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Multiple anthelmintic resistance in gastrointestinal nematodes of Caprines on Mountain Research Centre for Sheep and Goat at Kashmir Valley, India

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ABSTRACT

The study was conducted to evaluate the status of anthelmintic resistance in Gastro-Intestinal Nematodes (GINs) of goats at an organized farm located in Kashmir, as there is no report of resistance against these parasites of goats from this temperate region, although it has been reported worldwide including India. Caprines reared at this farm exhibited reduced efficacy to multiple anthelmintics following treatments with Fenbendazole (FBZ), Closantel and Ivermectin (IVM) in Faecal Egg Count Reduction Test (FECRT). The results suggested that the overall efficacy was highest for IVM at 83.5% and 90.0% on 7th and 14th day post-treatment, respectively and least for FBZ at 44.3% and 62.5%, respectively, whereas the corresponding figures for closantel were 68.3% and 86.2%, respectively. The pre-treatment faecal culture revealed *Haemonchus contortus*, *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* as predominant strongyles, however, in post-treatment samples, only *H. contortus* was observed. Further, the infective larvae were subjected to Allele specific PCR (AS-PCR) for accurate diagnosis of BZ resistance. The AS-PCR revealed 52% of *H. contortus* were homozygous resistant (rr) and 17% were heterozygous (rS) on day "0" before treatment and 100% homozygous resistant (rr) on 7th day post treatment. In both *T. colubriformis* and *T. circumcincta*, 100% population was homozygous susceptible (SS) at day "0" before treatment. The overall frequency of resistant (r) allele for *H. contortus* was 60.5% and for susceptible allele (s) was 39.5%. For *T. colubriformis* and *T. circumcincta* the frequency of susceptible allele (s) was 100%. The survey indicated that the GINs of goats on the farm have developed multiple anthelmintic resistance to FBZ, closantel and IVM and the condition is alarming in the farm. Moreover surveillance studies about status of anthelmintic resistance in other farms (Govternment as well as Private) of Kashmir valley should be carried out at large scale to develop effective and sustainable control strategies against GI Nematodes.

1. Introduction

The Gastro-Intestinal Nematodes (GINs) of small ruminants are effectively controlled by chemical based anthelmintics, however, continuous and improper use of anthelmintics have led to wide spread selection of resistant nematodes within populations

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(Falzon et al., 2013). The anthelmintic resistance has been reported worldwide including India (Garg and Yadav, 2009; Maharshi et al., 2011; Jeyathilakan et al., 2013). The first report of anthelmintic resistance in India was by Varashney and Singh (1976) against phenothiazine and thiabandazole in *H. contortus* of sheep from Rishikesh (Uttarakhand). Subsequently anthelmintic resistance was reported in goat GINs from different parts of the country against different anthelmintics (Uppal et al., 1992; Gill, 1996; Ram et al., 2007; Godara et al., 2011; Jaiswal et al., 2013; Chandra et al., 2015), however, there is no report from north most temperate region, Kashmir, of India, barring three reports in sheep GINs (Nasreen et al., 2007; Itoo et al., 2012; Shahardar et al., 2014). In Jammu and Kashmir total small ruminant population is 6,195,000 (Anonymous, 2007) with 267,000 goats in the Kashmir province (Anonymous, 2011). GINs are one of the major constraints for small ruminant production throughout the world including India. In spite of significant production losses, which may run into millions of rupees (Shah and Chaudhry, 1995), the problem is neglected due to its chronic and insidious nature (Sanyal, 1998). Therefore, the present study was aimed to investigate the status of fenbendazole (FBZ), closantel and ivermectin (IVM) resistance in GINs of goats reared at an organized farm of a Research Center of Kashmir Valley by Faecal Egg Count Reduction Test (FECRT) and additionally molecular diagnosis of benzimidazole (BZ) resistance by Allele-specific Polymerase Chain Reaction (AS-PCR) was undertaken. The benzimidazole group of drugs has been used for decades to control GINs (Campbell, 1999) and the phenomenon of resistance to this anthelmintic group has been widely observed and described (Conway, 1964; Green et al., 1981; Echevarria et al., 1996).

For evaluation of anthelmintic efficacy, different i*n vivo* and i*n vitro* tests are recommended (Coles et al., 1992, 2006) and the FECRT is the most commonest method used for detection of anthelmintic efficacy (Presidente et al., 1985). The method has proved its efficacy but can only reliably detect resistant worm population when more than 25% of the parasites are resistant (Roos et al., 1995). AS-PCR can detect BZ resistant GINs even when less than 1% of the worms are resistant (Coles et al., 2006).

2. Materials and methods

2.1. Study area and parasite collection

The present study was conducted at Mountain Research Centre for Sheep and Goat (MRCSG), Shuhama (Alusteng), Srinagar (Kashmir). The farm is situated at $34^0$05/N Latitude and $74^0$50/E Longitude and has a temperate climate with the upper reaches receiving heavy snowfall during winter. The summer temperature varies between 10°C to 34°C with good little rain, however, relative humidity is generally high. The precipitation occurs throughout the year and no month is particularly dry. The hottest month is July (mean minimum temperature 6°C, mean maximum temperature 32°C) and the coldest is January (mean minimum temperature −15°C, mean maximum temperature 0°C).

