Indian Journal of Animal Sciences **95** (1): 13–17, January 2025/Article
https://doi.org/10.56093/ijans.v95i1.157162

PCR based identification of different strongyle species in goats of western Uttar Pradesh, India

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Received: 23 September 2024; Accepted: 18 November 2024

ABSTRACT

Strongyle infection in goats poses a significant threat to the agricultural industry leading to substantial economic losses through decreased productivity and increased mortality rates. The current study's goal is to determine the molecular- based methods for identification of different kind of strongyle eggs from naturally infected goats from four selected districts of Western Uttar Pradesh. A total of 360 faecal samples were collected, with 32.22% testing positive for strongyle infection. The positive faecal samples were pooled separately and subjected to larval culture, followed by DNA extraction from the L3 larvae. Polymerase chain reaction (PCR) analysis of the ITS-2 region of the rDNA confirmed the presence of *Haemonchus contortus*, *Trichostrongylus* and *Oesophagostomum* genera. The prevalence rates of *Haemonchus contortus*, *Trichostrongylus* spp. and *Oesophagostomum* spp. were determined to be 54.16%, 31.66% and 14.16% respectively. The finding of this study highlighted *Haemonchus contortus* as the predominant species among the identified strongyle spp. These findings provide valuable insights into the distribution of different types of strongyle infection in goats, which can help in management of parasites and their control in goats.

Keywords: Faecal culture, Goat, *Haemonchus*, *Oesophagostomum*, PCR, *Trichostrongylus*

Goat farming in rural areas has the potential to significantly reduce poverty and create employment opportunities for small, marginal and landless farmers (Kochewad *et al.* 2023). Goats are commonly suffering from gastrointestinal parasites leading a significant economic loss in term of reduced body weight gain and mortality (Das *et al.* 2017). India ranks first globally in goat milk production and second in goat meat and skin production. Furthermore, 75% goats are reared by the marginal and landless households with less than 2.0 hectare of land (Tripathi *et al.* 2020). The impact of Strongyle infections depends on the degree of infection and other associated risk factors like species, age, season and intensity of worm load (Singh *et al.* 2016). Gastrointestinal nematode (GIN) infection is a major problem leading to economic losses in ruminant farming. Various gastrointestinal parasites (GIPs) including nematodes, cestodes, trematodes, and protozoa affect goats (Paul *et al.* 2016). The species of strongyles, such as *Haemonchus contortus*, *Trichostrongylus colubriformis, Trichostrongylus axei* and *Oesophagostomum columbianum* cannot be differentiated from each other on the basis their morphological characters of eggs (Airs *et al.* 2023). According to Nisbet *et al.* (2016), *Haemonchus contortus* is a significant and globally distributed strongyle species that is present in sheep and goats.

Traditional faecal examination methods for identifying eggs or larvae based on morphology can be challenging for species differentiation due to identical or minor variations in morphological features. Molecular methods are applied for the confirmation of species of strongyle worm based their genetic materials (Nisbet *et al.* 2016). Although morphometric- based identification is more economical, molecular methods are still the gold standard for accurately identifying nematode species (Nath *et al.* 2021). Because of its high intra-specific sequence divergence, the ITS2 is frequently used to identify different strongyle spp. (Sato *et al.* 2014). There is little information available for morphological identification of diagnostic stages of strongyle species in goats. The current study used PCR techniques with DNA extracted from larvae samples to identify strongyle nematodes at the genus and species level in goats from western Uttar Pradesh, India that had similar morphological characteristics.

