Veterinary Letters
ISSN 2581-2416
DOI: https://dx.doi.org/10.29244/avl.9.4.113-114
https://journal.ipb.ac.id/index.php/arshivetlett

Molecular detection of *Haemonchus contortus* on spotted deer (*Axis axis*)

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ABSTRACT: Spotted deer (*Axis axis*) as a protected endemic animal has the potential to be developed to provide economic value. However, in general, deer are often infected by endoparasitic diseases caused by various factors such as direct contact with infected areas to transmission through feed. As a result of endoparasitic infections, infected animals can experience malnutrition, physiological disorders, and even death. This study aims to identify nematode worm species using molecular diagnosis in spotted deer. Molecular identification of nematode species in deer feces was carried out by fecal sample collection and cultivation, larvae isolation, DNA extraction, PCR process, and electrophoresis. Molecular analysis using specific primer targeting the ITS gene. The PCR results showed the single band at 260 bp. Based on the result, the nematode species that has infected the spotted deer was *Haemonchus contortus*. These findings provide the first molecular-level confirmation of endoparasite infection in spotted deer in Indonesia, expanding the existing diagnostic information for wildlife health monitoring.

Keywords:
deer, *Haemonchus contortus*, PCR, infection, endoparasites

■ INTRODUCTION

Deer are ruminant species that are highly susceptible to endoparasitic infections, which are a major health concern in wild and captive populations. Endoparasites reside within the host body and may be transmitted through infected animals or contaminated water (Hartono *et al.* 2019). The shared grazing areas of wild ruminants and livestock increase the risk of pasture contamination with infective nematode larvae, enabling cross-species transmission (Wirawan *et al.* 2019).

Haemonchus contortus is one of the most prevalent gastrointestinal nematodes infecting small ruminants in Indonesia, accounting for 80% of the reported infections (Yuswandi & Yuniar 2015). The hematophagous activity of this parasite in the abomasum causes anemia, reduced growth, production losses, and mortality in cases of heavy infection. Information on the occurrence and identification of *H. contortus* in wild ruminants, particularly spotted deer, remains limited.

Recent advances in molecular diagnostics have highlighted the reliability of PCR in identifying parasitic nematodes at the species level. However, no studies in Indonesia have reported the molecular identification of parasitic nematodes in spotted deer using PCR-based techniques. This study aimed to identify the parasitic nematode species infecting spotted deer using molecular approaches.

■ MATERIALS AND METHODS

Sample collection, larval culture, and isolation: Fecal samples were collected from seven spotted deer (one male and six females) at Waru Land, Ciampea, Bogor, Indonesia. All procedures were approved by the Institutional Animal Ethics Committee (109/KEH/SKE/IX/2023). The samples were examined and cultured for infective larvae following Shaikenov *et al.* (2004) and Hambal *et al.* (2016).

Third-stage larvae (L3) were recovered using the Baermann technique, washed with distilled water, and isolated in 0.2 mL microcentrifuge tubes with 10 µL distilled water. One larva was stained with Lugol's iodine for identification, and the other was reserved for molecular analysis. The positive control used L3 larvae from cultured Haemonchus contortus from local slaughterhouses.

DNA extraction: Genomic DNA was extracted using lysis. 10 µL of lysis solution (25 µL Direct PCR buffer, 1 µL of 1 M DTT, and 1 µL proteinase K) was added to each larval tube. Samples were incubated at 60°C for 60 min, then at 95°C for 10 min for enzyme inactivation.

PCR amplification used *H. contortus*-specific primers: forward HcBotuF1 (5′-TGT CGA ACA AAC TCG TC-3′) and reverse HcBotuR2 (5′-TGT GTC TCT ACC GCC CGA GT-3′), as per Amarante *et al.* (2017). Each 30 µL PCR reaction included 1.5 µL of each primer, 15 µL GoTaq Green Master Mix, 9 µL nuclease-free water, and 3 µL DNA template.