In this study, about 124 Boer and Bakerwal cross bred goats suffering from GIN infection (Mean egg per gram EPG =277) were randomly selected irrespective of age, sex and weight. The representative faecal samples were collected per-rectally. The farm had a history of irregular deworming and no fixed regimes of deworming were followed and there were frequent complaints of drug failure. The basic history of different anthelmintics used at the said farm for the last five years is presented in Table 1.

2.2. Experimental design for FECRT

The therapeutic efficacy of different anthelmintic drugs *viz,* FBZ, closantel and IVM was determined using the FECRT as per the guidelines of World Association for Advancement of Veterinary Parasitology (WAAVP) (Coles et al., 1992, 2006). The animals were divided into four groups (Group I, II, III and IV). First three groups comprising of thirty one animals each were treated with anthelmintics and fourth group comprising of same number of animals was kept as untreated infected control (Table 2). Per rectal

Table 1
Frequency of various Anthelmintics used at MRCSG

Year	Name of the drug	Frequency of application per year
2010	Albendazole	Once
	Closantel	Once
	Ivermectin	Once
2011	Albendazole/Fenbendazole	Thrice
	Closantel	Once
	Ivermectin	Once
2012	Fenbendazole	Twice
	Closantel	Twice
	Ivermectin	Twice
2013	Fenbendazole	Twice
	Closantel	Once
	Ivermectin	Twice
2014	Fenbendazole	Twice
	Closantel	Four times
	Ivermectin	Nil
2015	Fenbendazole	Twice
	Closantel	Nil
	Ivermectin	Once

Table 2
Schedule of drug trial

Group	Drug used	Dosage /kg body weight	Route of adminstration
1.	Fenbendazole	5 mg	Oral
2.	Closantel	7.5 mg	Oral
3.	Ivermectin	200 μg	Subcutaneous
4.	Infected Control	–	–

copro samples were collected from each experimental goat and placed into individual labelled polythene bags on day '0' before treatment and on 7th and 14th day post treatment. The samples were brought to the Divisional laboratory and processed for EPG determination using a modified McMaster technique (Coles et al., 1992). The therapeutic efficacy of all the three anthelmintics was determined by the formula: FECR (%) = 100 (1-Xt/Xc) and percentage efficacy of each drug was calculated using the formula: PE (%) = 100 [(MC-MT)/MC]; where, 'Xt' is average EPG of treatment group and 'Xc' is average EPG of control group. MC = mean EPG in the control group, MT = mean EPG in the treated group (Coles et al., 1992).

2.3. Genomic DNA extraction and AS-PCR

The faecal samples collected from Group I animals on day "0" before administration of FBZ and on 7th day post treatment were subjected to coproculture (MAFF, 1977) and the larvae were collected and identified as per the morphological keys (MAFF, 1986; Van Wyk and Mayhew, 2013). The genomic DNA extraction from larvae and AS-PCR were done based on the method employed by Silvestre and Humbert (2000) and Coles et al. (2006). Briefly, the larvae were washed 3–4 times with distilled water and approximately 100 larvae were exsheathed by incubation for 5–20 min in a petridish containing 4ml of larval suspension and 180μl of sodium hypochlorite (aqueous solution, 3.5% active chlorine). Single exsheathed larva was placed in a PCR tube and freeze thawed three times at -80°C and 37°C. DNA was extracted by adding 7μl of extraction buffer (10mM Tris-HCl, 5mM EDTA and 5mg/ml proteinase K) and incubating tubes at 56°C for 8 h. Proteinase K was inactivated by incubating the lysate in a thermal cycler at 99°C for 20 min. Genomic DNA lysate of larvae was used for amplification of truncated β-tubulin isotype-1 gene (Coles et al., 2006).

The preparation of reaction mixtures including primers and reaction conditions of PCR were followed as per the method of Coles et al. (2006). The β-tubulin PCR amplicons were used as templates for nested PCR and the amplified nested product was digested with the restriction enzyme *Rsa*I (MBI fermentas) for accurate species determination of *H. contortus, T. colubriformis* and *T. circumcincta* (Silvestre and Humbert, 2000). Species specific primers were used for AS-PCR using nested PCR product as templates for diagnosis of FBZ resistance (Coles et al., 2006). The amplicons were separated by 1.5% agarose gel electrophoresis.

2.4. Statistical analysis

The Chi-square test was used for statistical analysis of the data as per Snedecor and Cochran (1994).

