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Gastrointestinal Nematode Population with Multiple Anthelmintic Resistances in Unorganized Sheep Farms from the Semi-arid Zone of Haryana

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ABSTRACT

In present study two organized and six unorganized farms of sheep from three agro-climatic zones of Haryana were surveyed to assess the prevalence of anthelmintic resistance in gastrointestinal nematodes to fenbendazole (@ 5 mg/kg b.wt. orally), closantel (@ 10 mg/kg b.wt. orally) and ivermectin (0.2 mg/kg, subcutaneous injection) by faecal egg count reduction test (FECRT). Per cent reduction in faecal egg counts by fenbendazole (FBZ), closantel (CLS) and ivermectin (IVM) in organized farms ranged from 53.24-60.60% (FBZ), 74.24-76.62% (CLS) and 63.63-69.69% (IVM) while unorganized farms 59.62-75.56% (FBZ), 79.19-92.47% (CLS) and 69.94-82.79% (IVM). Over all per cent reduction in faecal egg counts in organized and unorganized farms ranged 53.24-75.56% (FBZ), 74.24-92.47% (CLS) and 63.63-82.79% (IVM), respectively. In all cases of anthelmintic resistance, *Haemonchus contortus* was the predominant parasite involved. Reduction in faecal egg counts indicate multidrug severe to moderate resistance in sheep farms of different agro-climatic zones of Haryana.

HIGHLIGHTS

- *H. contortus* resistant to anthelmintics.
- Fenbendazole showed severe to moderate resistance, while Closantel & Ivermectin showed moderate resistance.

Keywords: Anthelmintic resistance, Closantel, Haryana, Sheep

Sheep farming are important source of income for low earning and landless farmers in India and contribute towards their livelihood. However, multifactor that restrict production and productivity in sheep, including nutritional problems and inadequate management practices in sanitary hygiene, especially the poor hygiene conditions that favor the increase of the population of gastrointestinal parasites, mainly *Haemonchus contortus*. The *H. contortus* is one of the important parasite due to its high resistance to anthelmintics (Idris *et al*., 2012). Anthelmintics reduce the adverse effects of these nematode parasites which is easy available option but anthelmintic resistance emergence in India is main reason of worry. In India, the first report of anthelmintic resistance in sheep dates back to 1976 against phenothiazine and thiabendazole resistant strain of *H. contortus* (Varshney and Singh, 1976). Since then a number of reports have been reported in sheep (Singh and Gupta, 2010; Kumar and Singh 2016 and Vohra *et al.,* 2019). Therefore, present study was undertaken to evaluate the efficacy of fenbendazole, closantel and ivermectin against gastrointestinal nematodes in sheep farms of different agroclimatic zones of Haryana to diagnosis and

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measure the anthelmintic resistance in parasitic population for effective control measures.

MATERIALS AND METHODS

Area of study

For present study we selected three agro-climatic zones which divide Haryana state into three climatic zones namely arid (Zone-I), semi-arid (Zone-II) and dry sub-humid (Zone-III). We collected samples from two organized and unorganized sheep farms from Zone-I. As if there were no organized farms in Zone-II and Zone-III we collected samples from two unorganized sheep farms from each zone. Samples were collected from three zones of Haryana as given below:

Zone I (Arid)

1. Sheep, Breeding farm LUVAS, Hisar (Organized) (SBFL)
2. Sheep, Central Sheep Breeding Farm, Hisar (SCSBF)
3. Sheep, Chirod village, Hisar (Unorganized) (SCV)
4. Sheep, Gujrani village, Bhiwani (Unorganized) (SGV)

Zone II (Semi-Arid)

1. Sheep, Sinsar village, Jind (Unorganized) (SSV)
2. Sheep, Budhakhera village, Kaithal (Unorganized) (SBV)

Zone III (Dry Sub-Humid)

1. Sheep, Samlehri village, Ambala (Unorganized) (SVA)
2. Sheep, Philkani village, Ambala (Unorganized) (SPV)

In all the selected organized farms, sheep were maintained under semi-intensive system i.e. in day-time they were allowed to graze in free-range field and during night-time they were kept in confinements. Routine deworming programs were being carried out for the control of GI helminth parasites in farms. The flocks of sheep in each farm which was not treated with any anthelmintic at least 8-10 weeks earlier were selected for present experimental trials. Unorganized farms of sheep were having no deworming and management record.

