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Research Article

EFFICACY OF INDIVIDUAL AND COMBINATION OF ANTHELMINTICS AGAINST GASTROINTESTINAL NEMATODES IN GOATS

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ABSTRACT

Faecal egg count reduction (FECR) test was conducted to determine the efficacy of individual and combination of anthelmintics against gastrointestinal nematodes in goats. The experiment was conducted in two parts in animals with eggs (>150) per gram faeces. In first part, goats (n=81) were divided into five groups (A-I, A-II, A-III, A-IV and A-V) having 12, 12, 17, 24 and 16 goats, respectively. Groups A-I, A-II, A-III and A-IV were treated with fenbendazole (@ 10mg/kg b.wt. orally), ivermectin (0.4 mg/kg b.wt subcutaneously), closantel (@ 15 mg/kg b. wt orally) and nilzan (@ 1ml /3kgb.wt. orally), respectively. Groups A-V served as control. Faecal egg counts of each animal in each group were ascertained on 0 day and 12th day post treatment (PT) by the modified Mc Master technique. FECR percentage in groups A-I, A-II, A-III and A-IV was 79.04, 87.01, 89.35 and 100%, respectively. Identification of infective larvae from both pre-and post-treatment faecal culture revealed the predominance of *Haemonchus contortus.* The results indicated the resistance of fenbendazole, ivermectin and closantel against *H.contortus.*

In the second part, 32 goats were divided into two equal groups (B-I and B-II) of 16 goats each. Group B-I was administered fenbendazole (@ 10mg/kg b.wt. orally) + ivermectin (@ 0.4 mg/kg b.wt. subcutaneously) and group B-II served as control. FECR was 98.6% on 12th day PT. Coproculture from pretreatment faecal culture revealed the predominance of *H.contortus* whereas no larvae were recovered from post-treatment faecal culture. Thus, the present study indicated that the combination of fenbendazole+ivermectin was more effective against resistant *H.contortus* than when these drugs were administered individually.

Key words: Anthelmintic combinations, fenbendazole, ivermectin, levamisole, nilzan, goat, *Haemonchus contortus*

Goat industry plays an important role in the economy of the country due to low initial input, less maintenance cost, minimum disease exposure and quick, high and profitable returns. Gastrointestinal (GI) parasites are responsible for significant production losses in grazing ruminants throughout the world (Sykes, 1994). In India, *Haemonchus contortus* is the most prevalent and pathogenic GI nematode and is responsible for high morbidity and mortality (Yadav, 1997). The control of this parasite is not only important but essential for profitable goat farming and it relies primarily on the use of anthelmintic drugs. Frequent and indiscriminate use of chemical compounds has resulted in fast emergence of anthelmintic resistance against this parasite. The high frequency of parasitism by GI nematodes and the increase of anthelmintic resistance are threatening goat industry (Beatriz *et al*., 2013). It is important to state that resistance has been recorded against all the broad spectrum classes used for their control throughout the world (Jabbar *et al*., 2008; Kumsa *et al*., 2010) including India (Singh and Yadav, 1997). No new class of anthemintics has been introduced in market in almost last 25 years (Kaplan, 2004). Therefore, regular monitoring of status of anthelmintic efficacy for the existing drugs is required, at least once in two years in an organized flock for suitable worm control programme. Further, to control the evolution of resistance, a simultaneous use of minimum two anthelmintics had also been proposed (McKenna *et al*., 1996; Andrews, 2000). The aim of the present study was to determine the efficacy of individual drug like fenbendazole, ivermectin, closantel and nilzan and the combination (fenbendazole+ ivermectin) against naturally occurring gastro-intestinal nematodes of goats.