3. Results and discussion

The percentage reduction in faecal egg counts with FBZ, closantel and IVM treatment against strongyle worms was recorded as 45.0 and 63.0%; 69.0 and 86.3%; and 84.0 and 91.0% on 7th and 14th day post-treatment, respectively (Table 3). These values suggest that GINs have developed resistance against all the three drugs *i.e.* FBZ, closantel and IVM as per Coles et al. (1992). During the present research high degree of resistance for FBZ against GINs was recorded in cross bred goats on 7th day post treatment whereas it was moderate on 14th day post treatment as the value of FECR percent was less than 60% on 7th day post-treatment and less than 90% but greater than 60% on 14th day post-treatment. Shahardar et al. (2014) had already reported slight resistance of sheep GINs to FBZ at MRCSG. This difference in the result at the same farm may be due to the past higher frequency of the use of FBZ in the studied farm for deworming, causing selection pressure among parasites and initiation of more resistance in GINs. Our observations are in line with the findings of Sharma et al. (2015) who reported 57.44% and 70.87% FECR percentage on 7th and 14th day post treatment, respectively against *H. contortus* in goats of Jammu region. There are many reports regarding BZ resistance in goats against GINs from others parts of the country like Uppal et al. (1992); Ram et al. (2007); Godara et al. (2011); Manikkavasagan et al. (2015). The anthelmintic resistance survey of Chandra et al. (2015) indicated that the status of BZ resistance is in alarming conditions in all parts of the Uttar Pradesh, India. In addition to MRCSG, Shahardar et al. (2014) reported moderate resistance to FBZ at Govt. sheep breeding farm, Poshnar (Handwara), Kashmir and at Govt. sheep breeding farm, Zawoora (Shopian), Kashmir.

Moderate resistance on 7th and 14th day post treatment was observed for closantel. The moderate resistance in *H. contortus* to closantel has also been reported by Wooster et al. (2001) in New South Wales and Fiel et al. (2011) in Argentina. Uppal et al. (1992) and Kadam et al. (2009) reported 100% efficacy of closantel against strongyle worms in UP, India and in Akola region of India, respectively. Shahana (2013) observed closantel efficacy to the tune of 98.80 and 97.60% against strongyle worms of sheep on 8th and 14th day post treatment, respectively at Budgam district of Kashmir Valley. In case of IVM the resistance was considered to be moderate on 7th day and slight on 14th day post-treatment as the value of FECR percent was greater than

Table 3
FECR and Efficacy of different Anthelmintics

Group	Mean EPG Day '00'	Mean EPG Day '07'	FECR %	Efficacy %	Mean EPG Day '14'	FECR %	Efficacy %
1.	$332.25^{a}\pm9.59$	$141.93^{a}\pm20.09$	45	44.3	$96.77^{a}\pm23.86$	63	62.5
2.	$283.87^{ab}\pm18.01$	$80.64^{b}\pm16.98$	69	68.3	$35.48^{b}\pm9.89$	86.3	86.2
3.	$254.83^{b}\pm12.98$	$41.93^{b}\pm11.14$	84	83.5	$25.80^{b}\pm9.23$	91	90.0
4.	$238.70^{b}\pm11.98$	$254.83^{c}\pm11.20$	--	--	$258.06^{c}\pm11.14$	--	--

Values with same superscript in a column do not vary significantly ($P>0.05$).

90% but less than 95% on 14th day post-treatment in the present study. IVM resistance in GINs of goats has been reported from different parts of India like Laha et al. (1999); Deepa and Devada (2006); Jaiswal et al. (2013). Our observations are almost similar to the findings of Shahardar et al. (2014), who reported slight resistance to IVM at Govt. sheep breeding farm, Poshnar (Handwara), Kashmir and moderate resistance at Govt. sheep breeding farm, Zawoora (Shopian), Kashmir.

On coprocultural examination of Group I animals, *Haemonchus* spp. (80.00%) was the predominant strongyle followed by *Trichostrongylus* spp. (12.00%), and *Teladorsagia* spp. (7.00%) with very less prevalence of *Chabertia ovina* on day "0" (Pre treatment), however, *H. contortus* was the only species recovered from faecal culture on 7th day post treatment (Fig. 1). All the exsheathed larvae were collected after observing under binocular stereomicroscope and further transferred individually in PCR tubes with the help of micropipette (Fig. 2). The first PCR amplicon used for nested PCR yielded approximately 820 bp product (Fig. 3).The digestion of nested PCR product with *Rsa*I enzyme resulted in different restriction patterns (Fig. 4). Restriction digests of *H. contortus* yielded three major fragments of 440, 190 and 140 bp, while digests of *T. colubriformis* yielded four major fragments of 390, 180, 100 and 40 bp. The restriction digests of *T. circumcincta* yielded three major fragments of 284, 189 and 182 bp. As per the restriction profiles, the samples were confirmed to be proportionally dominated by *H. contortus* (80–85%), followed by *T. colubriformis* and *T. circumcincta* before treatment. However, after treatment, all the larvae were confirmed to be *H. contortus* indicating BZ resistance in *H. contortus* and not in other strongyle worms.

