Radhika (2005). Nonsheathed microfilaria that had acid phosphatase activity at the anal pore and central body region (CB) is also confirmed as *Dirofilaria repens* (Fig.2) as reported by Valcarcel *et al.* (1990). Nons

heathed microfilaria with uniform acid phosphatase activity in the body, but less intense activity cranial to the excretory pore (EP) agrees with orginal description of *Acanthocheilonema reconditum* (Fig.3) (Chalifoux and Hunt, 1971). Histochemical differentiation of sheathed microfilariae revealed two distinct patterns. Sheathed microfilariae which exhibited acid phosphatise activity at amphids (AM), excretory (EX) and anal vesicles (AN) and phasmids (PS) (Fig.4) were similar to the pattern of *Brugia malayi* (Kobasa *et al.*, 2004). Sheathed microfilaria showing intense enzyme activity uniformly along the entire body of the organism is *Brugia pahangi* (Fig.5) which agrees with the observation made by Kobasa *et al. (*2004).

Histochemical staining to detect acid phosphatase activity require fresh sample to yield optimal results and need expertise to identify and confirm the species (Yen and Mak, 1978). Although it require fresh sample, it is the easiest method of differentiation of various species of microfilariae.

SUMMARY

Various species of canine microfilariae *viz Dirofilaria repens* , *Acanthocheilonema reconditum, Brugia malayi* and *Brugia pahangi* can be easily differentiated using acid phosphatase staining.

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