

Short Communication

DETECTION OF MODERATE ANTHELMINTIC RESISTANCE IN CLOSTANTEL AND MACROCYCLIC LACTONE IN ORGANIZED CENTRAL SHEEP BREEDING FARM OF HARYANA, INDIA

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ABSTRACT: This study was performed to evaluate the efficacy of currently used anthelmintics in organized central breeding sheep farm (CSBF) in Hisar, Haryana using faecal egg count reduction test (FECRT). A total of 60 sheep were chosen for this study and divided equally into four groups. Sheep were tested with closantel (@10mg/kg body weight) and two derivatives of macrocyclic lactone, moxidectin and doramectin (@0.2mg/kg body weight) while the control group did not receive any treatment. Sheep were naturally infected with gastro-intestinal nematodes and having EPG of faeces more than 150 counts before treatment were selected. Rectal faecal samples were collected on zero (pre-treatment) and 14th day (post-treatment) from animals of all groups and EPG of faeces were carried out by modified McMaster technique. In FECRT, reduction of faecal egg count (FEC) of less than 95%, indicate anthelmintic resistance (AR) in one or more nematode genera for the given anthelmintic. Per cent of FEC on 14th day revealed that closantel, moxidectin and doramectin had 64%, 69% and 76% efficacy, respectively. Coprocultures from pre- and post-treated sheep revealed predominance *Haemonchus contortus* larvae. The result indicated development of moderate anthelmintic resistance against three anthelmintics, leading to change of anthelmintic drugs and regular monitoring for possible anthelmintic resistance in the farm.

Key words: Anthelmintic resistance, Closantel, Macrocyclic lactones, Small ruminants, FECRT.

The small ruminant industry faces huge economic loss due to high prevalence of gastrointestinal nematodes (GIN) in tropical and sub-tropical counties like India. The state of Haryana lies in the northern region of India. Gastrointestinal nematodes cause economic loss in terms of wool, meat, milk, skin, manure and costs of control measures and death of animals. Adoptions of suitable control measures against these GINs are important for small ruminants, targeted farmer groups and for preventing economic loss of a country. Continuous and long-term use of anthelmintics without any preceding testing remains the mainstay of prevention in many herds. Anthelmintic resistance can be defined as the ability of parasites to survive doses of drugs that would normally kill parasites of the same species and stage. It is inherited and selected because the survivors of treatments pass

genes for resistance onto their offspring (Geary *et al.* 2012). The use of the same anthelmintics in long term, its underdosing, overdosing, frequently repeated treatments and lack of quarantine has led to worldwide anthelmintic resistance (AR) in gastrointestinal nematodes which alarming situation for small ruminants farming in Haryana, India. Several reports of AR against closantel and macrocyclic lactone from different parts of Haryana and India (Gupta *et al.* 2003, Buttar *et al.* 2012, Bihagi *et al.* 2020) as well as from different countries (Verissimo *et al.* 2012, Pena-Espinoza *et al.* 2014, Garcia *et al.* 2016). Thus, for maintaining the efficacy of the available drugs, regular monitoring of the status of anthelmintic resistance is required, at least once in two years. Therefore, the present study was planned to investigate the current status of anthelmintic resistance

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and there were no studies against moxidectin and doramectin in CSBF, Hisar Haryana. So we used the anthelmintic resistance method *i.e.*, *in vivo* faecal egg count reduction test (FECRT) for checking the efficacy of closantel, moxidectin and doramectin treatment in sheep.

Study pattern

Central sheep breeding farm (CSBF) have 2456 acres of land for rearing, breeding and upgrading sheep and goat. In total they have 2500 sheep of either Corriedale cross Sonadi or Rambouillet cross Nali ewe or ram. We used 60 Corriedale cross Sonadi ewes (above 3 months) for the present survey which were not dewormed from last three months.

