Case Report

MOLECULAR CONFIRMATION OF *SPIROCERCA LUPI* INFECTION IN A GERMAN SHEPHERD DOG

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ABSTRACT

A six-year-old German shepherd dog was presented with the history of vomiting and anorexia. Live worms were recovered from the vomit and subjected to morphological and molecular identification. Morphologically the worm was identified as Spirocerca lupi and the molecular analysis revealed the 689bp amplicon specific to COX I gene of Spirocerca lupi. This communication records the first molecular report of Spirocerca lupi in dog from Cauvery Delta region of Tamil Nadu.

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INTRODUCTION

Spirocerca lupi is a globally distributed nematode of carnivores, particularly canids, with higher prevalence in warmer climates (Bailey, 1972). Infection occurs following ingestion of dung beetles carrying infective third-stage larvae or paratenic hosts such as birds, lizards and rodents (Soulsby, 1982). Adult worms localize in the esophagus and may cause dyspnea, regurgitation, vomiting, chronic disease or sudden death due to aortic rupture. Larval migration and prolonged tissue persistence produce characteristic lesions including esophageal nodules, granulomas, aortic scars and aneurysms, considered pathognomonic (Merwe *et al*., 2008). Early diagnosis is often difficult, with most cases detected only during advanced stages of disease. This report describes the morphological and molecular detection of *S. lupi* infection in a German Shepherd from the Cauvery Delta region of Tamil Nadu.

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MATERIALS AND METHODS

A 6-year-old GSD dog was presented to the Small Animal Referral Clinic of Veterinary Clinical Complex,Veterinary College and Research Institute, Orathanadu,

TANUVAS with the history of inappetence, vomiting and panting for the past 5 days. During clinical examination, the dog vomited with 2live red worms (Fig. 1). The worms were collected in 70 % ethanol and faecal sample were submitted to Department of Veterinary Parasitology, VCRI, Orathanadu for morphological identification. The worms were processed and cleared in lactophenol (Soulsby, 1982) and mounted in DPX for further morphological identification. DNA was extracted from the worms collected using the Qiagen DNeasy® tissue kit and stored at -20oC for future use. The COI gene was targeted in PCR using species-specific primers (FP: 5′-TGATTGGTGTTTTTGGGTAA-3′ ; RP: 5′-ATAAGTACGAGTATCAATATC-3′) under the following cyclic conditions with initial denaturation of 94°C for 5 min, denaturation at 94°C for 30 sec, annealing at 56°C for 30 sec, extension at 72°C for 30 sec for 36 cycles and final extension at 72°C for 5 min. The PCR amplicons were electrophoresed in 1.5% agarose gel and visualized under UV transilluminator.

RESULT AND DISCUSSION

The male worm measured approximately 3.5 cm in length and 0.8 mm in width, whereas the female measured 4.2 cm in length and 1.0 mm in width. The male worm exhibited a distinct hexagonal oral opening at the anterior end (Fig. 2), surrounded by six pseudo-lips. The oesophagus was divided into a short anterior muscular portion and a long posterior glandular segment with a total oesophageal length of approximately 3.1 mm. The posterior end of the male was ventrally curved, bearing prominent caudal alae with parallel longitudinal cuticular striations. Pre-and post-cloacal papillae also were evident. The copulatory structures consisted of two unequal spicules (Fig. 3). The left spicule was longer, slender, needle-shaped, mildly sclerotized and ended in a tongue-shaped (Fig. 4), knobbed distal tip with longitudinal grooves visible under magnification. In contrast, the right spicule was shorter and broader, terminating in a smooth, rounded tip. The female worm shared a similar anterior morphology with a hexagonal oral aperture and six pseudo-lips(Fig. 5). The posterior end of the female tapered to a conical tail with a subterminal anus and the vulva was located at the middle level of the body (Fig. 7); the reproductive system was didelphic with eggs in the uterus (Fig. 6). The eggs were gelatin capsule shaped having fully developed larvae inside the egg (Fig .8) with the morphometry of approximately 30–38 × 11–15 µm.The COX I gene was amplified with the amplicon size of 689 bp (Fig .9).

Spirocerca lupi is predominantly reported from warm climatic regions and has been documented in several states of India including Tamil Nadu (Anataraman and Krishna, 1966), Kerala and other southern regions (Ramachandran *et al*., 1984). The present study identified the parasite morphologically by the characteristic hexagonal oral opening with six pseudo-lips, divided muscular and glandular oesophagus and unequal spicules, consistent with the descriptions of Soulsby (1982) and Hoa *et al.* (2021). Transmission occurs through ingestion of infected dung beetles

(Scarabaeidae), while chickens, rabbits, lizards and rodents act as paratenic hosts harboring infective L3 larvae (Veeraselvam *et al.*, 2024). The persistence of larvae in these hosts contributes to the maintenance and spread of canine spirocercosis(Merwe *et al*., 2008). Clinical manifestations commonly include dysphagia, regurgitation, vomiting, weight loss and dyspnea, whereas aberrant migration may occasionally result in haemothorax, paraparesis and aortic thromboembolism (Merwe *et al.*, 2008; Dvir *et al.*, 2001).

