



Molecular Identification of Microfilariae in Native Cattle in Thepha District, Songkhla Province

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Background

Microfilariae are blood-borne nematode larvae transmitted by mosquito vectors and are widely distributed in tropical and subtropical regions. In cattle, adult worms typically reside in the peritoneal cavity. Infections are generally asymptomatic; however, aberrant larval migration to the central nervous system can result in paralysis. Morphological diagnosis has limited capacity to differentiate species, posing a challenge for identifying the specific species circulating within a region. Therefore, molecular techniques employing highly specific primers are required for accurate species identification, which is essential for the development of effective prevention and control strategies.

Objective

To identify microfilarial species in native cattle in Thepha District, Songkhla Province, based on the mitochondrial cytochrome c oxidase subunit 1 (*cox 1*) gene.

Methods

Blood samples were collected from three asymptomatic native cattle at a farm in Thepha District, Songkhla Province. All cattle had previously tested positive for microfilariae using Woo's method and Giemsa-stained blood smear examination. Genomic DNA was extracted from blood samples, and polymerase chain reaction (PCR) amplification targeting the *cox1* gene according to Casiraghi et al. (2001) was performed, followed by DNA sequencing. Primer list is shown in table 1. The obtained sequences were analyzed using BLASTn and compared with reference sequences in the NCBI GenBank database to confirm species identity.

Table 1. Lists of primers used in this study

Target gene	Primer names	Oligonucleotide primer (5' --> 3')	Product sizes (bp)
Mitochondrial cytochrome c oxidase subunit 1 (COX 1)	cox1intF	5'- TGATTGGTGGTTTTGGTAA -3'	682
	cox1intR	5'- ATAAGTACGAGTATCAATATC -3'	

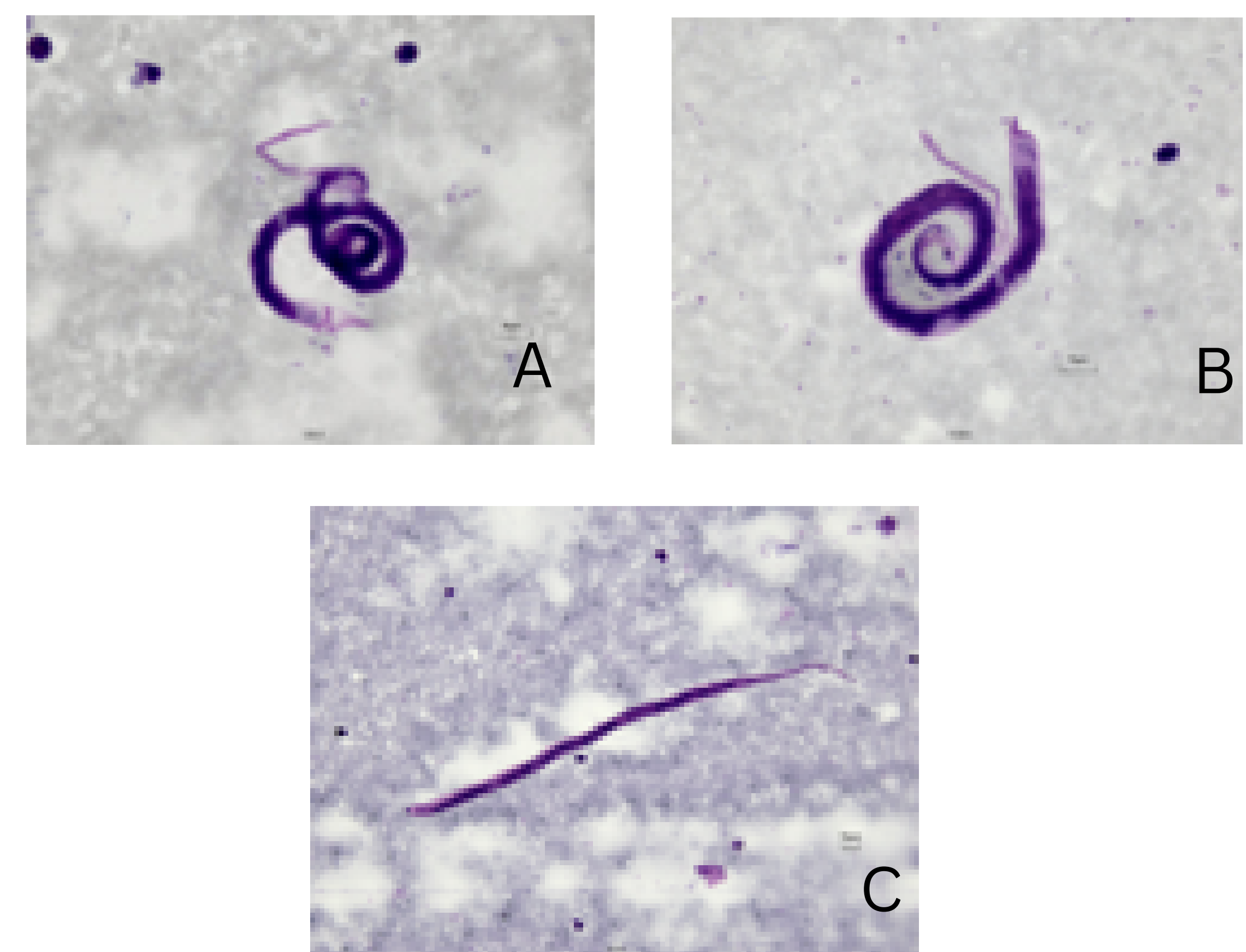
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Results

The morphology of microfilariae in Giemsa-stained blood smears is shown in Figure 1. An approximately 682 bp amplicon of the *cox1* gene was successfully obtained from all three microfilaria-positive samples. All sequences showed high similarity to GenBank entries assigned to *Setaria* spp. Two sequences exhibited 100% identity with *Setaria digitata* (GenBank accession no. KY284626). The third sequence showed high similarity to both *Setaria marshalli* (LC719467) and *Setaria labiatopapillosa* (NC044071), with sequence identities of 99.66% and 99.38%, respectively.

Figure 1. Morphology of microfilariae in Giemsa-stained blood smears. (A, B) *Setaria digitata* (100 % sequence similarity); (C) microfilariae closely related to *Setaria marshalli* (99.66 %) and *Setaria labiatopapillosa* (99.38 %).



Conclusion

Molecular techniques based on the *cox1* gene effectively identified microfilarial species in asymptomatic cattle; however, closely related species, *Setaria marshalli* and *Setaria labiatopapillosa*, remain difficult to distinguish. Integrating morphological analysis of adult worms would improve diagnostic accuracy. Despite the limited sample size, this study provides preliminary molecular evidence of *Setaria* diversity in Thepha District, Songkhla Province, and provides baseline data for future studies and surveillance.

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