Copro-polymerase chain reaction for molecular identification of *Haemonchus contortus* in goats

L. M. Thamilbharathi[1], R. Radhika[2*], M. N. Priya[2], Binu K. Mani[3], K. Anbarasu[1] and K. Devada [4]

Department of Veterinary Parasitology,
College of Veterinary and Animal Sciences, Mannuthy, Thrissur- 680 651
Kerala Veterinary and Animal Sciences University
Kerala, India.

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Abstract

Haemonchus contortus commonly called as stomach worm or wire worm of ruminants inhabit the abomasum and is considered as one of the economically important gastrointestinal strongyles in goats. In the present study, H. contortus was identified by PCR using the primers targeting partial 5.8S and partial internal transcribed spacer region 2 (ITS-2). Adult worms were identified morphologically and genomic DNA was extracted using DNeasy Blood and Tissue kit (QIAGEN, Germany). Gradient PCR protocol was standardised using the extracted genomic DNA. Ten-fold serial dilution of adult DNA was used to analyse the minimum detection limit and the products were amplified upto tenth dilution. Cross reaction of primer sets was checked using the DNA extracted from predominant adult strongyles like Oesophagostomum columbianum and Trichostrongylus colubriformis and no cross reaction was seen at the optimum annealing temperature (60.7°C)

Keywords: *Haemonchus contortus, goats, PCR, ITS-2*

Short Running Title: Molecular identification of *Haemonchus contortus* in goats

1. MVSc scholar
2. Assistant Professor
3. Assistant Professor, Department of Veterinary Microbiology
4. Professor and Head
**Corresponding author: thamil.vet93@gmail.com Ph. 9500387316*

Haemonchus contortus belong to Trichostrongylidae family and are commonly called as stomach worm or wire worm of ruminants. The adult worms attach to the abomasal mucosa of small ruminants and due to its haematophagus nature it causes anaemia, jowl oedema and even death in young ones. Adult worms are identified based on morphological features. But identification of nematode species based on features of strongyle egg is difficult during coprological examination. Coproculture aided in species identification but it takes seven to ten days to identify the infective larvae (Fletcher, 1993). Hence, molecular identification was undertaken in this study as a tool for species level identification.

Table 1. Composition of reaction mix for PCR to identify infective larvae of *H. contortus*

Components	Quantity (µL)
10 X PCR buffer (without $MgCl_2$)	2.5
dNTP (10 mM each)	0.50 (200µM each)
Primer forward	1 (10 pmol)
Primer reverse	1 (10 pmol)
Magnesium chloride (25 mM)	1.50 (1.5mM)
Taq polymerase (5 IU/µL)	0.20 (1U)
DNA template	5
Nuclease free water	13.3
Total	25

After performing gradient PCR, the amplicons were subjected to agarose gel electrophoresis in 1.5 per cent agarose gel at 80V, 400mA for 35 min and the gel was visualised in Gel Doc^{TM} EZ imager and documented using Image lab software. The amplicons were purified and sequenced at AgriGenom labs private limited, Cochin using Sanger's di-deoxy nucleotide chain termination method.

Male tail end had well developed bursa with elongate lateral lobes which was supported by an asymmetrical dorsal lobe. Dorsal lobe was placed on the left lateral lobe which was supported by an inverted Y shaped dorsal ray. Whereas, female had barber's pole appearance which was due the coiling of white ovaries wound around the red intestine (Soulsby, 1982). *Haemonchus contortus* has been identified as the predominant strongyle species in goats in different places including Kerala (Deepa, 2005), North-West India (Kumar *et al*., 2008) Malaysia (Chandrawathani *et al*., 2009), Kashmir (Irfan-ur-Rauf-Tak *et al*., 2013) and Ethiopia (Chalchisa *et al*., 2015).

Sensitivity of *H. contortus* primer sets was checked using ten-fold serial dilution and the ability of primers to amplify minimum DNA concentration was analysed. The initial concentration of DNA used for sensitivity study was 4.7 ng/µL and ten-fold serial dilution was performed. PCR products were amplified upto tenth dilution which showed that the primer pairs could amplify DNA with minimum concentration of 4.7 ag/ µL (attogram per microlitre) (Fig. 1.).

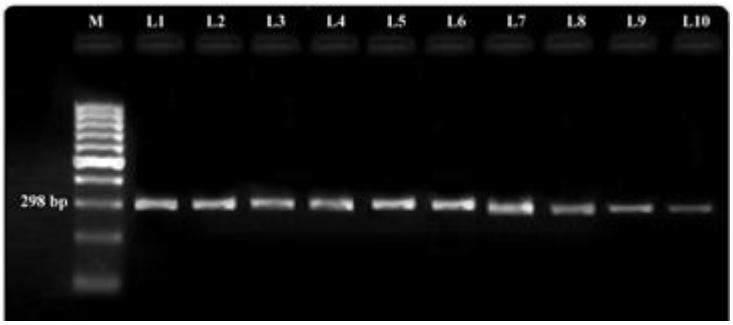

Fig. 1. Amplicons of *H. contortus*
Lane M: 100 bp ladder
Lane 1-10: Ten-fold serial dilution *H. contortus* DNA

The specificity of primer was cross checked with DNA of other important strongyles like *Oesophagostomum columbianum* and *Trichostrongylus colubriformis* to detect the cross amplification between species.

Summary

The study forms the basis for developing copro-polymerase chain reaction for detecting *H. contortus* infection in goats. Specific detection of this pathogen from clinical samples would aid in initiating timely control measures.

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Conflict of interest - The authors declare that they have no conflict of interest.

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