Haryana Vet. (June, 2015) **54 (1)**, 11-14

Research Article

STATUS OF ANTHELMINTIC RESISTANCE OF LEVAMISOLE AND IVERMECTIN AND EFFICACY OF THEIR COMBINATION AGAINST GASTROINTESTINAL NEMATODE IN SHEEP

SATYAVIR SINGH[1], SUKHDEEP VOHRA[1], SARVAN KUMAR[2]* and J.S. POONIA[3]
[1]Department of Veterinary Parasitology; [2]Department of Veterinary Pathology
[3]Department of Animal Genetics and Breeding, College of Veterinary Sciences
Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar-125 004, India

Received: 22.01.2015, Accepted: 21.05.2015

ABSTRACT

Faecal egg count reduction test (FECRT) was conducted to determine the efficacy of levamisole and ivermectin alone and in combination against gastrointestinal nematodes in sheep. The experiment was conducted in two parts in animals with eggs (>150) per gram faeces. In first part, sheep (n=45) were divided into three groups (A-I, A-II and A-III) having 15 animals in each group. Groups A-I and A-II were treated with levamisole (7.5mg/kg b.wt. subcutaneously) and ivermectin (0.2 mg/kg b.wt. subcutaneously), respectively. Group A-III served as untreated control. Faecal egg counts of each animal in each group were ascertained on 0 and 11th day post treatment (PT) by modified Mc Master technique. Percent FECR in groups A-I and A-II was 92.5 and 91.34%, respectively. Identification of infective larvae from both pre- and post treatment faecal culture revealed the predominance of *Haemonchus contortus*. The results indicated the partial resistance of levamisole and ivermectin against *H. contortus.* In the second part, 30 sheep were divided in two equal groups (B-I and B-II) of 15 sheep each. Group B-I was administered levamisole (@7.5 mg/kg b. wt. subcutaneously)+ivermectin (0.2 mg/kg b. wt. subcutaneously) and group B-II served as control. FECR was 99.37% on 11th day PT in group B-I. Coproculture from pre-treatment faecal samples revealed the predominance of *H. contortus* whereas no larvae were recovered from post treatment (PT) faecal culture in group B-I. Thus, the present study indicated that the combination of levamisole+ivermectin was more effective against resistant *H. contortus* than when these drugs were administered individually.

Key words: Anthelmintic combinations, *Haemonchus contortus,* ivermectin, levamisole, sheep

Gastrointestinal (GI) parasites are a major source of economic loss to sheep producer's worldwide (Halliday *et al*., 2012). Among these, *Haemonchus contortus* is the most prevalent and pathogenic GI nematode responsible for high mortality and morbidity (Kumar *et al*., 2015). Currently, helminths are kept in check using a variety of either natural or synthetic anthelmintics. Frequent and indiscriminate use of these chemical compounds contributed to rapid emergence of anthelmintic resistance (Singh and Yadav, 1997). Therefore, for maintenance of efficacy of the available anthelmintics, regular monitoring of the status of anthelmintic resistance is required, at least once in two years; this is an integral part of worm control programme. Further, to control the evolution of resistance, simultaneous use of a minimum of two anthelmintics had been proposed (Andrews, 2000). Parasitologists generally agree that if delaying resistance is the prime objective, it is better to use a combination of two or more effective broad spectrum anthelmintics than using them alone. Hence the present study was designed to determine the efficacy of levamisole and ivermectin individually and subsequently their combination against naturally occurring gastro-intestinal nematodes of sheep.

*Corresponding author: drsrvn38@gmail.com

MATERIALS AND METHODS

During April 2014, a study was conducted at the Sheep Breeding Farm of the University to determine the efficacy of levamisole and ivermectin alone and their combination against gastrointestinal nematodes of naturally infected sheep using faecal egg count reduction test (FECRT). In the first experiment, sheep (n=45; 2 to 5 years of age), naturally infected with GI nematodes and egg counts (>150), were selected for the study. The selected animals had not been administered any anthelmintic in the past two months. These animals were weighed, identified and were divided into three groups (A-I, A-II and A-III) of 15 animals each. Groups A-I and A-II were administered subcutaneously levamisole (Zoetis India Limited, Haridwar, Uttarakhand) @ 7.5mg/kg b.wt. and ivermectin (Virbac Animal Health, Mumbai) @ 0.2mg/kg b.wt., respectively. Group A-III served as untreated control. Faecal egg count of each animal was ascertained on 0 day and 11th 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±2^{0}C for 7 days were made to recover infective larvae (L_3) from each group on day 0 and 11th PT. The infective larvae were identified as per criteria of Keith (1953).

