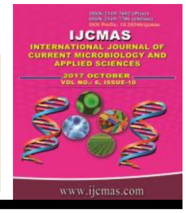
Int.J.Curr.Microbiol.App.Sci (2017) *6*(10): 790-796



International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 10 (2017) pp. 790-796**
Journal homepage: http://www.ijcmas.com

Original Research Article

https://doi.org/10.20546/ijcmas.2017.610.095

Emergence of Anthelmintic Resistance in *Haemonchus contortus* on Organized Sheep and Goat Farms of Sub-Himalayan Region of Northern India

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Keywords

Anthelmintic resistance, *Haemonchus contortus*, Sheep, Goats, Fenbendazole, Tetramisole HCl, Ivermectin.

Article Info

Accepted:
07 September 2017
Available Online:
10 October 2017

ABSTRACT

The present study was planned to know the status of anthelmintic resistance in gastrointestinal (GI) nematodes of two organized sheep and one goat farms of sub-Himalayan region of northern India using faecal egg count reduction test (FECRT). Three anthelmintics, fenbendazole (FBZ), tetramisole HCl (TMS) and ivermectin (IVM) of benzimidazole, imidazothiazole and macrocyclic lactone groups, respectively were tested at each farm. Resistance was detected against FBZ and TMS in all selected farms with FECRT% ranging from -170 to 63 and 18 to 91, respectively. However, GI nematodes were found susceptible to IVM in all farms with FECRT% ranging from 97 to 100. Pre-treatment coproculture revealed *Haemonchus contortus* as the predominant GI nematode followed by *Oesophagostomum columbianum* and *Trichostrongylus colubriformis*. Besides this, the larvae of *Ostertagia* spp. were also recorded in sheep of Kedarkatha farm. Post-coproculture results revealed the presence of 100% percent larvae of *H. contortus* only in animals treated with FBZ and TMS. This is the first report of *Haemonchus* being resistant to both FBZ and TMS at organized farms simultaneously from sub-Himalayan region of northern India.

Introduction

In India, small ruminants contribute in providing economic security to small, landless and marginal farmers. Parasitic diseases are important cause of production losses in small ruminants the world over. Of these, gastrointestinal (GI) nematodosis is a common parasitic infection of small ruminants in India including sub-Himalyan region of northern India (Yadav *et al.*, 2009). It is caused by mixed infections of GI nematodes. Among the various GI nematodes, *Haemonchus contortus* is the predominant parasite found throughout the year and is considered as the most pathogenic GI nematode responsible for impaired productivity in small ruminants throughout the world (Khalafalla *et al.*, 2011).

Anthelmintics are administered to animals even when they show non-specific clinical signs like diarhhoea or are found positive on faecal examination without estimation of intensity of infection. This has led to indiscriminate and frequent use of the drugs resulting in the emergence of drug resistance (Barton, 1980). Efficacy of various anthelmintics must be monitored regularly so that proper selection of anthelmintic can be done otherwise there will be huge economic losses due to cost of anthelmintics, sustained parasitic load due to ineffective worm control strategies and increased selection of resistant worms. The growing importance of these anthelmintic-resistant nematodes and the need for reliable information on their occurrence and spread has increased to rule out their occurrence in a proper area.

In India, the first report of anthelmintic resistance in *H. contortus* to phenothiazine and thiabendazole has been reported by Varshney and Singh (1976) in sheep from Central Sheep and Wool Research Station, Pashulok, Rhikish, Uttar Pradesh (now in Uttarakhand). Then after, various reports on emergence of anthelmintic resistance have been documented from different parts of India (Yadav, 1990; Laha *et al.*, 1999; Garg *et al.*, 2007; Jaiswal *et al.*, 2013; Rialch *et al.*, 2013; Pandey and Vatsya, 2013). However, there is scanty information on the prevalence of anthelmintic resistance from organized sheep and goat farms of sub-Himalayan region of northern India. So, the present study was planned with the objective to know the status of anthelmintic resistance against GI nematodosis at two organized sheep and one goat farms of sub-Himalayan region of northern India.