Allele specific PCR yielded 550 and 750 bp products in susceptible larvae in case of *T. colubriformis* and *T. circumcincta,* however, it was 603 and 750 bp & 223 and 750 bp products in susceptible and resistant larvae, respectively in case of *H. contortus* (Fig. 5). A total of

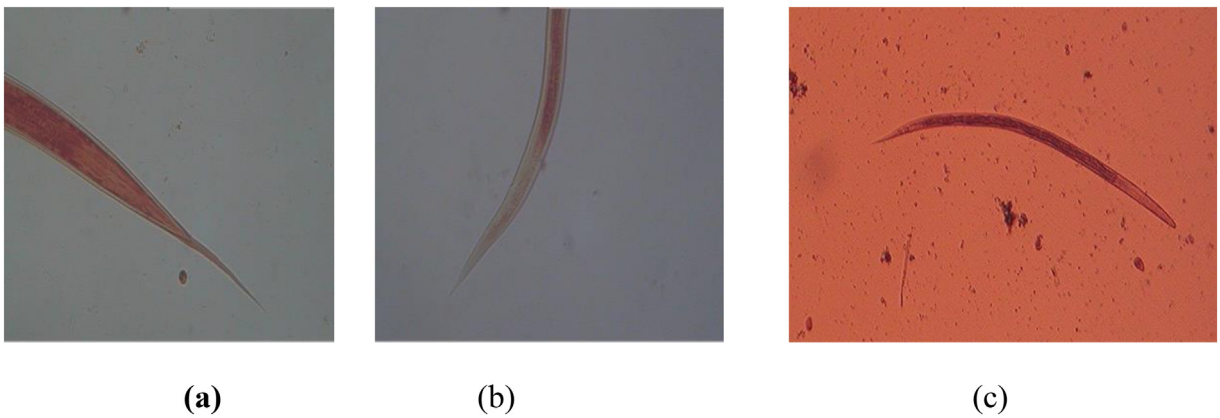

Fig. 1. (a) Posterior end of *Haemonchus* spp. (kinked tail) (b) Posterior end of *Trichostrongylus* spp.(Short tip and resembles pencil tip) (c) *Teladorsagia* spp. (long sheath which tappers to relatively blunt point).

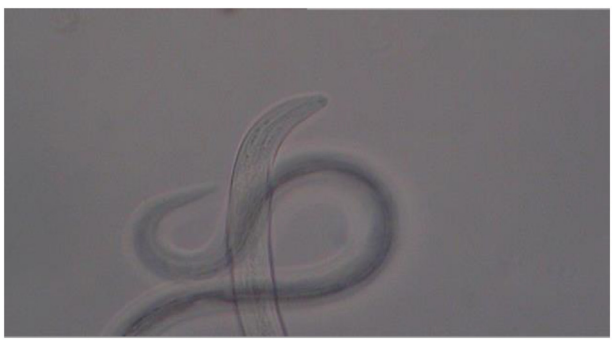

Fig. 2. Exsheathment of L3 larvae.

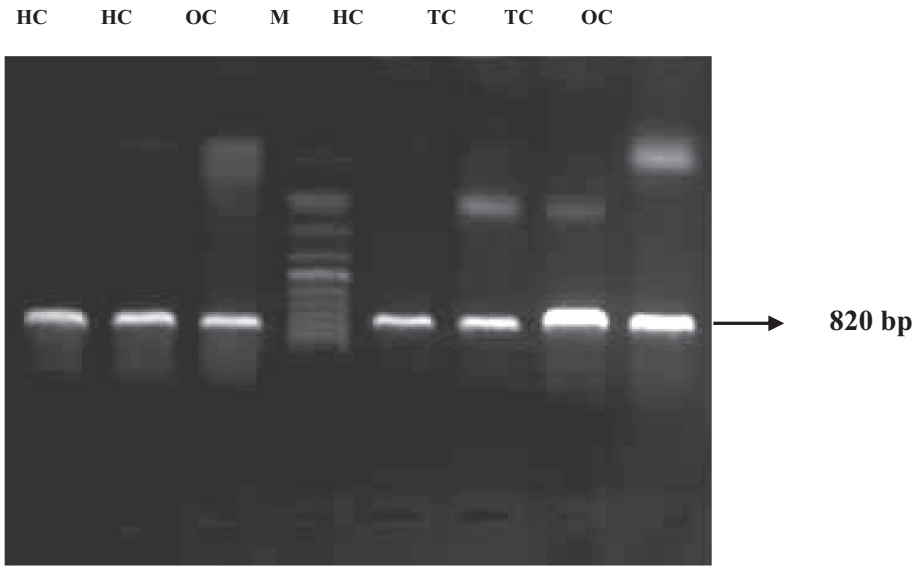

Fig. 3. Nested PCR for the amplification of beta-tubulin isotype-1 gene. Lane M: 100 bp plus DNA ladder (Fermentas). HC- *Haemonchus contortus.* OC- *Teladorsagia circumcincta.* TC- *Trichostrongylus colubriformis.*

200 *H. contortus* larvae were genotyped for BZ resistance pre- and post-treatment. The results indicated that before administration of FBZ, 52% of *H. contortus* larvae were homozygous resistant (rr,TAC/TAC), 31% were homozygous susceptible (SS,TTC/TTC) and 17% were heterozygous (rS, TAC/TTC) on MRCSG. However, after administration of FBZ on 7th day 100% larvae were homozygous resistant (rr). In total, 12 larvae of *T. colubriformis* and 7 larvae of *T. circumcincta* were genotyped. The genotyping of larvae showed that all the larvae of *T. colubriformis* and *T. circumcincta* were homozygous susceptible (SS) (Table 4).