Later, to determine the efficacy of combinations of levamisole and ivermectin, 30 sheep naturally infected with GI nematodes and had an EPG of faeces more than 150 counts prior to treatment were used for FECRT. These animals were weighed, identified, their EPG estimated and then divided into two groups (B-I and B-II) of 15 animals each. Group B-I was given levamisole and ivermectin in combination in the doses as specified earlier. Group B-II served as untreated control. Faecal egg counts of each animal were ascertained on 0 day and 11th day PT as mentioned earlier. 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 (Coles *et al.*, 1992). The combination of drugs was considered to be fully effective when it reduced the egg counts by more than 95% and lower and upper confidence limits were higher than 90%.

RESULTS AND DISCUSSION

The results revealed that levamisole caused 92.5% reduction in faecal egg counts on 11th day PT while FECR % due to ivermectin was 91.34% (Table 1) indicating mild resistance against levamisole and ivermectin. Group A-III had significantly ($P<0.05$) higher faecal egg counts on 11th day PT than groups A-I and A-II (Table 1).

Table 2 shows the effect of anthelmintics on different genera of GI nematodes of sheep at surveyed sheep farm. Based on faecal culture, the principal nematode larvae recovered in the two treatment groups (A-I and A-II) comprised mainly of *H. contortus*. In India, strains of *H. contortus* resistant to various groups of anthelmintics have been reported in small ruminants from different agroclimatic zones (Singh and Yadav, 1997; Swarnkar *et al*., 1999; Singh *et al*., 2013).

The result based on larval culture showed that the worms surviving levamisole treatment were mostly *H. contortus* (95%) followed by *Strongyloides papillosus* (3%). Previously, Singh and Yadav (1997) reported 24% efficacy of levamisole on this farm. Hence, there was partial reversion to susceptibility as a result of discontinuing the use of levamisole for 17 years on the farm studied. Earlier, studies by Martin *et al.* (1988) reported reversion to benzimidazole susceptibility particularly after use of levamisole. Singh and Gupta (2009) reported partial reversion to susceptibility of fenbendazole and levamisole resistant strain of *H. contortus* at an organized sheep farm after switching over to ivermectin and closantel for 12 years. However, Zajac and Gipson (2000) reported complete reversion to susceptibility of levamisole after withdrawal of drug for three years in sheep. Das and Singh (2010) observed complete reversion to susceptibility of fenbendazole resistant *H. contartus* after withdrawal of drug for 10 years. It is clear from the present study that even if the drug is withdrawn for a prolonged period under permanent grazing system, reversion to complete susceptibility is delayed. In developed countries, where free range grazing system and other animal husbandry practices like rotational grazing etc. are followed, reversion takes place earlier (Martin *et al.*, 1988; Zajac and Gipson, 2000).

The results based on larval culture in sheep given ivermectin alone showed that the worms surviving ivermectin treatment were mostly *H. contortus* (98%) followed by *S. papillosus* (2%). Thus, the present study revealed the presence of ivermectin resistant *H. contortus* population in sheep which was detected for the first time on this farm. Ivermectin resistance to GI nematodes in sheep had also been reported by many workers from different countries (McMohan *et al*., 2013; Holm *et al*., 2014). *H. contortus* has been reported to be the main species involved in drug resistance. Earlier, Singh and Yadav (1997) and Das and Singh (2005) found ivermectin and doramectin, respectively to be 100% effective against *H. contortus* on this farm. Since then macrocyclic lactone class anthelminitcs are being used. Continuous use of this anthelmintic (2-3 times per year) for the last 10 years at this farm could be the reason for development of resistance.