Received: 21.08.2025 | **Revised:** 26.09.2025 | **Accepted:** 30.09.2025

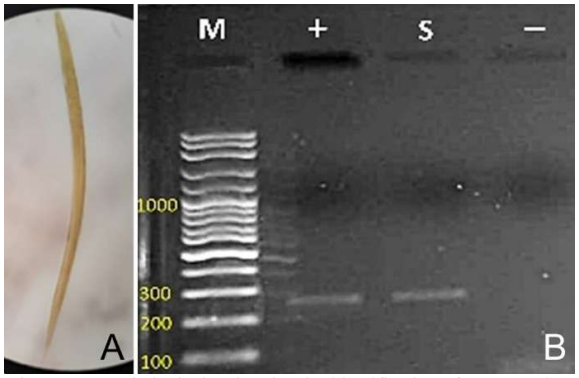

Figure 1. Morphological and molecular identification of *Haemonchus contortus* larvae from spotted deer. (A) Infective third-stage larvae (L3) from faecal cultures. (B) Agarose gel electrophoresis of PCR products showing the 260 bp amplicon of *Haemonchus contortus*. M, DNA ladder; +, positive control; S, sample; −, negative control.

The PCR conditions: denaturation at 95°C for 2 min, followed by 40 cycles of denaturation at 95°C for 15 s, annealing at 55°C for 15 s, and extension at 72°C for 15 s, with final extension at 72°C for 7 min. Products were separated by electrophoresis on 1% agarose gel in 1× TAE buffer. Using DM2100 ExcelBand 100 bp DNA ladder as marker, electrophoresis was run at 100 V for 25 min. Positive amplification was confirmed by bands at 260 bp.

■ RESULTS AND DISCUSSION

The results of larval isolation from fecal cultures are presented in Figure 1(A). Microscopic examination revealed larvae with elongated cylindrical bodies, enlarged anterior regions with narrow, rounded heads, and tapered tails. The infective third-stage larvae (L3) showed characteristic features, including a defined cuticle, esophagus, intestine, and protective sheath, which were consistent with the genus Haemonchus. These observations align with those of Anggraini (2010), who noted that infective Haemonchus larvae are elongated and enveloped by a protective sheath that enables survival and mobility during environmental migration.

The results of the PCR amplification and agarose gel electrophoresis are shown in Figure 1(B). The L3 larval isolate produced a distinct DNA band corresponding to 260 bp, which was identical in size to that observed in the positive control. This amplicon size is consistent with the molecular identification of *Haemonchus contortus* using the same primer set, as reported by Amarante *et al.* (2017). In contrast, no amplification was observed in the negative control, indicating the absence of contamination during the PCR process and confirming the reliability of molecular analysis.

The successful amplification of the target fragment demonstrated the high specificity of the primers for *H. contortus*, supporting earlier findings that these primers consistently amplify DNA from samples containing this species (Amarante *et al.* 2017). Molecular confirmation of *H. contortus* is particularly relevant given the high level of genetic diversity exhibited by this parasite, which enables its adaptation to diverse climatic conditions and a wide range of host species (Laing *et al.* 2013).

Haemonchus contortus infects small ruminants in various climatic regions (Besier *et al.* 2016). Studies have documented this parasite in several ruminant species, including red deer in New Zealand; however, sheep and goats remain the primary hosts (Tapia-Escárate *et al.* 2019). The detection of *H. contortus* in spotted deer in Indonesia highlights the potential role of wild ruminants as reservoir hosts, which may enable cross-transmission of parasites to domestic livestock. These findings emphasize the need for wildlife health surveillance in parasite control strategies, particularly in areas where wild and domestic ruminants share grazing resources.

■ CONCLUSION

PCR methods with HcBotuF1 and HcBotuR2 primers can be used to help diagnose *Haemonchus contortus* infection in deer.

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