

Original Research Article

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Prevalence of Gastrointestinal Strongyles of Goats in Mid Himalayas of Uttarakhand, India

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ABSTRACT

Gastrointestinal nematodes (GIN) in ruminants are one of the major impediments in livestock production and causes enormous economic losses. Accurate identification of GIN is essential for studying epidemiology and envisage control programme. The present study describes the generic composition of strongyle affecting goats of mid Himalayan Uttarakhand by polymerase chain reaction based restriction fragment length polymorphism (PCR-RFLP). The faecal samples (N=2231) of goats were collected from different places of Uttarakhand over the period of three years (2016-2018) and screened for strongyle infection by salt floatation. The positive samples were pooled subjected for larval culture. The infective third stage larva obtained from the culture were used for PCR-RFLP using *RsaI* restriction enzyme on β -tubulin isotype-I gene. The floatation results were indicated that the strongyle infection was present throughout year and the intensity of infection was moderate to severe from mid rainy season to autumn (Mid July to October). The prevalence of GIN infection was 95.78% (2137/2231) and egg per gram was 1982 ± 452 . The PCR-RFLP clearly differentiated three common strongyle species of goats such as *Haemonchus contortus*, *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*. Based on PCR-RFLP, the predominant strongyle infections are *H. contortus* and *T. circumcincta* in high altitude of Uttarakhand. The results obtained in the present study suggest that the GIN infection is common among goats of mid Himalayan region of Uttarakhand and further it enlighten on timely intervention before the development of clinical disease.

Keywords

β -tubulin isotype-1,
Goats, PCR-RFLP,
Trichostrongyles

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Introduction

Livestock agriculture is subject to severe economic losses from gastrointestinal nematodes (GIN) infections, in the form of direct losses due to blood and tissue feeding,

reduced reproductive efficiency, stunted growth and predisposing for other infections (Waller, 2006). If the infection persists without any intervention, GIN can cause significant losses in animal production, ranging from reduced body weight to the

death of the animals, particularly young stock (Soulsby, 1982). The major species of nematodes infecting the GI tract of ruminants belong to the family *Trichostrongylidae*. The economically important trichostrongyles of small ruminants in tropical and sub-tropical regions like India are *Haemonchus contortus*, *Trichostrongylus* spp., *Oesophagostomum* spp. and *Bunostomum trigonocephalum*.

In temperate region, addition to above four species, *Teladorsagia circumcincta*, *Chabertia ovina* and *Nematodirus* spp., are also pose a major threat to successful livestock production (Dhar *et al.*, 1982; Waller, 2006; Tariq *et al.*, 2008, 2010; Annual report, GIP, 2014).

The strongyle infection in ruminants is acquired by ingesting grasses contaminated with third stage infective larvae (L₃). The eggs or larvae of these species cannot be differentiated accurately; therefore proper identification is must for epidemiological aspects and formulation of control strategies (Gasser *et al.*, 2009).

The polymerase chain reaction linked random fragment length polymorphism (PCR-RFLP) on ribosomal genes (Hoste *et al.*, 1998) and β -tubulin (Silvestre and Humbert, 2000) have demonstrated for accurate identification trichostrongyles. A detailed survey is essential to understand the implication of gastrointestinal nematodiasis in goats of mid Himalayan Uttarakhand.

Currently, very little data exist for nematode infections of ruminants mainly based on morphological identification method. The paucity of data has necessitated this study with primary objective to provide data on prevalence of gastrointestinal strongyles of goats of mid Himalayan Uttarakhand in different seasons.

Materials and Methods

Sample collection

The present study was carried out on grazing goats in the districts Almora, Nainital, Champawat, Pithoragarh, Bageshwar, Uttarakasi and Rudraprayag of Uttarakhand. These areas are coming under the mid Himalayan region of Uttarakhand. The faecal samples of goats were collected randomly from both sexes and different age groups of goats over the period of three years (February 2016 to December 2018) in all seasons [spring (March to June), monsoon (July to September), autumn (October to November) and winter (December to February)]. The samples were collected per rectum in labeled containers and processed in the laboratory.