Extensive use of anthelmintics has been reported to be one of the reasons for development of resistance (Coles, 1999). It has been observed that frequent use of the same group of anthelmintic may result in the development of resistance (Singh and Yadav, 1997; Das and Singh, 2005). The selection pressure exerted by regular use of anthelmintic also aids the development of anthelmintic resistance. Coles *et al*. (1999) have reported the development of anthelmintic resistance even when only two or three treatments were given annually. Frequent use of ivermectin/doramectin without alternation with other drugs has also been reported as the reason for the fast development of resistance in *H. contortus* in Scotland and the US (Howell *et al*., 2008). Ivermectin resistance is recorded for the first time in sheep of this farm and second time from India.

The results of this study revealed that levamisole and ivermectin combination reduced the faecal egg count by 99.37% on 11th day PT (Table 1). The combination of anthelmintics was found to be completely effective against all the GI nematodes as no larvae were detected in the PT faecal culture group (B-I) when compared with control group (B-II) (Table 2). This indicated that this

Table 1

Response to individual and combination of anthelmintics administered in sheep naturally infected with gastrointestinal nematodes at an organized farm

Group	No. of sheep	Faecal egg counts on days PT (Mean±S.E.)		Percentage reduction and variance on 11th day PT		Confidence limit at 95%	
		0 day	11 day	%	Variance	Upper	Lower
A-I	15	2956.67±539.21	220±054.53	92.50	0.073	95.73	87.09
A-II	15	2183.33±262.66	256.67±068.13	91.34	0.082	95.18	84.43
A-III	15	2933.33±318.80	2963.33±317.01	-	-	-	-
B-I	15	3506.67±764.84	20±013.16	99.37	0.457	99.84	97.50
B-II	15	3316.67±491.52	3200±492.27	-	-	-	-

PT=Post-treatment; Group A-I=Treated with levamisole @ 7.5 mg/kg b.wt. subcutaneously; Group A-II=Treated with ivermectin @ 0.2 mg/kg b.wt. subcutaneously; Group A-III=Untreated control; Group B-I=Treated with levamisole (@ 7.5 mg/kg b.wt. subcutaneously)+ivermectin (0.2 mg/kg b.wt. subcutaneously); Group B-II=Untreated control

Table 2

Effect of levamisole and ivermectin individually and in combination on different genera of gastrointestinal nematodes of sheep at an organized farm

Group	Anthelmintic	Species	Percent larval composition on day	
			0	11
A-I	Levamisole	*Haemonchus contortus*	92	95
		Trichostrongylus sp.	1	2
		Oesophagostomum sp.	1	-
		Bunostomum sp.	1	-
		Strongyloides papillosus	5	3
A-II	Ivermectin	*H. contortus*	90	98
		Trichostrongylus sp.	4	-
		Oesophagostomum sp.	-	-
		Bunostomum sp.	-	-
		S. papillosus	6	2
A-III	Untreated	*H. contortus*	89	91
		Trichostrongylus sp.	3	2
		Oesophagostomum sp.	1	2
		Bunostomum sp.*S.*	2	1
		papillosus	5	4
B-I	Levamisole +	*H. contortus*	94	-
	Ivermectin	*Trichostrongylus* sp.	1	-
		Oesophagostomum sp.	1	-
		Bunostomum sp.	1	-
		S. papillosus	3	-
B-II	Untreated	*H.contortus*	91	92
		Trichostrongylus sp.	2	1
		Oesophagostomum sp.	1	1
		Bunostomum sp.	2	1
		S. papillosus	4	5

Group A-I=Treated with levamisole @ 7.5 mg/kg b.wt. subcutaneously; Group A-II=Treated with ivermectin @ 0.2 mg/kg b.wt. subcutaneously; Group A-III=Untreated control; Group B-I=Treated with levamisole (@ 7.5 mg/kg b.wt. subcutaneously)+ivermectin (0.2 mg/kg b.wt. subcutaneously); Group B-II=Untreated control

combination was effective against resistant *H. contortus* species. Previously, Anderson *et al.* (1998) had proposed that use of mixture of drugs from different chemical families to be 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, Andrews (2000) had also proposed a simultaneous use of a minimum of two anthelmintics to control the evolution of resistance.