Faecal egg count reduction test (FECRT)

Faecal egg count reduction test was carried according to the method described by the 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. Sheep from group S1 remained untreated as a control, group S2 was treated with fenbendazole (Panacur vet @ 5 mg/kg b.wt. orally, MSD), group S3 with ivermectin (Zenvet, 0.2 mg/kg b.wt. subcutaneous injection, INTAS) and group S4 with closantel (Zycloz, 10 mg/kg b.wt. orally, Zydus). After 14th day, rectal sample was taken from all sheep treated with anthelminthic, except 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). A composite larval culture was made for each group (0 day and 14th day post treatment) by mixing 5 g of faeces from each individual sample. Pooled faecal cultures were kept at 27±20°C for 7 days to recover infective third stage larvae i.e. L_3 from each group. The infective larvae were identified as per criteria of Keith (1953). Faecal egg count reduction percentage and confidence intervals (95%) were determined following the method of the World association for the advancement of veterinary parasitology (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).

RESULTS AND DISCUSSION

The results obtained on the basis of FECRT have been summarized in table 1. The emergence of anthelmintic resistance (AR) varied with different anthelmintics in different villages/farms. There is clear indication from table 1 that there is severe to moderate resistance for FBZ and moderate resistance for CLS and IVM in sheep of Haryana. The predominant larvae recovered from faecal culture before and after treatment were of *Haemonchus contortus* which can be seen in Fig. 1. Few larvae of *Trichostrongylus* spp., *Oesophagostomum* spp., *Strongyloides* spp. and *Bunostomum* spp. were also recovered from the faecal cultures before treatment.

In the organized sheep farms the efficacy of fenbendazole against gastrointestinal nematodes (GIN) are in agreement with Kumar and Yadav (1994), Rialch *et al*. (2013),

Table 1: Per cent reduction in faecal egg counts on treatment with fenbendazole, closantel and ivermectin in sheep of Haryana

Farm/Village	Fenbendazole (FBZ)				Closantel (CLS)				Ivermectin (IVM)			
	% FECR	95% CL		Result	% FECR	95% CL		Result	% FECR	95% CL		Result
		U	L			U	L			U	L	
Zone I												
SCSBF	53.24	75.26	31.69	**R**	76.62	89.03	53.15	**R**	63.63	80.40	64.12	**R**
SBFL*	60.60	78.66	27.27	**R**	74.24	86.24	51.76	**R**	69.69	84.22	41.77	**R**
SCV	66.66	82.26	37.35	**R**	87.50	95.32	66.58	**R**	76.38	89.39	47.41	**R**
SGV	59.62	77.0	28.56	**R**	84.61	92.91	66.58	**R**	76.92	88.27	54.59	**R**
Zone II												
SSV	65.82	79.68	42.51	**R**	82.27	92.63	57.36	**R**	74.68	87.84	47.28	**R**
SBV	75.26	89.57	41.31	**R**	92.47	98.47	62.81	**R**	82.79	94.23	48.67	**R**
Zone III												
SVA	63.58	86.57	11.19	**R**	79.19	92.84	39.50	**R**	69.94	88.92	18.40	**R**
SPV	66.66	83.75	31.61	**R**	85.29	95.58	51.04	**R**	76.47	90.86	39.36	**R**
Overall range % FECR	53.24-75.56				74.24-92.47				63.63-82.79			

*Organized farm, % FECR = % Faecal egg count reduction, CL = Confidence limit at 95% level, U = Upper confidence limit, L = Lower confidence limit, R=Resistant.

Pena-Espinoza *et al*. (2014) and Garcia *et al*. (2016) who reported severe to moderate resistance 60%, 59%, 54.95%, 54% and 59.3% in Hisar, sub-Himalyan region of northern India, Denmark and Colombia, respectively.