Parasite examination

The occurrence and intensity of infection was determined by qualitative (salt floatation) method (Soulsby, 1982). The positive samples were subjected for modified McMaster method for quantification of parasitic eggs (MAFF, 1977; Soulsby, 1982; Fowler, 1986). The microscopy positive faecal samples were pooled based on location and subjected for faecal culture to obtain infective third stage larvae.

DNA isolation

The genomic DNA from single larva was extracted based on the method employed by Silvestre and Humbert (2000) and Chandra *et al.*, (2015). In short, the infective larvae obtained from the culture were cleaned 3-4 times with distilled water and were exsheathed by sodium hypochlorite (aqueous solution, 3.5% active chlorine). Single exsheathed larva was picked by using micropipette in a PCR tube and digested by adding 5 μ l of digestion buffer (50mM Tris-

HCl, 10mM EDTA and 5 mg/ml proteinase K). The larva was incubated at 56 °C for 4 hrs. The lysate was further incubated at 99°C for 20 minutes to inactivate Proteinase K.

Primary PCR amplification of β -tubulin isotype 1 gene

Genomic DNA from third stage larvae (5 μ l) was used as template for amplification of β -tubulin isotype-1 gene in reaction mixture of 25 μ l containing 10 pmol of each primers (Pn1- 5' GGC AAA TAT GTC CCA CGT GC 3' and Pn2- 5' GAT CAG CAT TCA GCT GTC CA 3'), 200 μ M of each dNTPs, and 1U of Taq DNA polymerase. Polymerase chain reaction was performed at initial denaturation at 98 °C for 2 min, then 20 cycles of 98 °C for 15 s, annealing temperature of 57 °C for 30 s, 68 °C for 1min, then a final step at 68 °C for 10 min. PCR products were used as template for nested PCR.

Nested PCR

One μ l of primary PCR products were used as template for nested PCR. The final reaction mixture of 25 μ l is containing 10 pmoles of each primer (Pn3- 5 GGA ACA ATG GAC TCT GTT CG 3' and Pn4- 5' GGG AAT CGA AGG CAG GTC GT 3), 2.0 mM MgCl₂, 200 μ M of each dNTPs, and 1U of Taq DNA polymerase. Polymerase chain reaction was performed at initial denaturation at 98 °C for 2 min, then 33 cycles of 98 °C for 15 s, annealing temperature of 57 °C for 30 s, 68 °C for 1 min, then a final step at 68 °C for 10 min.

RFLP with *RsaI* enzyme

For species identification, 10 μ l of of nested PCR product was digested with restriction enzyme *RsaI* for 1.5 h at 37°C. The digested amplicons were resolved in 2.5% agarose gel electrophoresis and documented.

Results and Discussion

A total of 2231 faecal samples were screened by salt floatation and faecal egg counts (EPG). The results were indicated that the endoparasitic infection was present throughout year (2137/2231, 95.78%) in mid Himalayan region of Uttarakhand and EPGs were in the range from 500-11460 (mean EPG 1982 \pm 452). The peak EPG was observed during and after monsoon. However, mild infections were maintained also in winter months (December to February). In agreement with present study, Ram *et al.*, (2007) also reported overall GIN prevalence of 93.86% in goats of Uttarakhand.

The nested PCR amplicon size was 774 bp (Fig.1) and the *RsaI* RFLP digested fragments of *H. contortus* showed major fragments of 441 bp, 190 bp and 155 bp, *T. colubriformis* revealed fragments of 395 bp, 177 bp and 98 bp and *Te.circumcincta* showed fragments of 284 bp, 182 bp and 132 bp (Fig.2). These banding patterns clearly differentiated commonly infecting GINs of goats in Uttarakhand. Earlier studies showed PCR-RFLP on ribosomal genes such as internal transcribed spacer 1 and 2 (ITS-1 and 2) (Hoste *et al.*, 1998; Heise *et al.*, 1999) and β -tubulin isotype 1 (Silvestre and Humbert, 2000) are very powerful tool for trichostrongyle species differentiation. The results of PCR-RFLP revealed that *H. contortus* (41-54%) and *Te.circumcincta* (24-37%) were the most common strongyle in goats of Uttarakhand, invariably from all districts (Table.1). However, other nematodes *Trichostrongylus* spp (9-13%), *Oesophagostomum* spp (7-14%) were also recorded. Earlier reports demonstrated GINs common in goats of Uttarakhand (Yadav *et al.*, 2008; Subramani *et al.*, 2014).

