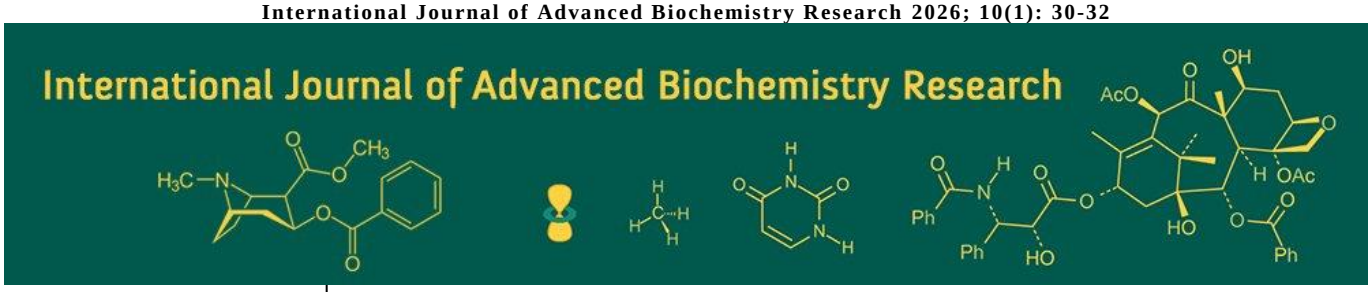

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Morphological identification of Gastrointestinal Nematode L3 Larvae in small ruminants

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Abstract
Gastrointestinal (GI) nematode infections remain one of the major constraints in small ruminant production, particularly in tropical regions. This study aimed to identify the third-stage (L3) larvae of strongyle nematodes recovered from sheep and goats in selected districts of Tamil Nadu using coproculture and morphological keys. A total of 24 farms across Pudukkottai, Sivagangai and Tiruchirapalli districts were included, and pooled faecal samples from each farm were subjected to coproculture. The infective larvae were identified based on established morphological characteristics. The predominant genera detected was *Haemonchus*. In addition to *Haemonchus, larvae of Trichostrongylus, Oesophagostomum* and *Bunostomum* were also detected, indicating that mixed strongyle infections are common under extensive and semi-intensive farming systems. The study highlights the distribution of GI nematode larvae in small ruminants and emphasizes the role of coproculture-based morphological identification in epidemiological investigations under field conditions.

Keywords: Coproculture, L3 larvae, Gastrointestinal nematodes, Sheep, Goats, Morphology

Introduction
Gastrointestinal nematode infections are of considerable importance in sheep and goat production worldwide. These parasites are major limiting factors of small ruminant productivity, leading to reduced weight gain, poor body condition, decreased milk and meat production and, in severe cases, mortality (Coop and Holmes, 1996; Urquhart *et al*., 1996; Pugh and Baird, 2012) [2, 10, 7]. Among the strongyle nematodes, *Haemonchus contortus* is one of the most pathogenic species owing to its blood-feeding habit, high fecundity and ability to rapidly contaminate pasture (Vlassoff and McKenna, 1994; Nginyi *et al*., 2001) [14, 6]. Accurate identification of gastrointestinal nematode species is essential for understanding parasite epidemiology and for designing appropriate control strategies (Coles *et al*., 2006) [1]. Traditionally, identification of gastrointestinal nematodes has been based on the morphological examination of adult worms recovered at necropsy (Soulsby, 1971) [9]. However, slaughter of animals solely for diagnostic purposes is neither practical nor routinely feasible. Routine faecal examination allows the detection of strongyle-type eggs, but these eggs are morphologically similar and do not permit differentiation of nematode genera. To overcome this limitation, coproculture is employed to obtain infective third-stage (L3) larvae, which can be identified to the genus level using established morphological keys. (Van Wyk *et al.*, 2004; Van Wyk and Mayhew, 2013) [12, 11].
Coproculture facilitates the isolation of infective third-stage (L3) larvae from faecal samples for subsequent microscopic identification. Allowing differentiation of major strongyle genera and remains a practical, cost-effective tool, particularly in resource-limited settings. Although molecular techniques provide higher precision, larval morphology still forms the basis of most routine epidemiological studies (Heise *et al.*, 1999; Veena *et al*., 2020) [3, 13].
In Tamil Nadu, small ruminant farming contributes significantly to rural livelihoods. However, data on the morphology-based identification of infective L3 larvae circulating in sheep and goat flocks remain limited. The present study was therefore undertaken to identify gastrointestinal nematode L3 larvae in sheep and goats from selected districts of Tamil Nadu using coproculture and and identified according to their morphological characteristics.

Materials and Methods

Study Area

The study was carried out in Pudukkottai, Sivagangai and Tiruchirappalli districts of Tamil Nadu, India. The selected blocks included Aranthangi and Viralimalai (Pudukkottai), Ilayangudi and Sivagangai (Sivagangai), and Musiri and Lalgudi (Tiruchirapalli). The climate of the region is predominantly tropical, suitable for the development and survival of gastrointestinal nematodes. Small ruminant rearing in these districts is largely extensive or semi-intensive.

Farm Selection and Sample Collection

A total of 24 farms (twelve sheep and twelve goat farms) were selected. From each farm, faecal samples were collected from 10 animals and pooled to obtain a composite sample. Pooled sampling was adopted to obtain a representative composite sample from each flock for coproculture and morphological identification of gastrointestinal nematode larvae.