Faecal egg count reduction test

Faecal egg count reduction test was carried according to the method described by the World Association for Advancement of Veterinary Parasitology (WAAVP) (Coles *et al.* 1992). Four groups of 15 sheep (S1, S2, S3 and S4) were formed and weighed and treated according to body weight on zero day (15 January 2020). Sheep from group S1 remained untreated as a control, group S2 was treated with Closantel (Zycloz[®], 10mg/kg oral solution, Zydus), group S3 with Moxidectin (Moxidecton[®] 1% injection, 0.2 mg/kg Intas) and group S4 with doramectin (Dectomax[®] 1% injection, 0.2 mg/kg, Zoetis). After 14th day (28 January 2020), the rectal samples were taken from all sheep treated with

anthelmintic, except the control group. Egg counts were performed using a modified McMaster method with a sensitivity of 50 (one egg in the counting chamber represents 50 eggs). Pooled faecal cultures were kept at 27±2°C for 7 days to recover infective third stage larvae *i.e.* L₃ from each group. The infective larvae were identified as per the criteria of Keith (1953). Faecal egg count reduction percentage and confidence intervals (95%) were determined following the method of the WAAVP using arithmetic mean egg counts. Resistance was considered to be present in the worm population when the egg count reduction following treatment was less than 95% and the confidence limits were less than 90% (Coles *et al.* 1992).

Result and analysis

The results revealed that closantel, moxidectin and doramectin reduced the faecal egg counts by 64,76 and 69% on 14th day, respectively which indicates moderate resistance. Faecal egg counts (Mean ± S.E.) on 0 and 14th day post-treatment (PT), per cent reduction in faecal egg counts (FECR%), variance, 95% upper confidence limits and larval composition treated with different anthelmintics are given in Table 1. The highest reduction of egg counts on 14th day was observed in moxidectin 76% with 95% upper confidence levels as 32.51. Doramectin reduced 69% of egg counts on 14th day with 95% upper confidence levels as 37.53. Clostantel reduced egg count 64% with 95% upper confidence levels as 32.78. These values suggest that GINs have developed

Table 1. Pre and post-anthelmintic treatment faecal egg count in sheep naturally infected with gastro-intestinal nematodes at Central Sheep Breeding Farm, Hisar of Haryana, India.

Group	Dose (mg/kg) and route of administration	Faecal egg counts on days (Mean ± S.E.)		Faecal egg counts reduction on 14 th day (Post-treatment)		Confidence limits at 95%	Per cent of larval composition on day	
		0	14	%	Variance (y ²)		Upper	0
Control	—	7300 ^a ±57.62	6900 ^b ±68.86	—	—	—	89 ^c , 8 ^d , 3 ^e	92 ^c , 5 ^d , 3 ^e
Clostantel	10, Oral	8900 ^a ±140.56	2480 ^a ±48.42	64	0.11	32.51	85 ^c , 9 ^d , 6 ^e	100 ^c
Moxidectin	0.2, Injection	7600 ^a ±67.94	1630 ^a ±34.48	76	0.12	37.53	86 ^c , 8 ^d , 6 ^e	
Doramectin	0.2, Injection	7700 ^a ±67.51	2160 ^a ±46.52	69	0.13	32.78	93 ^c , 5 ^d , 2 ^e	

(*Means with the same superscripts are not significantly different (p<0.01) column-wise. c = *H. contortus*, d = *Trichostrongylus* spp. and e = *S. papillosus*).

moderate resistance against all the three drugs *i.e.* closantel, moxidectin and doramectin. The composition of larvae for all three drugs on 14th day was having 100% *H. contortus*.

Closantel showed (64%) moderate resistance in sheep on 14th day post-treatment with the predominance of *H. contortus* L₃ larvae. Our findings are in agreement with Gupta *et al.* (2003), Fiel *et al.* (2011) and Ploeger and Everts (2018) who reported moderate resistance in Hisar, Argentina, and Netherlands. While in contrast Meenakshisundaram *et al.* (2014), Vohra *et al.* (2019) and Eye *et al.* (2020) reported high efficacy in Tamil Nadu, Hisar and Mongolia, respectively. The reason for moderate resistance against closantel may be due to continuous use of closantel from last 2 years as recorded from management history of farms and its use against liver fluke since the emergence of triclabendazole resistance in liver fluke in the late 1990s (Moll *et al.* 2000). Closantel high efficacy in Haryana reported by Vohra *et al.* (2019) was in unorganized sheep farm where regular deworming is not practised. So proper use and continuous investigation are advised for closantel return to 100% efficacy which will avoid its shift from moderate to severe resistance in small ruminants.

