

# COMPARISON OF VARIOUS METHODS FOR THE DETECTION OF MICROFILARIA OF *SETARIA* IN THE BLOOD OF CATTLE

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Among parasitic diseases, filariasis in man and livestock constitutes a major health problem in tropical countries. Detection of microfilaria in blood of infected animals plays a major role in control and eradication of filarial diseases. There is an increased demand for simple, sensitive, specific and rapid diagnostic methods to effectively study microfilariosis in detail. In this study, blood samples from cattle were screened by direct wet mount preparation, Giemsa stained blood smear, Modified Knott's method (MKM) and Citrate Saponin acid method (CSAM) for microfilaria of *Setaria*. Blood samples from 500 cattle were screened during April 2001 to October 2002. Male animals screened were from Karnataka Meat and Poultry Marketing Corporation Limited slaughter house, Bangalore and cows screened were from postmortem studies conducted at Department of Pathology, Veterinary College, Bangalore.

Blood samples (10 ml from each animal) were collected in clean and sterile glass vials with EDTA and Sodium citrate as anticoagulants separately. A wet film was prepared and examined under low magnification for microfilaria. Thick blood smears made and air dried at room temperature were fixed in methanol and stained with Giemsa's stain (Diluted 1:20 in buffered distilled water) for 30 minutes. The slides were then washed in buffered water, air dried and examined.

MKM was done as per Lindsay (1965). Two ml of blood was mixed with 10 ml of formalin solution, centrifuged and sediment was examined after adding 0.1 per cent methylene blue or 0.2 per cent methyl green stains. In CSAM, ten ml of blood and two ml of freshly prepared two per cent Sodium citrate solution in 0.85 per cent saline was mixed well and centrifuged at 1500 rpm for 10 minutes. The top plasma layer was aspirated and discarded and packed erythrocytes and buffy coat were transferred to freshly prepared Saponin solution (10 ml). Saponin solution was prepared by adding 0.25 g of Saponin powder (M/s Himedia, Mumbai) to 50 ml of 0.85 per cent saline solution. This was then centrifuged and to the sediment, two drops of one percent acetic acid solution was added and mixed well with an applicator stick and spread over slide surface.

The slides were dried and then dipped in Methylene blue phosphate solution (composition: 0.5 g of calcium phosphate in 20-0 cm<sup>3</sup> of aqueous solution of 1.0 x 10<sup>-3</sup> mol dm<sup>-3</sup> of methylene blue) and were rinsed in two changes of distilled water and then placed in Giemsa's stain. One percent Brilliant cresyl blue and 0.5 per cent Methylene blue were also used for staining microfilaria instead of Methylene blue phosphate solution. After eight to ten minutes, slides were washed in distilled water and microfilariae were studied under high power magnification.

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Among 500 cattle screened, 49 were found to be positive for microfilaria. Out of 49 cattle which were microfilaraemic, 32 were positive in wet film. 29 in Giemsa's stained blood smear, 44 in MKM and all 49 were positive in CSAM. Twenty-nine samples were positive in all methods (Table 1)

Among different stains, 0.1 percent methylene blue and Giemsa's stains were found to be the best to appreciate sheath and to study different structures of microfilaria with better clarity when compared to Methylene blue phosphate and one percent Brilliant cresyl blue.

All microfilariae observed were sheathed. They were long and slender with blunt anterior and tapering posterior ends. A transparent structureless sheath extending beyond the body proper was always present. Anteriorly, the sheath immediately enclosed the head leaving a very short sheath space. Cephalic end (head) was round to elongate and cephalic space was prominent. Nerve ring was little obliquely placed and was followed by an excretory apparatus consisting of an excretory pore and excretory cell. Central viscus (central body) was very long and clearly visible. Germ cells and anal pore were situated posteriorly. Tail was long and tapering gradually from anal pore

to its tip. At posterior end, caudal sheath extended well beyond tail for a variable length. Some microfilariae were found with separated sheath. Detailed morphometry of microfilariae is presented in Table 2. Species identification was made as per Sloss et al 1994.

Microfilariae assumed different shapes, some were either straight, coiled or forming a figure of "8" structure etc., and showed a distinct cephalic space and tapering caudal end. In one heavy microfilaraemic sample, microfilariae recovered by CSAM were clustered with tail ends together and formed a powder puff like appearance when examined immediately after recovery. Repeated Wet smear examination of blood samples preserved under refrigeration revealed presence of live microfilariae showing progressive undulating snake like movement up to sixth day of collection.