Materials and Methods

Location of study area

The study was carried out at two organized sheep and one organized goat farms located in sub-Himalayan region of northern India. Among the two sheep farms, one organized sheep farm was located at Kedarkatha (1,293 m above sea level), Chamoli district and the second one was situated at Samaliti (1,004 m above sea level), Bageshwar district. However, goat breeding farm selected in the study was located at Gwaldam (1,940 m above sea level), Chamoli district of Garhwal region. Each farm had its own pasture land on which the animals were grazed from early morning till late evening. All animals were dewormed 6-8 times in a year. Fenbendazole and tetramisole Hcl had been used for 8-10 years.

Selection of animals

Faecal samples from both sheep and goats of individual farms were collected and examined by modified Mc Master egg counting technique. Animals of the individual farm having faecal egg count more than 200 e. p. g. were included in the present study. The animal flocks selected were not administered any kind of anthelmintic treatment for last three months.

Anthelmintic treatment of animals

Sixty sheep and forty goats of either sex, aged between 1-3 years and naturally infected with mixed infections of gastrointestinal nematodes (GIN) at each farm were selected, numbered and weighed. The selected sheep were randomly divided into four groups *viz.* GI, GII, GIII and GIV of 15 animals each. However, goats were randomly divided into four groups *viz.* GI, GII, GIII and GIV of 10 animals each.

Three anthelmintics of three different classes *viz*. Benzimidazole (fenbendazole), imidazothiazole (tetramisole Hcl) and macrocyclic lactone (ivermectin) were tested in animals of each farm. Animals of GI, GII and GIII groups were treated with commercially available fenbendazole (Fentas-Intas Pharmaceuticls Ltd. Ahmedabad) @ 5 mg/kg body wt. orally (sheep) and @ 10 mg/kg body wt. orally (goats), tetramisole hydrochloride (Nilverm-Virbac Animal

Health India Pvt. Ltd., Mumbai) @ 15 mg/kg body wt. orally and ivermectin (Neomac-Intas Pharmaceuticls Ltd. Ahmedabad) @ 0.2 mg/ kg body wt. sub-cutaneously (s/c), respectively. GIV animals were kept as untreated infected control.

Faecal Egg Count Reduction Test (FECRT)

Faecal samples of each animal of treated and untreated groups were collected directly from rectum in separate polythene faecal bag on day 0 and 10 days post-treatment (DPT).

All the collected samples were kept in ice bag and brought to the laboratory and processed for the estimation of egg per gram of faeces (e.p.g.) using modified Mc Master technique (Coles *et al.*, 1992).

Coproculture and larval identification

Pooled faecal samples of each group of an individual farm were cultured for speciation of nematodes on day 0 of treatment and on 10 DPT. GI nematode larvae were identified on the basis of their morphological characters as described by Soulsby (1965).

Statistical analysis

The percent reduction in faecal egg counts was determined as per the guidelines of World Association for the Advancement of Parasitology. Data was also analysed for the calculation of variance of counts (S), variance of reduction and 95% upper and lower confidence limit (Coles *et al.*, 1992).

Arithmetic mean ($X= \sum X/N$) for post treatment FEC

Variance of count $[S^2=\{X^2 (\sum X)^2/N\} N-1]$

% FECR [100 {1-Xt/Xc }]

Variance of reduction on log scale $Y^2=S_t^2/N_tX_t^2+ S_c^2/ N_cX_c^2$

Calculation of 95% confidence limits-

Upper confidence limit 100 $[1-Xt/Xc^{\exp(-2.048 Y^{2)}}]$

Lower confidence limits100 $[1-Xt/Xc^{\exp(+2.048\, ó Y^{2)}}]$

Where X_t = arithmetic mean epg count of treated groups, X_c = arithmetic mean epg count of control group, S_t^2= variance of treated group, S_c^2= variance of control group, N_t= number of animals in treated group and N_c = number of animals in control group.