Coproculture

Coproculture was performed following the method described by Sathianesan and Peter (1970) [8]. Fresh pooled faecal samples were crushed and mixed with water to achieve suitable moisture content. The cultures were incubated at room temperature (25-28 °C) for 7-10 days. Infective larvae were recovered from the culture surface and condensation fluid using a Pasteur pipette.

Morphological Identification of L3 Larvae

Larval suspensions were examined microscopically after adding a drop of 1 % iodine staining solution. Observations were made under 10× and 40× magnification. Identification of larvae was carried out using standard morphological keys described by Van Wyk *et al*. (2004) [12] and Van Wyk and Mayhew (2013) [11]. The diagnostic features considered included head shape, oesophageal length, sheath tail extension (STE), tail morphology and presence or absence of a terminal filament.

Results

Coproculture of pooled faecal samples from 24 farms yielded infective third-stage (L3) larvae belonging to multiple gastrointestinal nematode genera. *Haemonchus* was the predominant genus detected across all farms, while larvae of *Trichostrongylus*, *Oesophagostomum* and *Bunostomum* were also detected in lower proportions. Larvae identified as *Haemonchus contortus* were characterized by a bullet-shaped head and a long sheath tail extension with a short terminal filament and a typical kink near the tail tip (Fig1). *Trichostrongylus colubriformis* larvae showed a rounded head, absence of a terminal filament and a sharply tapered sheath tail extension (Fig 2). *Oesophagostomum columbianum* larvae were identified by their square-shaped head and long caudal filament (Fig 3). *Bunostomum trigonocephalum* larvae exhibited a small body size, bullet-shaped head and uniform iodine staining (Fig 4). *Strongyloides papillosus* larvae were occasionally observed and were identified by their long oesophagus and characteristic forked tail (Fig 5). Mixed infections were common, with *Haemonchus* larvae dominating both sheep and goat flocks. Sheep farms showed a relatively higher proportion of *Haemonchus* and *Trichostrongylus* larvae, whereas goats exhibited more frequent recovery of *Oesophagostomum* larvae.

Fig 1: *Haemonchus contortus* L3 larvae: A. Cranial end showing bullet shaped head B: Caudal end showing short filament with kinked tail

Fig 2: *Trichostrongylus columbroormis* L3 larvae: A. Cranial end showing round head B: Caudal end without filament

Fig 3: *Oesophagostomum columbianum* L3 larvae: Caudal end showing long filament

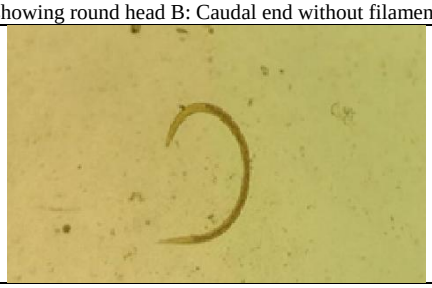

Fig 4: *Bunostomum phlebatomum* L3 larvae: Cranial end showing bullet shape, Caudal end showing tail extension with filament

Fig 5: *Strongyloides Papillosus* L3 larvae: One third the length of oesophagus

Discussion

The present study demonstrated that *Haemonchus contortus* was the predominant gastrointestinal nematode species affecting sheep and goats in the selected districts of Tamil Nadu. This finding is consistent with previous reports from tropical regions, where *Haemonchus* spp. dominate owing to favourable environmental conditions and their high biotic potential (Nginyi *et al*., 2001; Keyyu *et al*., 2002; Kumsa and Abebe, 2009; Veena *et al*., 2020) [6, 4, 5, 13]. The dominance of *H. contortus* is of major concern due to its blood-feeding nature and ability to cause severe anaemia and production losses (Coop and Holmes, 1996; Pugh and Baird, 2012) [2, 7]. Haemonchus was the predominant genus detected. In addition to *Haemonchus*, larvae of *Trichostrongylus*, *Oesophagostomum* and *Bunostomum* were also detected, indicating that mixed strongyle infections are common under extensive and semi-intensive farming systems. Although these genera were less abundant than *Haemonchus*, their presence reflects the diversity of nematode fauna circulating in the study area and their potential contribution to subclinical and chronic production losses (Vlassoff and McKenna, 1994) [14]. The occasional detection of *Strongyloides papillosus* suggests environmental contamination, particularly in moist soil and housing conditions, and may be of greater importance in young or immunocompromised animals (Soulsby, 1971; Pugh and Baird, 2012) [9, 7]. From a methodological point of view, infective third-stage (L3) larvae were isolated by coproculture and identified solely on the basis of their morphological features. The present findings support earlier studies indicating that coproculture combined with larval morphology is an effective and economical method for identifying major gastrointestinal nematode genera under field conditions (Van Wyk *et al*., 2004; Van Wyk and Mayhew, 2013; Veena *et al*., 2020) [12, 11, 13]. While morphological identification has limitations in distinguishing closely related species, it remains a practical approach for routine epidemiological surveys. The predominance of *H. contortus* observed in this study highlights the need for regular monitoring of gastrointestinal nematodes and implementation of strategic parasite control measures in Tamil Nadu. Adoption of targeted selective treatment, improved grazing management and farmer awareness programs may help reduce parasite burden and improve small ruminant productivity.

Conclusion

The study revealed that *Haemonchus contortus* is the predominant gastrointestinal nematode infecting sheep and goats in selected districts of Tamil Nadu. Coproculture-based morphological identification proved to be a practical and effective tool for determining the composition of infective L3 larvae under field conditions. Continuous surveillance and integrated parasite management strategies are essential for sustainable small ruminant production.

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