Based on the results of this study it may be concluded that anthelmintics should be used judiciously and the anthelmintic resistance may be evaluated atleast once in two years. The chemicals to which the parasites have developed partial resistance should be discontinued or used in combination with chemicals having different mode of action to eliminate the resistant worms. Combinations of anthelmintics from different chemical families may be efficacious against resistant nematode strains where the failure of individual drug is present. This finding has great economic significance as anthelmintic resistance in GI nematodes against two broad spectrum families (benzimidazole, imidazothiazoles and macrocyclic lactones) has been reported throughout the world and no new anthelmintics with different mode of action are expected in the market in the near future as development and release of new anthelmintic may take 6-8 years. The cost involved in this process was estimated to be approximately US$230 million (McKellar, 1994).

ACKNOWLEDGEMENTS

Thanks are due to the Scientist Incharge and supporting staff at Sheep Breeding Farm, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar for their help and cooperation.

REFERENCES

Anderson, N., Martin, P.J. and Jarrett, R.G. (1998). Mixture of anthelmintics: A strategy against resistance. *Australian 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.

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.

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

Das, M. and Singh, S. (2010). Effect of withdrawl of anthelmintics on fenbendazole and morantel resistance status of *Haemonchus contortus* in sheep and goats. *Haryana Vet.* **49**: 22-24.

Halliday, A.M., Lainson, F.A., Yaga, R., Inglis, N.F., Bridgett, S., Nath, M. and Knox, D.P. (2012). Transcriptional changes in *Teladorsagia circumcincta* upon encountering host tissue of differing immune status. *Parasitol.* **139**: 387-405.

Holm, S.A., Sorensen C.R.L., Thamsborg, S.M. and Enemark H.L. (2014). Gastrointestinal nematodes and anthelmintic resistance in Danish goat herds. *Parasite* **21**: 37.

Howell, S.B., Burke, J.M., Miller, J.E., Terrill, T.H., Valencia, E., Williams, M.J., Williamson, L.H., Zajac, A.M. and Kaplan, R.M. (2008). Prevalence of anthelmintic resistance on sheep and goat farms in the southeastern United States. *J. American Vet. Med. Assoc.* **233**: 1913-1919.

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

Kumar, S., Jakhar, K.K., Singh, S., Potliya, S., Kumar, K. and Pal M. (2015). Clinico-pathological studies of gastrointestinal tract disorders in sheep with parasitic infection. *Vet. World* **8(1)**: 29-32.

Martin, P.J., Anderson, N., Brown, T.H. and Miller, D.W. (1988). Changes in resistance of *Ostertagia* spp. to thiabendazole following natural selection or treatment with levamisole. *Int. J. Parasitol.* **18**: 333-340.

McKellar, O.A. (1994). Chemotherapy and delivery systems-helminths. *Vet. Parasitol*. **54:** 249-258.

McMahon, C., Bartley, D.J., Edgar, H.W.J., Ellison, S.E., Barley, J.P., Malone, F.E., Hanna, R.E.B., Brennan, G.P. and Fairweather, I. (2013). Anthelmintic resistance in Northern Ireland (I): Prevalence of resistance in ovine gastrointestinal nematodes, as determined through faecal egg count reduction testing. *Vet. Parasitol*. **195(1–2)**: 122-130.

Singh, S. and Gupta, S.K. (2009). Studies on development of reversion to susceptibility of fenbendazole and levamisole resistant *Haemonchus contortus* strain in sheep. *Haryana Vet.* **48**: 100-102.

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.

Swarnkar, C.P., Singh, D., Khan, F.A. and Bhagwan, P.S.K. (1999). Multiple anthelmintic resistance in *Haemonchus contortus* of sheep. *Indian J. Anim. Sci*. **69**: 547-549.

Zajac, A.M. and Gipson, T.A. (2000). Multiple anthelmintic resistance in a goat herd. *Vet. Parasitol.* **87**: 163-172.