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MATERIALS AND METHODS

During September, 2012, a study was conducted at the Goat Breeding Farm of the University to determine the efficacy of fenbendazole, ivermectin, closantel, nilzan and the combination of fenbendazole+ivermectin against gastrointestinal nematodes of naturally infected goats using faecal egg count reduction test (FECRT). In first experiment, 81 goats (2 to 5 years of age) which were

naturally infected with gastrointestinal nematodes and had eggs per gram (EPG) of faeces> 150 counts prior to treatment were used for FECRT. The selected animals had not been administered any anthelmintic during the previous two months. (Ivermectin, closantel and rafoxanide had been used during 2006-2012 (Table 3). These animals were weighed, identified, their EPG estimated and divided into five groups (A-I, A-II, A-III, A-IV and A-V) of, 12, 12, 17, 24 and 16 animals each. The number of animals in each group varied depending upon the availability and convenience of farm management. Animals of Groups A-I, A-II, A-III and A-IV were treated with fenbendazole (Panacur, Intervet India Pvt. Ltd., Pune) @ 10mg/kg b. wt. orally, ivermectin (Virbac Animal Health, Mumbai) @ 0.4 mg/kg b.wt. subcutaneously, closantel (Zydus Animal Health Ltd., Ahmedabad) @ 15 mg/kg b. wt. orally and nilzan (Virbac Animal Health, Mumbai) @ 1ml/3 kg b. wt. orally, respectively. Group A-V served as control. Faecal egg count of each animal was ascertained on 0 day and 12th day post treatment (PT) by the modified Mc Master technique to an accuracy of one egg counted representing 50 EPG. Pooled faecal cultures at 27±2ºC for 7 days were made to recover infective larvae, L_3, from each group on day 0 and 12th day PT. The infective larvae were identified as per criteria of Keith (1953).

In another experiment, 32 goats were divided into two groups (B-I and B-II) of 16 animals each. Group B-I was administered fenbendazole (10mg/kg b.wt. orally) +ivermectin (0.4 mg/kg b.wt subcutaneously). Group B-II served as control. Faecal egg counts of each animal were ascertained on 0 day and 12th day PT by the modified Mc Master technique. Pooled faecal cultures were made to recover infective larvae, L_3, from each group on day 0 and 12th day PT for the identification of infective larvae and estimation of their percentage. Percent reduction in faecal egg counts and confidence intervals (95%) were calculated by the method of the World Association for the Advancement of Veterinary Parasitology (WAAVP, Coles *et al.,* 1992). Resistance was considered to be present in the worm population against an anthelmintic when the egg count reduction following treatment was less than 95% and the lower and upper confidence limits were less than 90%.

RESULTS AND DISCUSSION

Feacal egg counts (Mean±S.E.) of individual anthelmintics on day 0 and 12th day post treatment (PT), percentage reduction in faecal egg count (FECR%), variance, upper and lower confidence limit (95%) are shown in Table 1. Fenbendazole, ivermectin, closantel and nilzan reduced the faecal egg counts by 79.04, 87.01, 89.35 and 100% respectively. The lower 95% confidence limit was 100% for nilzan and less than 90% for fenbendazole, ivermectin and closantel indicating resistance. The nematode larvae recovered from faecal culture in all the four treatment groups comprised mainly of *H. contortus* (Table 2). Earlier, Singh and Yadav (1997) estimated the efficacy of fenbendazole and ivermectin to be 76 and 100%, respectively on this farm. There after the use of fenbendazole continued along with other drugs though rarely, as per availability and convenience of management. Since 2001 ivermectin was proposed to be used in this farm along with closantel and had been continuously being used along with doramectin which also belong to same

Table 1
Response to individual and combination of anthelmintics administered in goats naturally infected with gastrointestinal nematodes at goat breeding farm

Group	Anthelmintic	No. of goats	Faecal egg count (Mean±S.E.) on day		Faecal egg count reduction on day 12 PT*		Confidence limit at 95%	
			0	12	%	Variance	Upper	Lower
A-I	Fenbendazole	12	1141.66± 343.22	504.16±101.79	79.04	0.079	88.23	62.70
A-II	Ivermectin	12	966.67± 166.70	312.5± 71.71	87.01	0.091	93.00	75.83
A-III	Closantel	17	2146± 412.04	256.25±78.67	89.35	0.127	94.87	77.88
A-IV	Nilzan	24	2393.75± 204.96	0	100	0.038	100	100
A-V	Control	16	2365.63± 493.68	2406.25± 472.34	0	-	-	-
B-I	Fenbendazole+ Ivermectin	12	2612.5± 676.44	34.38± 13.78	98.61	0.207	99.45	96.48
B-II	Control	12	2390.62± 488.92	2484.37± 479.21	0	-	-	-