AS-PCR, qPCR and pyrosequencing have been found to be more accurate in early diagnosis of BZ resistance as compared to FECRT or EHA (Silvestre and Humbert, 2000; Coles et al., 2006; Barrere et al., 2013a, 2013b; dos Santos et al., 2014; Chandra et al., 2015). Molecular techniques could save time and money before going for application of BZ group of anthelmintics in a farm against which resistance has developed throughout the world (Barrere et al., 2013b). In the present investigation, PCR-RFLP and AS-PCR was employed as a tool for molecular diagnosis of species identification and BZ resistance. In the first step

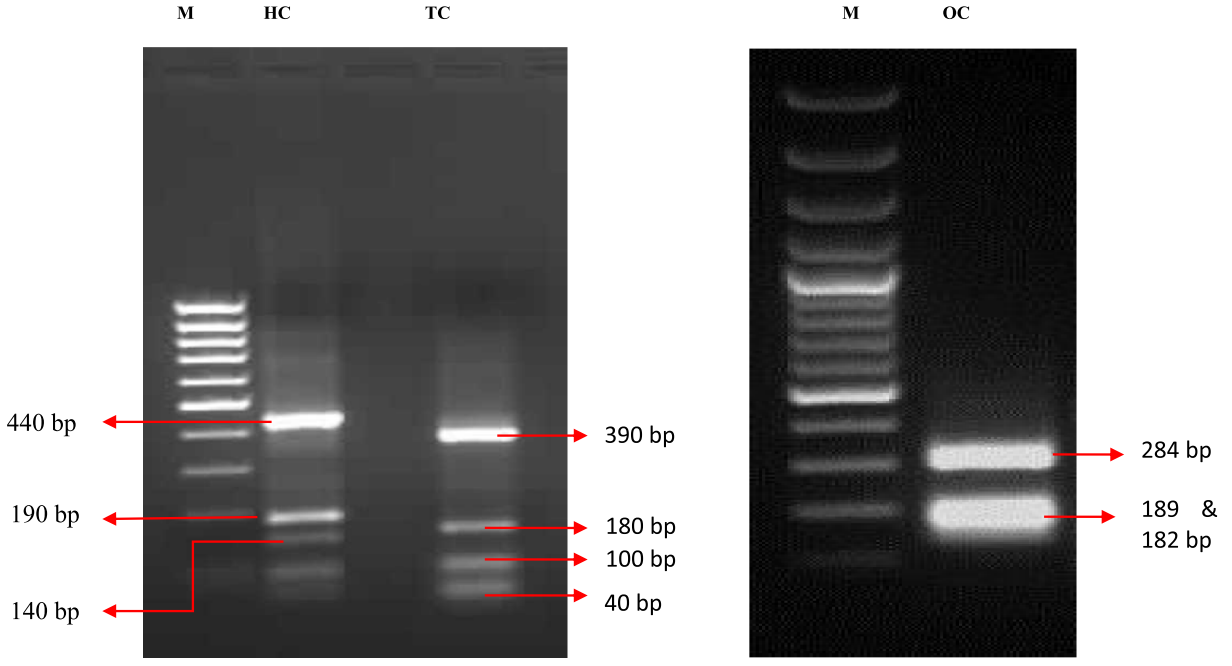

Fig. 4. Restriction enzyme digestion using *RsaI* for the identification of strongyle species. M: 100 bp DNA Ladder M: 100 bp plus DNA Ladder. HC: *H. contortus*: 440, 190 and 140 bp OC: *T. circumcincta:* 284, 189, 182 bp. TC:*T. colubriformis*: 390, 180, 100 and 40 bp.