MATERIALS AND METHODS

Study area and sample collection: The climate of this region is sub-tropical with maximum temperature of about

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42^0C during summer (April to October) and minimum temperature of about 7^0C during winter (November to March). The monsoon generally begins during the last week of June and ceases by the end of September. The average annual rainfall in this region is about 862.7 mm and the annual relative humidity varies from 67 to 83%. During March 2023 to February 2024, a total of 1117 goat faecal samples were collected one time from four districts namely Meerut (312), Baghpat (300), Saharanpur (248), and Muzaffarnagar (257) from western regions of Uttar Pradesh, India. Macroscopically, the color, consistency, odor, and presence of immature and adult worms in the faeces were assessed. Fifty grams of faeces were collected from each goat, placed in plastic bags, stored in icebox and transferred to Department of Veterinary Parasitology for analysis of parasitic eggs and larvae. The of faeces of goat were examined under 10× and 40× objectives of a compound microscope (Nikon Eclipse E200, Tokyo, Japan).The strongyle eggs were identified based on their distinctive morphological features (Soulsby 1982).

Faecal examination: The faecal samples were examined by direct faecal wet smear and faecal flotation technique (Soulsby 1982).

Coproculture: Coproculture was performed according to methods described by Roberts and Sullivan (1950) for positive samples. In shorts, around forty (40) grams of faecal samples were finely crushed with a mortar and pestle and along with a glass rod to obtain the required consistency. The appropriately textured feces were then placed evenly in a small petri dish and it was placed in a comparatively larger petridish containing a small quantity of water. The big petridish was placed under another petridish to reduce evaporation and kept in a BOD incubator (Biogen Scientific) at 27ºC for 7 days. The larvae that had moved after hatching were collected from the water of a larger petri dish. At the end of 7 days L3 stage of larvae was recovered from petridish after incubation (Muchiut *et al.* 2021). The infectious larvae that moved to the water on the outside of the petri dish were removed using a pipette and then centrifuged at 1500 rpm for 3 minutes. The sediment with larvae was utilized for extracting DNA.

Molecular characterization: DNA template preparation: Five larvae from the sediment were selected for DNA extraction. The larvae were exsheathed by incubating them in a 3.5% sodium hypochlorite for 20 minutes. The exsheathed larvae were then rinsed 3 to 5 times in distilled water before undergoing DNA extraction (Veena *et al.* 2020).

DNA extraction: Genomic DNA was extracted from the infective larvae with the help of QIAamp® Blood Mini Kit (Qiagen) according the manufacturer's instruction with some slight changes like freeze and thaw. The DNA was kept at -20°C after extraction for identifying the genus and species of the strongyle worm.

PCR amplification: PCR amplifications were conducted using a thermocycler (Bio-Rad). The 25 µL reaction mixture comprised of 12.5 µL Master mix (Promega), 1 µL of 10 pmol of each primer (Xploregen Discoveries Pvt. Ltd. Bengaluru, Karnataka, India), 2 µL of DNA template and 9.5 µL of nuclease free water was prepared for amplification. To visualize the amplicon 5 µL of PCR product and 10 µL of 100 bp ladder (BR Biochem, New Delhi) were loaded into 1.5% agarose gel electrophoresis unit using TAE buffer (Bio-Rad, Japan) and ethidium bromide (0.5µg/ mL of gel from 10mg/mL of stock). The gel was then run at 90 Volts for duration of 60 minutes in horizontal gel electrophoresis (HiMedia, Mumbai). The resolution pattern was analyzed using Gel Doc (Bio-Rad, Molecular Imager® Gel Doc™ XR). Details of primer against targeted genes with cyclic conditions are given in table 1.

RESULTS AND DISCUSSION

A total of 1117 faecal samples were screened using direct faecal wet smears and flotation methods to identify different types of strongyle eggs. Out of 1117 samples, 360 (32.22%) faecal samples were positive for strongyle eggs. In the current study, the overall prevalence of strongyle infection in goats was recorded as 32.22%. Similar findings were also observed previously as 31% in 5 agroecological regions of Zimbabwe during both dry and wet seasons (Zvinorova *et al.* 2016), 32.63% prevalence in two districts of Meghalaya of India (Das *et al.* 2017), 26.63% prevalence