The dog was managed with a combination of supportive, antiemetic, antimicrobial and specific anthelmintic therapy. Fluid therapy was instituted using isotonic crystalloids to correct dehydration and maintain electrolyte balance. Antiemetic therapy was initiated with Ondansetron administered at 0.2 mg/kg body weight intravenously twice daily for five days to control vomiting. Gastroprotective therapy included pantoprazole at 1 mg/kg body weight intravenously once daily, followed by oral administration and Prednisolone was given at 0.5 mg/kg body weight orally once daily for five days to reduce peri-nodular inflammation. Broad-spectrum antimicrobial coverage was provided using amoxicillin-clavulanate at 15 mg/kg body weight administered daily intravenously for seven days to prevent secondary bacterial infection. Specific therapy against spirocercosis was initiated using ivermectin at the dose rate of 600 µg/kg body weight subcutaneously at weekly intervals for a minimum duration of eight weeks.The animal was maintained on a soft, easily digestible diet offered in small frequent feedings to minimize esophageal irritation. Clinical response was monitored based on reduction in vomiting, improvement in appetite and general condition and the treatment was advised to be continued until complete clinical and radiographic resolution of lesions to prevent recurrence. This case highlights the morphological and molecular confirmation of *S. lupi* infection in a dog from Cauvery delta region of Tamil Nadu.

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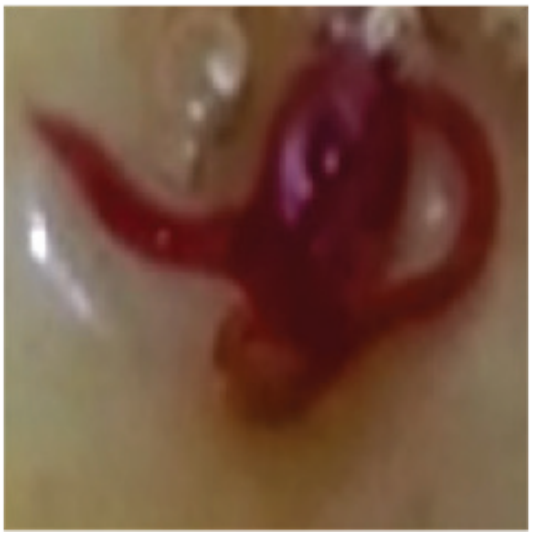

Fig. 1. Worms collected in the vomitus of dog

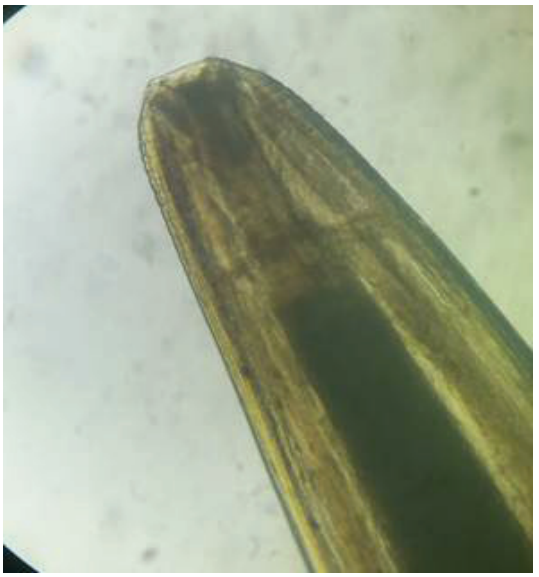

Fig. 2. Head end – Male – S. lupi(x100)

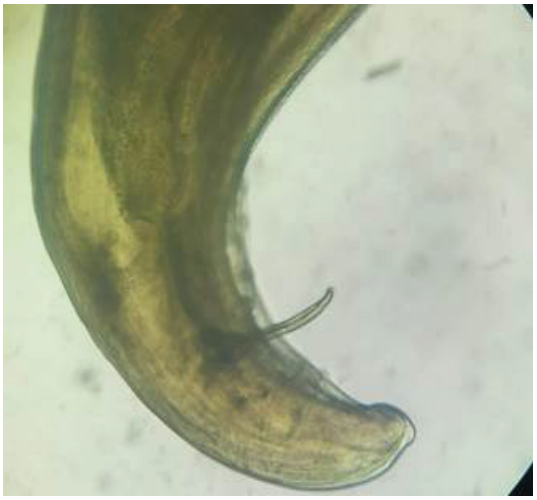

Fig. 3. Posterior end of male S. lupi with spicules (arrow) (x100)

Fig. 4. Left spicule slender, needle-shaped, mildly sclerotized, with a tongue-shaped distal end (x400)