*Per cent reduction in faecal egg counts was calculated by the method of the WAAVP (Coles *et al.,* 1992) using the formula: FECRT % = 100 (1- Arithmatic mean of treated group/Arithmatic mean of control group). PT=Post-treatment

anthelmintic class as ivermectin i.e. macrocyclic lactones during last 11 years (2-4 times per year). Anthelmintic effect of fenbendazole remained unchanged in the present study as the use of this drug continued along with other drugs though rarely. Jackson (1993) reported that when the predominant resistance species has a high biotic potential and is also highly pathogenic as in case of *H. contortus*, then the risk associated with reintroduction of the drug is very high. Further, Jackson and Coop (2000) reported that reintroduction of the drug can result in a rapid return to resistance state.

Table 2
Effect of individual and combination of anthelmintics on different genera of gastrointestinal nematodes of goats

Group	Anthelmintic	Species	Per cent larval composition on day	
			0	12
A-I	Fenbendazole	*Haemonchus contortus*	91	95
		Trichostrongylus sp.	2	2
		Oesophagostomum sp.	1	--
		Bunostomum sp.	1	--
		Strongyloides papillosus	5	3
A-II	Ivermectin	*Haemonchus contortus*	89	94
		Trichostrongylus sp.	2	1
		Oesophagostomum sp.	1	--
		Bunostomum sp.	2	1
		Strongyloides papillosus	6	4
A-III	Closantel	*Haemonchus contortus*	90	92
		Trichostrongylus sp.	2	2
		Oesophagostomum sp.	1	1
		Bunostomum sp.	2	1
		Strongyloides papillosus	5	4
A-IV	Nilzan	*Haemonchus contortus*	90	--
		Trichostrongylus sp.	3	--
		Oesophagostomum sp.	1	--
		Bunostomum sp.	2	--
		Strongyloides papillosus	4	--
A-V	Control	*Haemonchus contortus*	91	92
		Trichostrongylus sp.	2	1
		Oesophagostomum sp.	1	1
		Bunostomum sp.	2	1
		Strongyloides papillosus	4	5
B-I	Fenbendazole +Ivermectin	*Haemonchus contortus*	91	--
		Trichostrongylus sp.	1	--
		Oesophagostomum sp.	1	--
		Bunostomum sp.	2	--
		Strongyloides papillosus	5	--
B-II	Control	*Haemonchus contortus*	93	92
		Trichostrongylus sp.	2	1
		Oesophagostomum sp.	1	1
		Bunostomum sp.	1	1
		Strongyloides papillosus	3	5

The present study revealed presence of moderate to slight resistance of *H. contortus* to ivermectin in goats. Factors such as extensive use of anthelmintic (Prichard, 1994; Coles, 1999; Sangster and Gill, 1999) and frequent usage of the same group of anthelmintic (Martin *et al*., 1982; Singh and Yadav, 1997; Das and Singh, 2005) may result in the development of anthelmintic resistance. Moreover, goats metabolize anthelmintics much more rapidly than other livestock (Hennessy, 1994; Conder and Campbell, 1995) and have lower bioavailability of drugs after oral administration than sheep. In the present study, history of deworming revealed that ivermectin was being used @ 0.2 mg/kg b.wt i.e. at half the dose required by the animal resulting in under dosing.

Closantel showed slight resistance to *H. contortus* in goat population at the University farm. Though the chemical was administered at recommended dose since 1996, 2-4 times a year (Table 3) yet the prolonged and frequent use of the drug may contribute to the selection of resistance population. Further, the reduced accumulation of drug in parasite body by mechanism such as reduced feeding, failure to dissociate the drug-albumin complex in the gut or increased efflux of closantel from resistant worms attribute to anthelmintic resistance (Rothwell and Sangster, 1997). Gupta *et al*. (2003) also reported *H. contortus* resistance to closantel in sheep of western Haryana.