Table 1. Primers, targeted genes, cycling conditions and product size

Parasite	Primer sequence	Target gene	Cycling conditions	Product size
H. contortus	F: 5' CAAATGGCATTTGTCTTTTAG 3' F: 5'TTAGTTTCTTTTCCTCCGCT 3'	ITS-2 (Bott *et al.* 2009)	94˚C, 30s 55˚C, 30s 72˚C, 30s 35 cycles	265 bp
Trichostrongylus spp.	F:5'CACGAATTGCAGACGCTTAG3' R: 5' CTAAATGATATGCTTAAGTTCAGC 3'	ITS-2 (Waghorn *et al.* 2013)	12 cycles 94˚C ,15s 60˚C, (decreasing by 0.5˚C per cycle) 15s 72˚C, 30s Then 25 cycles 94˚C 15s 54˚C,15s, 72˚C, 30s	398 bp
Oesophagostomum spp.	F: 5' TCG ACT AGC TTC AGC GAT G 3' R: 5' CCA AAG CAT TCT TAG TCG CT 3'	ITS-2 (Kumar *et al.* 2018)	94˚C, 30s 53˚C, 30s 72˚C, 30s 36 cycles	333bp

in AnLemo, Hadiya Zone Southern Ethiopia (Sebro *et al.* 2022). Contrary to present findings, the higher prevalence of Strongyle was reported previously as 97.5% in Uganda (Kalule *et al.* 2023), 69.27% in Balaghat, Narsinghpur and Chhindwara in Madhya Pradesh, India (Singh *et al.* 2014), 65% from Malwa region of Madhya Pradesh, India (Rajpoot *et al.* 2017). This variation in the prevalence of Strongyle nematodes in goats was based on differences in various factors such as environmental and host factors. To confirm the existence of strongyle larvae at the genus and species levels PCR was performed on L3 larvae obtained from faecal cultures. The presence of *Haemonchus contortus, Trichostrongylus* and *Oesophagostomum* were confirmed by the PCR results (Table 2). In the present study, the prevalence rate was highest for *Haemonchus contortus* (54.16%), followed by *Trichostrongylus* spp. (31.66%) and lowest for *Oesophagostomum* spp. (14.16%).

Table 2. Prevalence of *Haemonchus contortus, Trichostrongylus* spp. and *Oesophagostomum* spp.

Genus / Species	No. of positive	Prevalence (%)
Haemonchus contortus	195	54.16
Trichostrongylus spp.	114	31.66
Oesophagostomum spp.	51	14.16

Haemonchus contortus was detected in 195 faecal samples using PCR. The amplified species specific product was of the expected size of 265 bp (Fig. 1). The presence of *Trichostrongylus* spp. was confirmed in 114 faecal samples, with the genus -specific product amplified to be expected size of 398 bp (Fig. 2). The PCR confirmed the presence of *Oesophagostomum* spp. in 114 faecal samples, with the genus specific product amplified to the expected size of 333 bp (Fig.3). Due to unavailability species specific primers of *Trichostrongylus* spp. and *Oesophagostomum* spp. during this study period.

Such work has not done previously in this study areas. Our result differed from that of previous report, who described the prevalence of *Haemonchus contortus* (47%), *Trichostrongylus* spp. (4%) and *Oesophagostomum columbianum* (0%) in goats from the Nyala Area South Darfur State, Sudan (Hassan *et al.* 2017). On the other hand, the prevalence of *Trichostrongylus axei* was reported as 87.5% in goats in Dakahlia Governorate, Egypt, using molecular methods (Elseadawy *et al.* 2021). However, another study reported that the prevalence of *H. contortus* (41 to 54%), *Trichostrongylus* spp. (9 to 13%) and *Oesophagostomum* spp. (7 to 14%) in goats in Uttarakhand, India (Sankar *et al.* 2020). Most of the nematodes infecting goats are belong to are group of strongyles worm in which *Haemonchus* spp. is prevalent than others GI parasites also reported by Zvinorova *et al.* (2016). Previous studies have also been reported the dominance of *Haemonchus* contortus among gastrointestinal helminths of goats (Tsotetsi and Mbati 2003, Ntonifor *et al.* 2013). The higher prevalence rate of *H. contortus* can be attributed to the high biotic potential of mature females resulting in fast