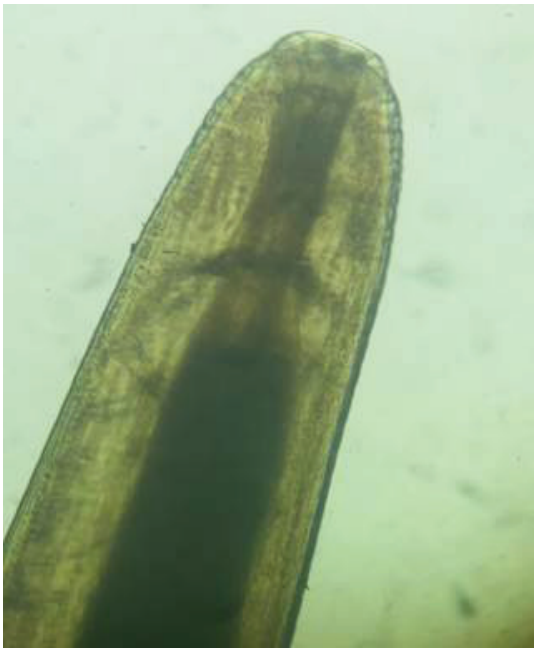

Fig. 5. Head end – Female – S. lupi(x100)

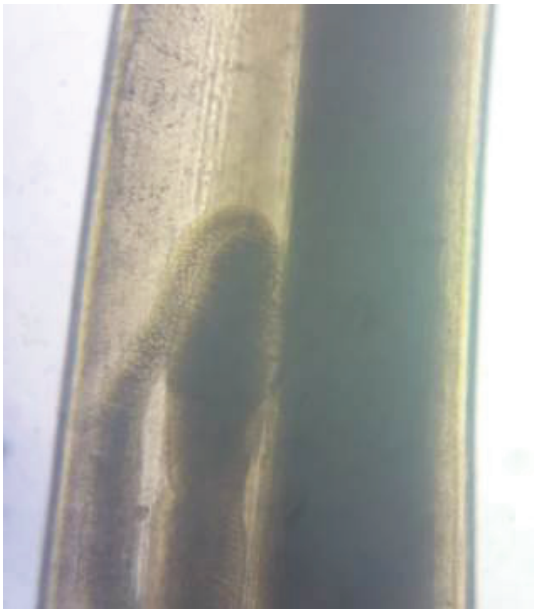

Fig. 6. Uterus with eggs (arrow) – Female – S. lupi(x100)

Fig. 7. Tapered conical tail with a subterminal anus – Female – S. lupi(x100)

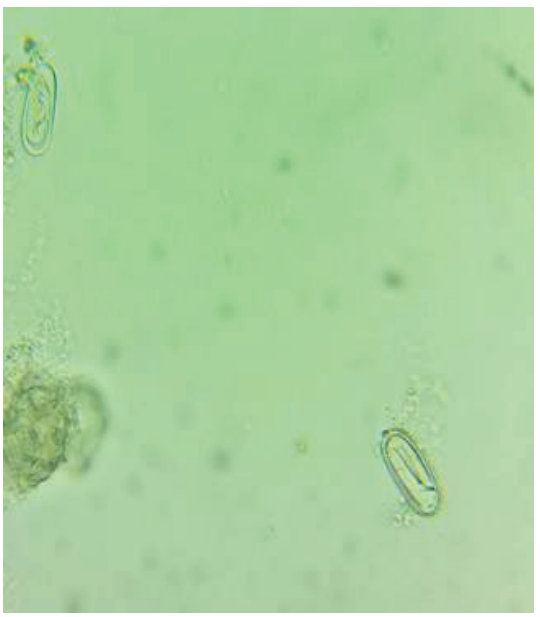

Fig. 8. Gelatin capsule shaped eggs with larva inside in Dog faeces(x400)

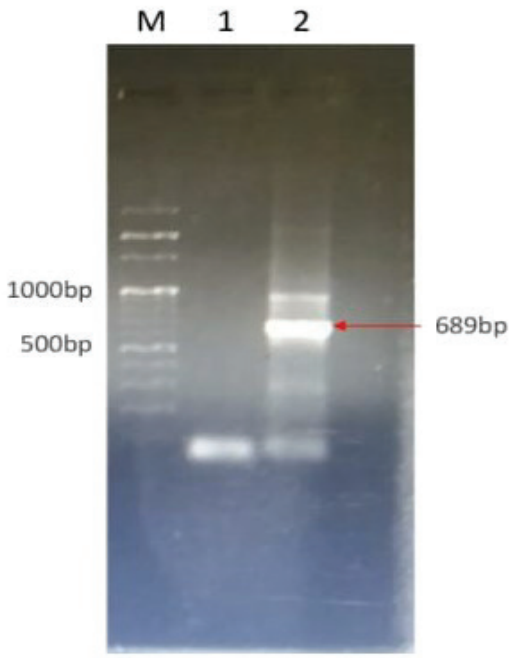

Fig. 9. Agarose gel (1.5%) electrophoresis of amplified DNA using species-specific PCR.
M: 100 bp DNA ladder; Lane 1: Negative control, Lane 2: Amplification of 689 bp fragment of mitochondrial cytochrome c oxidase I gene of Spirocercalupi

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