Interpretation of data

In FECRT, anthelmintic resistance was confirmed, if (i) the percent reduction in epg count was less than 95 % and (ii) the lower 95 % confidence interval was less than 90 %. If only one of the two criteria was met, the resistance was suspected.

Results and Discussion

The results of faecal egg count reduction test and confidence limit of the present study are presented in Table 1. The animals of GI, GII and GIII of each farm were treated with FBZ, TMS and IVM, respectively. In animals of GI, GII and GIII at Shamaliti sheep breeding farm, the percentage reduction in FEC recorded was -170, 18 and 97 with a lower 95% CI of 0, 0 and 93, respectively. However, the percentage reduction in FEC of -10, 24 and 100 with a lower 95% 0, 0 and 100, respectively was recorded in animals of GI, GII and GIII at Kedarkatha sheep breeding farm. While, the percentage reduction in FEC of 63, 91 and 100 with a lower 95% CI of 27, 82 and 99 was recorded in animals of GI, GII and GIII, respectively.

The coproculture and larval composition of each farm are presented in Table 2. In all selected organized sheep and goats farms, larval composition on pretreatment coproculture examination revealed *H. contortus* as predominant GI nematode (68-82%) followed by *O. columbianum* (11-20%) and *T. colubriformis* (5-15%). Besides this, the larvae of *Ostertagia* spp. (2-7%) were also recorded in sheep of Kedarkatha farm. In all treated animals, hundred percent larvae of *H. contortus* only were observed on 10DPT at each farm.

Based on FECRT data, fenbendazole and tetramisole resistance was observed at all the tested farms. However, GI nematodes in both sheep and goats of the farms were found susceptible to ivermectin. The results of post coproculture examination confirmed that *H. contortus* was resistant to drugs of benzimidazole and imidazothiazole groups in all selected organized sheep and goat farms.

In the present study, the percentage reduction in FEC in FBZ and TMS treated animals were less than 95% and lower 95% confidence levels in treated animals were less than 90%.

The post-treatment coproculture examination revealed the presence of only *H. contortus* third stage larvae. It was indicated that the strain of *H. contortus* was resistant to FBZ (benzimidazoles) and TMS (imidazothiazole). This is the first report of *H. contortus* being resistant to both FBZ and TMS at organized farms simultaneously from this part of India.

Animals of each farm were treated with FBZ and TMS since last 8-10 years and also received these drugs 6-8 times in a year. FBZ resistant *H. contortus* in small ruminants has also been reported by various workers from different parts of India (Yadav, 1990; Yadav *et al.,* 1996; Singh and Yadav, 1997; Swarnkar *et al.,* 1999; Swarnkar and Singh, 2012). Benzimidazole resistant *H. contortus* in field flocks of sheep and goats of sub-Himalayan region of northern India has also been reported by Laha *et al.,* (1999) and Rialch *et al.,* (2013). TMS resistant *H. contortus* has also been reported by Makvana and Singh (2009), Maharshi *et al.,* (2011) and Swarnkar and Singh (2012) from India. The prevalence of FBZ and TMS resistance might be due to frequent use of same anthelmintic for a long period (Maingi *et al.,* 1996). In India, this type of resistant *H. contortus* associated with frequent use of different anthelmintics has also been reported by Uppal *et al.,* (1992), Yadav and Uppal (1993) and Garg *et al.,* (2007).

IVM was found highly effective against mixed infections of GI nematodes at each farm with % FECR ranging from 97 to 100. Similar observation has also been reported by Uppal *et al.,* (1992), Laha *et al.,* (1999) and Garg *et al.,* (2007). However, Makvana and Singh (2009) and Jaiswal *et al.,* (2013) observed ivermectin resistant gastrointestinal nematodes in sheep and goats, respectively from Gujrat and Uttar Pradesh, India, respectively. The susceptibility of IVM in the selected farms might be due to less frequent use of IVM in routine deworming programme.