Moxidectin and doramectin also showed moderate resistance with predominance of *H. contortus* L₃ larvae on 14th day PT. Moderated resistance of doramectin was also reported by Borgsteede *et al.* (2007) in sheep of Netherlands while moxidectin resistance was reported by Van den Brom *et al.* (2013) in Dutch sheep flocks and Ploegera and Everts (2018) sheep of Netherlands. The reason for this may be the ivermectin which showed 100% efficacy against *H. contortus* reported by Singh and Yadav (1997) in organized farms. Since then, macrocyclic lactone class was proposed to be used in this farm. Doramectin, ivermectin and moxidectin had been used continuously during the last 5 years (4-5 times per year) which also belong to the same anthelmintic class, *i.e.*, macro-cyclic lactones. Thus, continuous use of these anthelmintics for the last 5 years at this farm could be the reason for development of resistance. Another reason may be introduction of new stock on the farm. Previous studies (Varady *et al.* 1993, Schnyder *et al.* 2005) have shown how the movement of livestock can introduce anthelmintic resistance in the new farms. There is a need of detailed study in India against moxidectin and doramectin in sheep to further compare the result.

The coproculture of pooled faeces from different groups and untreated control on day 0 and 14 were performed. A total of 400 infective larvae in each group

(S1, S2, S3 and S4) were counted. The infective larvae were identified as per the criteria of Keith (1953). The result showed different genera of GINs of sheep with the predominance of *H. contortus* followed by *Trichostrongylus* spp. and *Strongyloides papillosus* larvae on day 0. After 14th days of treatment, *H. contortus* was the only species found to survive in all treatment groups. The strain of *H. contortus* resistance to various anthelmintics in sheep has already been reported by Fleming *et al.* (2006) and Singh *et al.* (2013).

The investigation against closantel, moxidectin and doramectin revealed moderate resistance in CSBF of Hisar. Our reports are unique as there are no reports of resistance against moxidectin and doramectin from India while the decrease in the efficacy of closantel drugs was observed. So, we recommended choice of anthelmintic in a farm should be based on the previous history of use of drug, frequency of use of drug and status of anthelmintic resistance. It should always be considered primarily to use an anthelmintic judiciously and the anthelmintic resistance may be estimated at least once in two years. The drugs which show partial resistance should be changed immediately and discontinued for some years so that the larval population resistant to the drug is diluted and the portion of susceptible larval population is increased in the sheep flocks. Moreover, further such types of studies are needful in the Haryana to monitor and validate resistance against closantel, moxidectin and doramectin.

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REFERENCES

- Bihaqi S J, Allaie IM, Banday MAA, Sankar M, Wani ZA *et al.* (2020) Multiple anthelmintic resistance in gastrointestinal nematodes of Caprines on Mountain Research Centre for Sheep and Goat at Kashmir Valley, India. *Parasite Epidemiol Control* 11: e00163.
- Borgsteede FH, Dercksen DD, Huijbers R (2007) Doramectin and albendazole resistance in sheep in The Netherlands. *Vet Parasitol* 144: 180-183.
- Buttar BS, Hari HS, Singh NK, Haque JM, Rath SS (2012) Emergence of anthelmintic resistance in an organized sheep farm in Punjab. *J Vet Parasitol* 26(1): 69-71.