The highest infection was observed during monsoon and post monsoon sessions (Fig. 3

and Table 2), however, mild infection was maintained during winters. This pattern can be assigned to variation in the rainfall and temperature in the weather that favors the spurt of infective larvae in the environment during monsoon (Soulsby, 1982; Taylor *et al.*, 2015). PCR-RFLP using *RsaI* on β -tubulin isotype -1 gene showed higher prevalence of *H. contortus* and *Te. circumcincta* and moderate infection of *Oesophagostomum* spp and *Trichostrongylus* spp. The prevalence of strongyles were lowest in the winter months followed by spring that might be due to

hypobiosis of larvae in the host thus, less number of infective larvae in the pasture. Earlier study found that heavy infection of haemonchosis on goats of high altitude of Uttarakhand (Ram *et al.*, 2007) which is well corroborating with present study. The study concluded that GINs are very common and chronic debilitating disease of goats in Uttarakhand. The present study findings enlighten further to determine epidemiological pattern of GIN infection and formulation of control strategy.

Table.1 Various GIN infection in goats from different districts (in %)

Places (no. of larvae)	<i>H. contortus</i>	<i>T. colubriformis</i>	<i>T.circumcincta</i>	Others (mainly <i>Oesophagostomum</i> spp)
Almora (470)	44	10	32	14
Nainital (871)	54	13	24	09
Pithoragarh (92)	47	11	30	12
Champawat (81)	43	9	34	14
Bageshwar (74)	51	11	31	07
Uttarakasi (56)	41	13	37	09
Rudraprayag (63)	42	12	37	09

Table.2 Season wise GIN infection in goats

Season	<i>Teladorsagia circumcincta</i>	<i>Haemonchus contortus</i>	<i>Trichostrongylus</i> spp	Others (mainly <i>Oesophagostomum</i> spp)
Winter	37-44%	31-36%	12-13%	20-22%
Summer	18-21%	56-64%	11-13%	13-16%
Rainy	19-23%	55-67%	14-16%	07-10%
Autumn	16-20%	40-47%	10-13%	20-23%

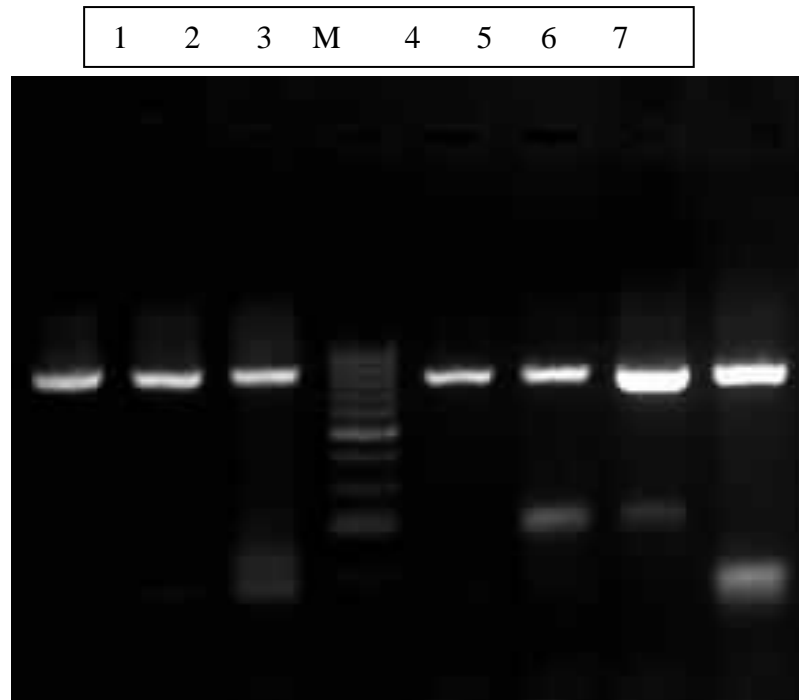


Fig.1 Results of nested PCR amplification of beta tubulin isotype 1 gene
Lane M- 100 bp plus Marker Lane 1-7: Nested PCR amplicons (774 bp)