The faecal examination of goat population also showed the presence of paramphistome eggs, resulting in selection of nilzan drug for efficacy trial. The administration of nilzan at recommended dose revealed 100% reduction in nematode population by FECRT test (Table 1). The drug had no history of use on the farm after 1999 (Table 3). The complete reversion to susceptibility of levamisole (class imidazothiazole) on this farm was reported by Singh *et al.* (2013). As levamisole present in nilzan also belongs to imidazothiazole class, so it was also 100% effective against gastrointestinal nematodes.

Various records of FECRT test like feacal egg counts (Mean±S.E.) on 0 and 12th day PT, FECR%, variance, upper and lower confidence limit in goats naturally infected with gastrointestinal nematodes and treated with combination of drug (fenbendazole + ivermectin) are shown in Table 1. The results revealed that fenbendazole+ivermectin reduced the faecal egg count by 98.6% on 12th day PT. The combination of anthelmintics was found to be completely effective against

Table 3
The management routines on the goat breeding farm

During the years	Total animals	Breeds	Grazing system	Anthelmintics used	No. of treatments/ year
1990-1995	147	Beetal and Black Bengal	Permanent	Fenbendazole, morantel and levamisole	5-6
1996-2000	170	Beetal and Black Bengal	Permanent	Morantel (upto 1997), levamisole (upto 1999), Fenbendazole and closantel	4
2001-2005	170	Beetal and Black Bengal	Permanent	Closantel, doramectin and rafoxanide	2-5
2006-2012	150	Beetal and Jakhrana	Permanent	Doramectin, rafoxanide, ivermectin and closantel	2-5

all the gastrointestinal nematodes as no larvae were detected in the PT faecal culture group when compared with control group (Table 2). This indicates that combination of fenbendazole+ivermectin was more effective against resistant *H. contortus* species than when these drugs were administered separately. These findings are in agreement with previous findings by Miller and Craig (1996) and Pomroy *et al*. (1992).

Previously, Anderson *et al.* (1998) had proposed that use of mixture of drugs from different chemical families is a valid strategy to delay the development of resistance and suggested that drug combinations may be efficacious against resistant nematode strains where the failure of individual drugs is documented. Further, McKenna *et al.* (1996) and Andrews (2000) had also proposed a simultaneous use of minimum two anthelmintics to control the evolution of resistance.

The present study thus revealed that combinations of anthelmintics from different chemical families may be efficacious against resistant nematode strains where the failure of individual drug is present. The finding has great economic significance as anthelmintic resistance in gastro-intestinal nematodes against three broad spectrum families (benzimidazole, imidazothiazoles and macrocyclic lactones) has been reported throughout the world.

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REFERENCES

Anderson, N., Martin, P.J. and Jarrett, R.G. (1998). Mixture of anthelmintics: A strategy against resistance. *Aust. Vet. J.* **65**: 62-64.

Andrews, S.J. (2000). The efficacy of levamisole, and a mixture of oxfendazole and levamisole, against the arrested stages of benzimidazole-resistant *Haemonchus contortus* and *Ostertagia circumcincta* in sheep.*Vet. Parasitol.* **88**: 139-146.

Beatriz, R.C., Sara VilarDantas, S. and Franklin, R.C. (2013). Dairy goat production in the Brazilian semiarid region: Integrated gastrointestinal nematodes control to overcome anti-helminthic resistance. *Pesquisa Veterinaria Brasileira*. **33**: 901-908.

Coles, G.C. (1999). Anthelmintic resistance and the control of worms. *J. Med. Microbiol*. **48**: 323-425.

Coles, G.C., Bauer, C., Borgsteede, F.H.M., Geerts, S., Klei, T.R., Taylor, M.A. and Waller, P.J. (1992).World Association for the Advancement of Veterinary Parasitology (WAAVP) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. *Vet. Parasitol.* **44**: 35-44.