Motility patterns of microfilaria were better appreciated and it was much easier to spot motile microfilariae without any confusion with artifacts by CSAM since they were alive, as Saponin does not kill microfilariae and destroys only erythrocytes, whereas in MKM they were dead and

**Table 1**  
**Results of four different methods in the detection of microfilariosis of *Setaria* in cattle (n=49 positive samples)**

Method	No Positive	% Efficacy
Wet film examination	32	65.30
Giemsa stained blood smear examination	29	59.18
Modified Knotts method	44	89.79
Citrate Saponin acid method	49	100

$$\chi^2 = 10.92 (P \leq 0.05)$$

differentiation of microfilariae from fibrin threads and other artifacts was found to cause problems in identification. Number of microfilariae recovered was nearly three to ten times more in CSAM and it also detected all positive samples compared to MKM. The microfilariae stained by methylene blue phosphate in CSAM were not clearly appreciable,

but one percent brilliant cresyl blue or 0.5 per cent methylene blue gave good clarity.

Satish (1996) on evaluating efficacy of MKM, Wet smear examination and Giemsa's stained blood smear for diagnosis of Setarial microfilariosis

**Table 2**  
**Morphometry of microfilaria (in  $\mu\text{m}$ ) of *Setaria* species**

Criteria measured	Average measurement		
	<i>S.digitata</i>	<i>S.cervi</i>	<i>S.labiato-papillosa</i>
Length of sheath	298.53 $\pm$ 5.27	261.28 $\pm$ 4.22	240.66 $\pm$ 6.11
Width of sheath	9.03 $\pm$ 0.25	7.74 $\pm$ 0.24	6.8 $\pm$ 0.22
Length of microfilaria without sheath	277.05 $\pm$ 4.66	255.54 $\pm$ 3.96	233.7 $\pm$ 4.19
Width of microfilaria without sheath	8.83 $\pm$ 0.58	6.06 $\pm$ 0.19	5.8 $\pm$ 0.18
Length of cephalic space	7.182 $\pm$ 0.92	8.28 $\pm$ 0.56	6.98 $\pm$ 0.29
Length of caudal space	24.97 $\pm$ 2.10	25.72 $\pm$ 2.25	22.87 $\pm$ 1.99
Length from anterior extremity to nerve ring	45.26 $\pm$ 1.91	51.66 $\pm$ 1.93	28.9 $\pm$ 2.04
Length from anterior extremity to excretory pore	67.01 $\pm$ 1.86	70.79 $\pm$ 1.77	58.6 $\pm$ 2.23
Length from anterior extremity to excretory cell	81.472.10	78.67 $\pm$ 1.84	67.55 $\pm$ 1.99
Length from anterior extremity to central viscus	104.32 $\pm$ 2.33	123 $\pm$ 2.06	98.66 $\pm$ 2.77
Length from anterior extremity to first genital cell	130.49 $\pm$ 1.96	147.92 $\pm$ 2.55	104.55 $\pm$ 2.49
Length from anterior extremity to anal pore	155.16 $\pm$ 2.57	193.19 $\pm$ 3.28	147.9 $\pm$ 3.44
Length of nerve ring	4.62 $\pm$ 0.19	5.67 $\pm$ 0.22	4.06 $\pm$ 0.18
Length of central viscus	39.20 $\pm$ 2.11	23.03 $\pm$ 2.09	21.55 $\pm$ 2.77
Length of Tail	33.97 $\pm$ 1.86	42.07 $\pm$ 1.21	44.3 $\pm$ 1.91

1 ocular division = 1.46  $\mu\text{m}$

The average measurements are MEAN  $\pm$  SE of 30 individual observations

in buffaloes, concluded that efficacies were 100, 27.7 and 8.3 per cent, respectively. In the present study, efficacies of same methods were 89.79, 65.3 and 59.18 per cent, respectively.

Morphological features and measurements of microfilaria for the three common species viz., *S. digitata*, *S. cervi* and *S. labiatopapillosa* were in agreement with earlier reports Balasubramaniam et al. (1973), Sonin (1977), Sastry et al. (1985) and Satish (1996).

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