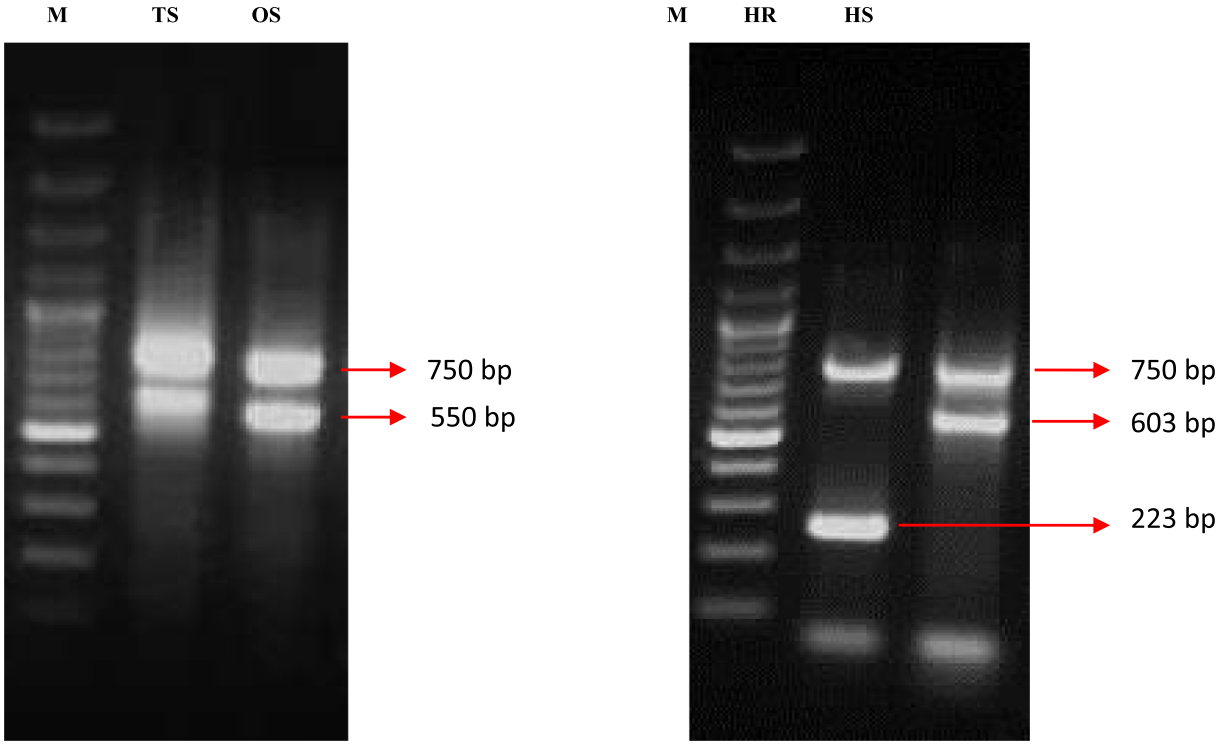

Fig. 5. Allele-Specific PCR for the detection of Benzimidazole resistance. Lane M: 100 bp plus DNA ladder (Fermentas). HS: *H. contortus* susceptible (750 bp and 603 bp). HR: *H. contortus* resistant (750 bp and 223 bp). TS: *T. colubriformis* Susceptible (750 bp and 550 bp). OS: *T. circumcincta* Susceptible (750 bp and 550 bp).

Table 4
Genotyping of GIN larvae (Pre- and Post- FBZ treatment)

Species	No. of larvae genotyped		Genotyping (%)				Gene frequency	
	Pre-Treatment	Post- Treatment	Pre-Treatment			Post- Treatment	Pre-Treatment	
			Resistant (rr)	Susceptible (SS)	Heterozygous (rS)		Resistant (r)	Susceptible (S)
H. contortus	100	100	52 (52%)	31 (31%)	17 (17%)	100 Resistant (rr)	0.605	0.395
T. colubriformis	12	00	00	12 (100%)	00	00	0.0	1.0
T. circumcincta	07	00	00	07 (100%)	00	00	0.0	1.0

the trichostrongylid nematode larvae were identified using PCR-RFLP and in the second step the detection of resistance in trichostrongylid species to BZ was determined using AS-PCR. In the present work this high proportion of resistant *H. contortus* larvae could be attributed to the fact that *H. contortus* larvae were present in a very large number which could have increased the selection of its resistant genotypes and thus outnumbering the genotypes of other two larvae *i.e. T. colubriformis* and *T. circumcincta*, thereby, increasing the selection pressure on the resistant *H. contortus* genotypes leading to their predominance. Similar studies on BZ resistance have also been conducted in Rajasthan (Tiwari et al., 2006); Uttarakhand (Garg and Yadav, 2009); Maharashtra (Ghalsasi et al., 2012) and UP, India (Chandra et al., 2015). The results of molecular detection of BZ resistance are in correlation with FECRT employed for determining efficacy of FBZ in the present study.

4. Conclusions

The results indicated that due to frequent and indiscriminate use of FBZ, closantel and IVM at MRCSG, the resistant population of GINs have increased and if not addressed quickly, can lead to development of completely resistant population of nematodes in due course of time. Studies have shown that usually 20-–30% of the animals suffer with heavy nematodosis but mass treatment is carried out by the farmers leading to great reduction in the *refugia* population (Swarnakar et al., 2010). Therefore, Targeted Selective Treatment (TST), FAMACHA may be applied to minimize the further development of resistance (van Wyk and Bath, 2002; van Wyk et al., 2006). Moreover further such types of studies are warranted in the Kashmir Valley to investigate the status of anthelmintic resistance, so that it can be controlled at an early stage before turning the situation grave.

Declaration of Competing Interest

The authors declare that there is no conflicts of interest.