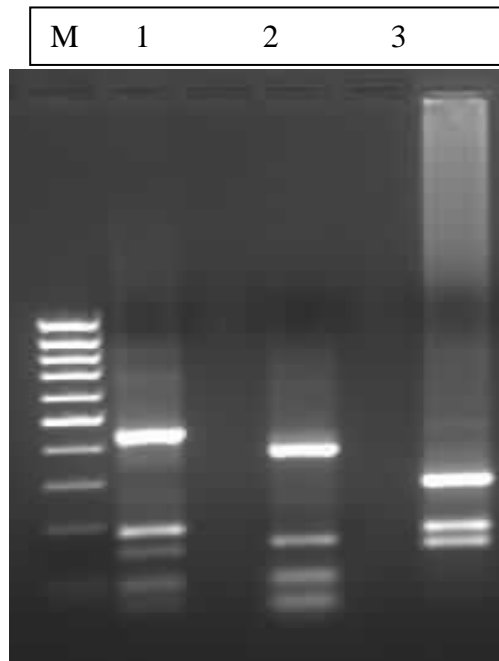


Fig.2 Results of PCR-RFLP for species identification
Lane 1- 100 bp plus Marker
Lane 2- *H. contortus* (441bp, 190bp, 155bp)
Lane 3- *T. columbianum* (395bp, 177bp, 98bp)
Lane 4 – *T. circumcincta* (284bp, 184bp, 132bp)

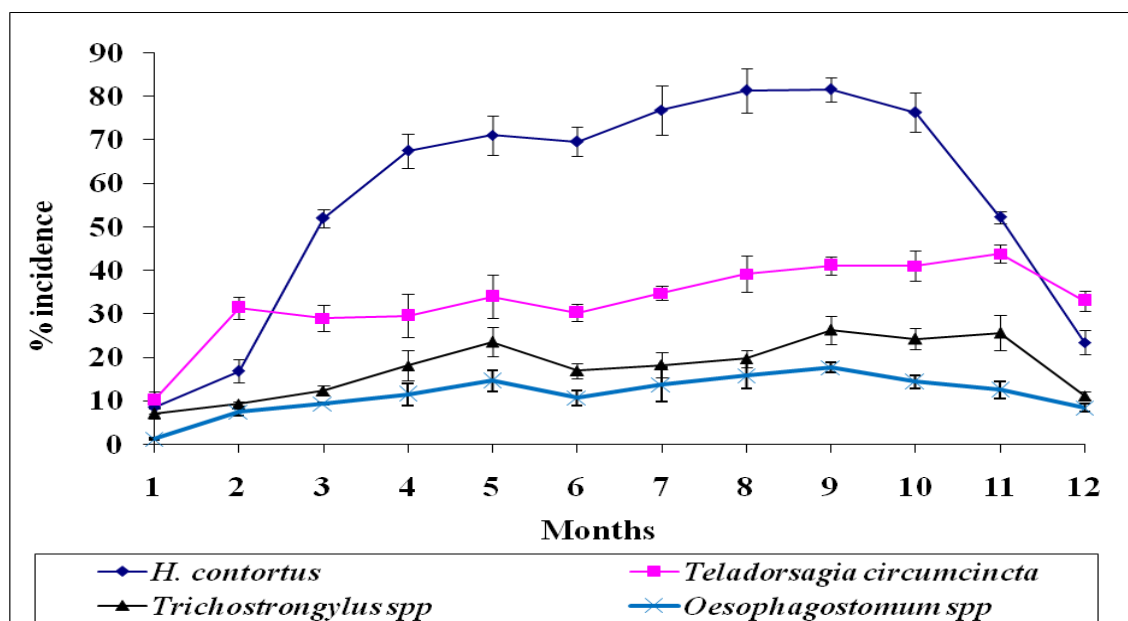


Fig.3 Monthly prevalence of common GIN of goats

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