Fig. 1: Infective third stage larvae of *Haemonchus contortus* kinked tail at 40x

The reason for severe to moderate resistance against fenbendazole in organized sheep farms may be due to use of drug continuously from last 2 years as shown in management history of farms, availability of drug and convenience of management. In unorganized sheep farms the efficacy of fenbendazole, against GIN are in agreement with Rialch *et al*. (2013), Garcia *et al*. (2016), Singh *et al*. (2016) and Kalkal *et al.* (2019) who reported severe to moderate resistance 65.3%, 60%, 73%, 69.10%, 59.3%, 66.99% and 62.17% in sub-Himalyan region of northern India, Colombia and Hisar, respectively. While in unorganized farms of sheep this may be due to use of this drug continued along with other drugs, as per availability, convenience and supply from government in veterinary hospitals. Overall efficacy of fenbendazole against GIN are in agreement with Pena-Espinoza *et al*. (2014), Garcia *et al*. (2016) and Singh *et al*. (2016) who reported severe to moderate resistance 54%, 51%-76.66% and 66.99% in Uttarakhand, Trinidad, sub-Himalyan region of northern India, Denmark, Colombia, Punjab and Hisar, respectively. Some workers Buttar *et al*. (2012), Meenakshisundaram *et al.* (2014) and Vohra *et al.* (2019) reported high efficacy which were 80.58%, 90.06-93.04% and 80.1% in Punjab, Tamil Nadu and Hisar, respectively. Once the benzimidazole resistant population of nematodes has developed, it continues to persist in the absence of any benzimidazole use over years in the field (Webb *et al*., 1979 and McKenna, 1990). Jackson (1993) reported that when the predominant resistant species have a high biotic potential and are also highly pathogenic as in case of *H. contortus*, then the risk associated with reintroduction of the drug is very high.

In (organized, unorganized and overall) sheep farms the efficacy of closantel against GIN are in agreement with Flavia da Silva *et al.* (2018), Ploegera and Everts (2018) and Parmar *et al.* (2020) who reported moderate resistance 84.4%, 80.1% and 91.24% in Brazil, Netherlands and Uttar Pradesh, respectively. While in contrast Meenakshisundaram *et al.* (2014), Vohra *et al.* (2019) and Eye *et al.* (2020) reported high efficacy which were 100%, 96.02% and 99% in Tamil Nadu, Hisar and Mongolia, respectively. The reason for moderate resistance against closantel in sheep of organized farms maybe due to continuous use of closantel from last 2 years as shown in 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). While in unorganized sheep farms there is moderate resistance this may due to its use against *H. contortus* and liver fluke, so proper use and continuous investigation is advised for closantel return to 100% efficacy which will avoid its shift from moderate to severe resistance in small ruminants.

In (organized, unorganized and overall) sheep farms the efficacy of ivermectin against GIN are in agreement with Singh and Gupta (2010), Kumar and Singh (2016) and Ploegera and Everts (2018) who reported moderate resistance 89-94%, 68.02% and 84.9% in Hisar and Netherlands, respectively. While in contrast Ahmed *et al.* (2017) and Islam *et al.* (2018) reported high efficacy which were 99.56% and 96.77% in Ethiopia and Bangladesh, respectively. When Singh and Yadav (1997) found that ivermectin was 100% effective against *H.contortus* in these farms. Since, then macrocyclic lactone class was proposed to be used in these farms and doramectin, ivermectin and moxidectin had been used continuously during the last 2 years (regularly from last 10 years) as shown in management history of farms which also belong to same anthelmintic class i.e. macrocyclic lactones. All macrocyclic lactones produce flaccid paralysis of the somatic muscles of the parasites and inhibit feeding by blocking pharyngeal pumping, hence have similar modes of action. Thus, continuous use of this anthelmintic form the last 2 years at this farm could be the reason for development of resistance. While in unorganized sheep farms there is also moderate resistance so, there is need to be vigilant while using ivermectin. It may be associated with the type and frequency of anthelmintic used by the local farmers. While History revealed that sheep were being treated with ivermectin by farmers since long for ecto and endo parasite. The treatments depended on the availability of anthelmintics and no record was maintained by the farmers. The role of frequent use of anthelmintics in the development of resistance has been reported previously (Barton 1980 and Martin *et al.,* 1982). The selection pressure exerted by regular use of anthelmintic is responsible for the development of anthelmintic resistance.

CONCLUSION

There is need of constant monitoring for AR for organized and unorganized farms to keep an eye on anthelmintics effectiveness before their use, where resistance has not already emerged. This in turn, helps in taking timely measures to prevent or to delay the occurrence of AR by minimum anthelmintic usage. It can be concluded from the study that there is multidrug severe to moderate AR against *H. contortus* of sheep in different agro-climatic zones of Haryana. Therefore, there is need to be more vigilant while using anthelmintics.

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