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References

Anonymous, 2007. Government of India/Economic Survey//2007.
MAFF, 1977. Manual of Veterinary Parasitological Techniques. In: Helminthology, Technical Bulletin No. 18. Ministry of Agriculture, London, UK.
MAFF, 1986. Manual of veterinary parasitological techniques. Fisheries and Food. Ref. Book 418. Ministry of Agriculture, London, UK.
Anonymous, 2011. Directorate of Animal Husbandry Kashmir. Government of J&K.
Barrere, V., Falzon, L.C., Shakya, K.P., Menzies, P.I., Peregrine, A.S., Prichard, R.K., 2013a. Assessment of benzimidazole resistance in *Haemonchus contortus* in sheep flocks in Ontario, Canada: comparison of detection methods for drug resistance. Vet. Parasitol. 198, 159–165.
Barrere, V., Keller, K., von Samson-Himmelstjerna, G., Prichard, R.K., 2013b. Efficiency of a genetic test to detect benzimidazole resistant *Haemonchus contortus* nematodes in sheep farms in Quebec, Canada. Parasitol. Int. 62, 464–470.
Campbell, W.C., 1999. Benzimidazole: Veterinary use. Parasitol. Today 6, 130–133.
Chandra, S., Prasad, A., Yadav, N., Latchumikanthan, A., Rakesh, R.L., Praveen, K., Khobra, V., Subramani, K.V., Misri, J., Sankar, M., 2015. Status of benzimidazole resistance in *Haemonchus contortus* of goats from different geographic regions of Uttar Pradesh. Indian Vet. Parasitol. 208, 263–267.
Coles, G.C., Bauer, C., Borgsteede, F.H.M., Geerts, S., 1992. World association for the advancement of veterinary parasitology (WAAVP) methods for the detection of anthelmintic resistance in nematode of veterinary importance. Vet. Parasitol. 44, 35–44.
Coles, G.C., Jackson, F., Pomroy, W.E., Prichard, R.K., Vonsamson-Himmelstjerna, G., Silvestre, A., Taylor, M.A., Vercruysse, J., 2006. The detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol. 136, 167–185.
Conway, D.P., 1964. Variance in effectiveness of thiabendazole against *Haemonchus contortus* in sheep. Am. J. Vet. Res. 25, 844–845.
Deepa, C.K., Devada, K., 2006. Anthelmintic Resistance of Gastro-Intestinal Nematodes in Goats. XVII- National congress of Veterinary Parasitology at Rajiv Gandhi College of Veterinary and Animal Sciences, Puducherrey-India.
dos Santos, J.M., Monteiro, J.P., Ribeiro, W.L., Macedo, I.T., Camurca-Vasconcelos, A.L., Luiz da Silva Vieira, Bevilaqua, C.M., 2014. Identification and quantification of benzimidazole resistance polymorphisms in *Haemonchus contortus* isolated in northeastern Brazil. Vet. Parasitol. 199, 160–164.
Echevarria, F., Borba, M.F.S., Pinheiro, A.C., Waller, P.J., Hansen, J.W., 1996. The prevalence of anthelmintic resistance in nematode parasite of sheep in southern Latin America Brazil. Vet. Parasitol. 62, 199–206.
Falzon, L.C., Menzies, P.I., Shakya, K.P., Jones-Bitton, A., Vanleeuwen, J., Avula, J., Stewart, H., Jansen, J.T., Taylor, M.A., Learmount, J., Peregrine, A.S., 2013. Anthelmintic resistance in sheep flocks in Ontario, Canada. Vet. Parasitol. 193, 150–162.
Fiel, C., Guzman, M., Steffan, P., Rodriguez, E., Prieto, O., Bhushan, C., 2011. The efficacy of trichlorphon and naphthalophos against multiple anthelmintic-resistant nematodes of naturally infected sheep in Argentina. Parasitol. Res. 1, 139–148.
Garg, R., Yadav, C.L., 2009. Genotyping of benzimidazole susceptible and resistant alleles in different populations of *Haemonchus contortus* from Himalayan and sub-Himalayan regions of north-West India. Trop. Anim. Health Prod. 41, 1127–1131.
Ghalsasi, P.P., Saste, S.R., Ghalsasi, P.M., Nimbkar, C., 2012. Emergence of benzimidazole resistance in nematodes of small ruminants in an organized farm and some small holder flocks in Phaltan Taluka, Maharashtra. J. Vet. Parasitol. 26, 95–98.
Gill, B.S., 1996. Anthelmintic resistance in India. Vet. Parasitol. 63, 173–176.
Godara, R., Sharma, R.L., Sodhi, S.S., 2011. Efficacy of fenbendazole, levamisole and ivermectin against gastrointestinal nematodes in Jamunapari goats. J. Parasit. Dis. 35, 219–221.
Green, P.E., Forsyth, B.A., Rowan, K.J., Payne, G., 1981. The isolation of a field strain of *Haemonchus contortus* in Queensland showing multiple anthelmintic resistances. Aust. Vet. J. 57, 79–84.
Itoo, S.A., Shahardar, R.A., Banday, M.A.A., Shahnawaz, M., Wani, Z.A., 2012. Tetramisole resistance of strongyle parasites in corridale sheep under organized farming system in Kashmir Valley. Anim. Sci. Rep. 6, 60–64.
Jaiswal, A.K., Sudan, V., Shanker, D., Kumar, P., 2013. Emergence of Ivermectin resistance in gastrointestinal nematodes of goats in a semi-organised farm of Mathura district-India. Vet. Arch. 83, 275–280.
Jeyathilakan, N., Radha, G., Gomathinavagam, S., John, L., 2013. Emergence of anthelmintic resistance in G.I nematodes of sheep in Tamil Nadu. J. Vet. Parasitol. 17, 159–160.
Kadam, Y., Waghmare, S.P., Kolte, A.Y., Mode, S.G., Pathak, V.P., Takarkhede, R.C., Kuralkar, S.V., 2009. Efficacy of oral ivermectin and closantel against haemonchosis in sheep. Ind. Vet. J. 86, 633–634.
Laha, R., Hemaprasanth, Harbola, P.C., 1999. Anthelmintic resistance in pashmina (cashmere) producing goats in India. Vet. Res. Comm. 23, 187–189.
Maharshi, A.K., Swarnkar, C.P., Singh, D., Mahonar, G.S., 2011. Correlation between status of benzimidazole resistance in *Haemonchus contortus* on bio and molecular assays. Ind. J. Anim. Sci. 81, 110–115.
Manikkavasagan, I., Binosundar, S.T., Raman, M., 2015. Survey on anthelmintic resistance to gastrointestinal nematodes in unorganized goat farms of Tamil Nadu. J. Parasit. Dis. 39, 258–261.
Nasreen, S., Jeelani, G., Sheikh, F.D., 2007. Efficacy of different anthelmintics against gastro-intestinal nematodes of sheep in Kashmir Valley. Vet. Scan. 2 (1) Article 13.
Presidente, P.J.A., Anderson, N., Waller, P.J., 1985. Methods for detection of nematode resistance to anthelmintics. Resistance of Nematodes to Anthelmintic Drugs, pp. 13–27 Melborne.
Ram, H., Rasool, T.J., Sharma, A.K., Meena, A.R., Singh, S.K., 2007. Comparative efficacy of different anthelmintics against fenbendazole-resistant nematodes of pashmina goats. Vet. Res. Comm. 31, 719–723.
Roos, M.H., Kwa, M.S.G., Grant, W.N., 1995. New genetic and practical implications of selection for anthelmintic resistance in parasitic nematodes. Parasitol. Today 11, 148–150.
Sanyal, P.K., 1998. Integrated gastrointestinal parasite management in dairy animals in Gujarat by self medication. J. Vet. Parasitol. 12, 17–20.
Shah, H.L., Chaudhry, R.K., 1995. Parasitism in dairy animals in india: present status and its impact. Proceedings of the workshop on control strategy against gastrointestinal parasites in dairy animals in India using medicated urea molasses blocks. National Dairy Development Board, Anand, India., pp. 1–5
Shahana, R.T., 2013. Prevalence and therapeutic studies on gastrointestinal helminth parasites of Sheep in Budgam District of Kashmir Valley. M.V.Sc. Thesis. Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir.
Shahardar, R.A., Itoo, S.A., Allaie, I.M., Wani, Z.A., 2014. Anthelmintic resistance in GI nematodes of sheep at farms of Kashmir Valley. J. Vet. Parasitol. 28 (2), 151–153.
Sharma, S., Kumar, S., Farooq, J., Rajesh, K., 2015. Comparative study of efficacy of fenbendazole and ivermectin against *Haemonchus contortus* in goats of Jammu region. J. Anim. Res. 5, 293–295.