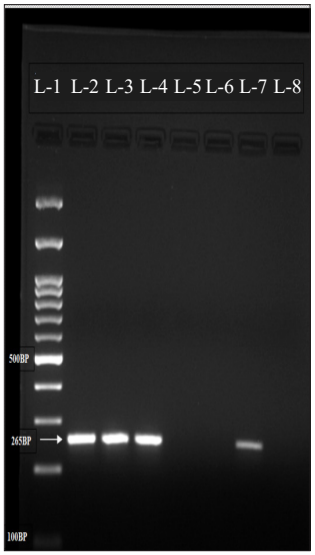

Fig. 1. Results of PCR amplification of ITS-2 of ribosomal DNA gene of *Haemonchus contortus* (265 bp). L1: DNA Ladder (100 bp); L2, L3, L4: Positive Samples; L5, L6: Negative Sample; L7: Positive Control; L8: Negative Control.

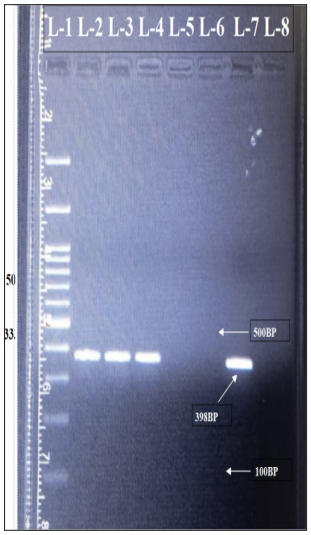

Fig. 2. Amplified product of ITS-2 of the ribosomal DNA gene of *Trichostrongylus* spp. (398 bp) Lane L1: 100 bp plus DNA ladder, L2, L3, L4: Positive samples, L5, L6: Negative sample L7: Positive control and L8: Negative control.

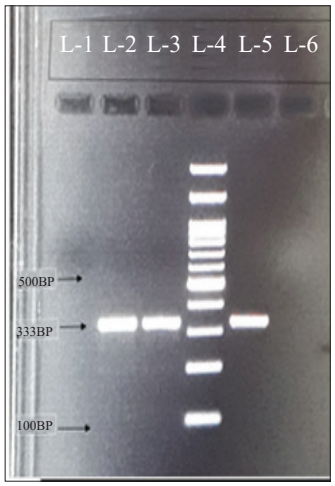

Fig. 3. Amplified product of ITS-2 of the ribosomal DNA gene of *Oesophagostomum* spp. (333 bp). Lane L4: 100 bp plus DNA ladder; L2, L3: Positive samples, L1: Negative sample, L5: Positive control and L6: Negative control.

contamination of pasture by the larvae (Roeber *et al.* 2013). Besides, climatic conditions play crucial role because the hatching, survivability and pathogenicity of parasites largely depends upon environmental condition throughout the World (Waller and Chandrawathani 2005).

Occurrences of gastrointestinal parasites are common in ruminants and responsible for significant economic losses. Identifying the hosts, species, and infection sites of these parasites is essential due to their diversity and varying susceptibility to anthelmintic treatments. The shape and

size of the eggs from different gastrointestinal parasites are not distinguishable. Furthermore, distinguishing the third stage of infective larvae can be difficult and prone to inaccuracies without expert assistance (Han *et al.* 2017). In this scenario, molecular techniques offer speedy and accurate, benefits for the identifying various nematode species (Callaghan and Beh 1994, Roeber *et al.* 2011). Also, sensitivity in identifying amplified DNA among pools of eggs, larvae, or adult worms has been observed in molecular methods (Santos *et al.* 2020).

In the present study, ITS-2 gene was selected for species identification due to its high specificity, more conservative regions and universal primers binding to the 5.8S and 28S ribosomal DNA genes in various nematodes (Heise *et al.* 1999). *Haemonchus contortus* and genra of *Trichostrongylus* and *Oesophagostomum* were identified in the goats from the study area by utilizing the molecular methods. The study concluded that *Haemonchus contortus* is predominantly prevalent among the goat population in the study area.

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