To avoid the failure of anthelmintic treatment in animals of these farms, it is also suggested that veterinarians of the respective farms should perform FECRT% test to check whether the drugs being used are effective.

Generally farmers or veterinarians treat the animals without accurate weighing resulting into under dosing of the drug. It can be easily avoided by taking the weight of heaviest animals of the flock and then calculate the dose of the drug for all the animals of the flock.

Table.1 Mean of faecal egg count, percent faecal egg count reduction, variance of count, 95% confidence limits in animals treated with different anthelmintics at various sheep and goat farms

Name of the farms	Groups	Arithmetic mean		%FECR	Variance of counts	95% confidence interval		Interpretation of data
		Day 0	10DPT			Upper limit	Lower limit	
Shamaliti sheep farm	GI	1940	2967	-170	7139167	-27	0	Resistance
	GII	910	897	18	1035524	65	0	Resistance
	GIII	1290	30	97	1357	99	93	Susceptible
	GIV	906	1100	-	1362500	-	-	No significance
Kedarkatha sheep farm	GI	380	497	-10	90524	39	0	Resistance
	GII	317	343	24	81381	61	0	Resistance
	GIII	460	0	100	0	-	100	Susceptible
	GIV	300	450	-	169643	39	0	No significance
Gwaldam goat farm	GI	1745	1175	63	934028	81	27	Resistance
	GII	3150	285	91	54472	94	82	Resistance
	GIII	2785	10	100	0	100	99	Susceptible
	GIV	2915	3135	-	3261139			No significance

Table.2 Coproculture examination of animals treated with various anthelmintics at different organized sheep and goat farms

Animals (Name of farms)	Groups	Percent larval composition							
		Pre-treatment (0 day)				10 days post treatment (10DPT)			
		H	Oe.	T	O	H	Oe.	T	O
Shamaliti sheep farm	GI	70	20	10	0	100	0	0	0
	GII	65	20	15	0	100	0	0	0
	GIII	82	10	8	0	100	0	0	0
	GIV	75	20	5	0	76	19	5	0
Kedarkatha sheep farm	GI	78	12	8	2	100	0	0	0
	GII	70	15	10	5	100	0	0	0
	GIII	73	17	6	4	100	0	0	0
	GIV	70	14	9	7	68	15	10	7
Gwaldum Goat farm	GI	68	17	15	0	100	0	0	0
	GII	76	14	10	0	100	0	0	0
	GIII	84	11	5	0	100	0	0	0
	GIV	81	13	6	0	80	15	5	0

H-*Haemoncus contortus*, Oe.-*Oesophagostomum columbianum*, T-*Trichostrongylus colubriformis*, O-*Ostertagia* spp.

Based on findings of the present study, it is suggested that FBZ and TMS cannot be used to control GI nematodosis in sheep and goats at the selected farms and it may be minimized either by the use of other effective anthelmintic or formulate another effective control strategies against GI nematodes.

Acknowledgements

Director Research, Experiment Station and Dean, College of Veterinary and Animal Sciences, G. B. Pant University of Agriculture and Technology, Pantnagar are also acknowledged for providing necessary facilities during the entire course of the study. Incharges of all the farms are also highly acknowledged for giving permission to carry out the present work.

Authors are highly thankful to Indian Council of Agricultural Research, New Delhi, India for giving sufficient funds to carry out the present research work in the form of "All India Network Progrmme on GI Parasitism (project grant code-137).

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How to cite this article:

Rajeev Ranjan Kumar, C.L. Yadav and Stuti Vatsya. 2017. Emergence of Anthelmintic Resistance in *Haemonchus contortus* on Organized Sheep and Goat Farms of Sub-Himalayan Region of Northern India. *Int.J.Curr.Microbiol.App.Sci.* 6(10): 790-796.
doi: https://doi.org/10.20546/ijcmas.2017.610.095