Silvestre, A., Humbert, J.F., 2000. A molecular tool for species identification and benzimidazole resistance diagnosis in larval communities of small ruminant parasites. Exp. Parasitol. 95, 271–276.
Snedecor, G.W., Cochran, W.G., 1994. Statistical Methods. 8th edition. Iowa State University Press, IOWA, USA.
Swarnakar, C.P., Singh, D., Solanki, V.K., 2010. Prevalence of gastrointestinal parasites of sheep in semi-arid Rajasthan: a field study. Ind. J. Small Rum. 16, 221–227.
Tiwari, J., Kumar, S., Kolte, A.P., Swarnkar, C.P., Singh, D., Pathak, K.M., 2006. Detection of benzimidazole resistance in *Haemonchus contortus* using RFLP-PCR technique. Vet. Parasitol. 138, 301–307.
Uppal, R.P., Yadav, C.L., Godara, P., Rana, J.S., 1992. Multiple anthelmintic resistance in a field strain of *Haemonchus contortus* in goats. Vet. Res. Comm. 16, 195–198.
Varashney, T.R., Singh, T.P., 1976. A note on development of resistance of *Haemonchus contortus* worm against phenothiazine and thiabendazole in sheep. Ind. J. Anim. Sci. 46, 666–668.
Wooster, M.J., Woodgate, R.G., Chick, B.F., 2001. Reduced efficacy of ivermectin, abamectin and moxidectin against field isolates of *Haemonchus contortus*. Aus. Vet. J. 79, 840–842.
van Wyk, J.A., Bath, G.F., 2002. The FAMACHA system for managing haemonchosis in sheep by clinically identifying individual animals for treatment. Vet. Res. 33, 509–529.
van Wyk, J.A., Mayhew, E., 2013. Morphological identification of parasitic nematode infective larvae of small ruminants and cattle: a practical lab guide. Onder stepoort. J. Vet. Res. 80, 539–553.
van Wyk, J.A., Hoste, H., Kaplan, R.M., Besier, R.B., 2006. Targeted selective treatment for worm management—how do we sell rational programs to farmers? Vet. Parasitol. 139, 336–346.