- Coles GC, Bauer C, Borgsteede FHM, Geerts S, Klei TR *et al.* (1992) World Association for Advancement of Veterinary Parasitology (WAAVP) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. *Vet Parasitol* 44: 35-44.
- Eye L, Wuen J, He X, Buyin B, Hai Y, Surong HASI (2020) Epidemiology and multidrug resistance of strongyle nematodes in Ordos Merino Sheep. *Research Square* 2: 234.
- Fiel C, Guzman M, Steffan P, Rodriguez E, Prieto O *et al.* (2011) The efficacy of trichlorphon and naphthalophos against multiple anthelmintic-resistant nematodes of naturally infected sheep in Argentina. *Parasitol Res* 1: 139-148.
- Fleming SA, Craig T, Kaplan RM, Miller JE, Navarre J *et al.* (2006) Anthelmintic resistance of gastrointestinal parasites in small ruminants. *J Vet Intern Med* 20: 435-444.
- Garcia CMB, Sprenger LK, Ortiz EB, Molento MB (2016) First report of multiple anthelmintic resistance in nematodes of sheep in Colombia. *Ann Brazil Acad Sci* 88(1): 397- 402.
- Geary TG, Hosking BC, Skuce PJ, von Samson-Himmelstjerna G, Maeder S *et al.* (2012) World Association for the Advancement of Veterinary Parasitology (WAAVP) Guideline: Anthelmintic combination products targeting nematode infections of ruminants and horses.
- Gupta SK, Chaudhri SS, Poonia JS (2003) Preliminary report of closantel resistance against *Haemonchus contortus* in sheep of western Haryana. *Indian J Anim Sci* 73: 1024-1026.
- Keith RK (1953) The differentiation of infective larvae of some nematode parasites of cattle. *Aust J Zool* 1: 223-235.
- Meenakshisundaram A, Anna T, Harikrishnan J (2014) Prevalence of drug-resistant gastrointestinal nematodes in an organized sheep farm. *Vet World* 7: 1113-1116.
- Moll L, Gaasenbeek CPH, Vellema P, Borgsteede FHM (2000) Resistance of *Fasciola hepatica* against triclabendazole in cattle and sheep in The Netherlands *Vet Parasitol* 91: 153-158.
- Pena-Espinoza M, Thamsborg SM, Demeler J, Enemark HL (2014) Field efficacy of four anthelmintics and confirmation of drug-resistant nematodes by controlled efficacy test and pyrosequencing on a sheep and goat farm in Denmark. *Vet Parasitol* 206: 208-215.
- Ploeger HW, Everts RR (2018) Alarming levels of anthelmintic resistance against gastrointestinal nematodes in sheep in the Netherlands. *Vet Parasitol* 262: 11-15.
- Schnyder M, Torgerson PR, Schonmann M, Kohler L, Herzberg H (2005) Multiple anthelmintic resistance in *Haemonchus contortus* isolated from South African Boer goats in Switzerland. *Vet Parasitol* 128: 285-290.
- Singh S, Vohra S, Kumar S, Poonia JS (2015) Status of anthelmintic resistance of levamisole and ivermectin and efficacy of their combination against gastrointestinal nematode in sheep. *Haryana Vet* 54: 11-14.
- Singh S, Yadav CL (1997) A survey of anthelmintic resistance by nematodes on three sheep and goat farm in Hisar (India). *Vet Res Commun* 21: 447-451.
- Van den Brom R, Moll L, Borgsteede FHM, Van Doorn DCK, Lievaart-Peterson K *et al.* (2013) Multiple anthelmintic resistance of *Haemonchus contortus*, including a case of moxidectin resistance, in a Dutch sheep flock. *Vet Rec* 173: 552.
- Varady M, Praslicka J, Corba J (1993) Multiple anthelmintic resistance of nematodes in imported goats. *Vet Rec* 132: 387-388.
- Verissimo CJ, Niciura SC, Alberti AL, Rodrigues CF, Barbosa CM *et al.* (2012) Multidrug and multispecies resistance in sheep flocks from Sao Paulo state, Brazil. *Vet Parasitol* 187: 209-216.
- Vohra S, Singh S, Kumar V (2019) Status of anthelmintic resistance of fenbendazole, closantel and levamisole against gastrointestinal nematodes in sheep of Haryana. *Int J Livest Res* 9: 91-96.

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