Conder, G.A. and Campbell, W.C. (1995). Chemotherapy of nematode infections of veterinary importance with special reference to drug resistance. *Adv. Parasitol.* **35**: 1-84.

Das, M. and Singh, S. (2005). Anthelmintic resistance to nematodes in sheep and goat farms in Hisar. *J. Vet. Parasitol.* **19**: 103-106.

Gupta, S.K., Chaudhri, S.S. and Poonia, J.S. (2003). Preliminary report of closantel against *Haemonchus contortus*in sheep of western Haryana. *Indian J. Anim. Sci.***73**: 1024-1026.

Hennessy, D.R.(1994). The disposition of antiparasitic drugs in relation to the development of resistance by parasites of livestock. *Acta Tropica*. **56**: 125-141.

Jabbar, A., Iqbal, Z., Saddiqi, H.A., Babar, W. and Saeed, M. (2008). Prevalence of multiple anthelmintic resistant gastrointestinal nematodes in dairy goats in a desolated tract (Pakistan). *Parasitol. Res*. **103**: 29-35.

Jackson, F. (1993). Anthelmintic resistance-the state of play. *Br. Vet. J.* **49**: 123-128.

Jackson, F. and Coop, R.L. (2000).The development of anthelmintic resistance in sheep nematodes. *Parasitol.* **120**: 95-107.

Kaplan, R.M. (2004). Drug resistance in nematodes of veterinary importance: a status report. *Trends Parasitol*. **20**: 477-481.

Keith, R.K. (1953). The differentiation of infective larvae of some nematode parasites of cattle. *Aust. J. Zool.* **1**: 223-235.

Kumsa, B., Debela, E. and Megersa, B. (2010). Comparative efficacy of albendazole, tetramisole, ivermectin against gastrointestinal nematodes in naturally infected goats in Ziway, Oromia

Regional State (Southern Ethiopia). *J. Anim. Vet. Adv.* **9**: 2905-2911.

Martin, P.J., Anderson, N.J., Jarrett, R.G., Brown, T.H. and Lord, G.E. (1982). Effect of preventive and suppressive control scheme on the development of thiabendazole resistance in *Ostertagia* spp. *Aust. Vet. J*. **58**: 185-190.

McKenna, P.B., Allan, C.M. and Taylor, M.J. (1996). The effectiveness of benzimidazole-levamisole combination drenches in the presence of resistance to both benzimidazole and levamisole anthelmintics in New Zealand sheep. *N. Z. Vet. J*. **44**:116-118.

Miller, D.K. and Craig, T.M., (1996). Use of anthelmintic combinations against multiple resistant *Haemonchus contortus* in Angora goats. *Small Ruminant Res.***19**: 281-283.

Pomroy, W.E., Whelan, N., Alexander, A.M., West, D.W., Stafford, K., Adlington, B.A. and Calder, S.M. (1992). Multiple resistance in goat-derived *Ostertagia* and the efficacy of moxidectin and combinations of other anthelmintics. *N. Z. Vet. J.* **40**: 76-78.

Prichard, R. (1994). Anthelmintic resistance. *Vet. Parasitol*. **54**: 259-268.

Rothwell J. and Sangster, N. (1997). *Haemonchus contortus*: the uptake and metabolism of closantel. *Int. J. Parasitol.* **27**: 313-319.

Sangster, N.C. and Gill, J. (1999). Pharmacology of anthelmintic resistance.*Parasitol.Today*. **15**: 141-146.

Singh, P., Singh, S. and Poonia, J.S. (2013). Status of anthelmintic resistance against gastrointestinal nematodes in an organized goat farm. *Vet. Pract*. **14**: 55-57.

Singh, S. and Yadav, C.L. (1997). A survey of anthelmintic resistance by nematodes on three sheep and two goat farms in Hisar (India). *Vet. Res. Commun*. **21**: 447-451.

Sykes, A.R. (1994). Parasitism and production in farm animals. *Anim. Prodn*. **59**: 155-172.

Yadav, C.L. (1997). Premature ovine births caused by *Haemonchus contortus*. *Indian Vet. J*. **74**: 983-984.