

EFFICACY OF FENBENDAZOLE, CLOSANTEL AND IVERMECTIN AGAINST GASTROINTESTINAL NEMATODES IN UNORGANIZED SHEEP FARMS FROM DRY SUB-HUMID ZONE OF HARYANA

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Received: 29.07.2022; Accepted: 19.01.2023

ABSTRACT

In the present study two unorganized sheep farms from semi-arid zones of Haryana were surveyed to assess the status of anthelmintics against gastrointestinal nematodes by faecal egg count reduction test (FECRT). A total of 120 sheep, 60 each from Samlehri village, Ambala (SVA) and Philkani village, Ambala (PVA) with at least 150 egg per gram (EPG) of faeces were selected. Animals were divided into four groups of 15 animals each in SVA (S1, S2, S3 and S4) and PVA (B1, B2, B3 and B4) farms. Group S1 and B1 were treated with fenbendazole (@ 5 mg/kg b.wt. orally), group S2 and B2 were treated with closantel (@ 10 mg/kg b.wt. orally), group S3 and B3 were treated with ivermectin (0.2 mg/kg, subcutaneous injection) and group S4 and B4 served as untreated control, respectively. Faecal samples were collected on zero and 14th day after treatment from all groups and egg counts were done by Modified Mc Master technique. Per cent reduction in faecal egg counts by fenbendazole, closantel and ivermectin in SVA was 62.58, 79.04 and 68.57 and in PVA was 66.66, 85.29 and 76.47, respectively indicating presence of moderate anthelmintic resistance to these drugs. The post-treatment coproculture of both the farms showed predominance of *Haemonchus contortus* larvae.

Keywords: Anthelmintic resistance, Closantel, Fenbendazole, Ivermectin, Sheep

How to cite: Kalkal, H. and Vohra, S. (2023). Efficacy of fenbendazole, closantel and ivermectin against gastrointestinal nematodes in unorganized sheep farms from dry sub-humid zone of Haryana. *Haryana Vet.* 62(SI-2): 15-17.

Small ruminants specially sheep are common source income for poor farmers which are landless. Gastrointestinal nematodes (GIN) constitute a major challenge to the health, welfare and productivity of sheep worldwide (Andrea *et al.*, 2011). Parasite which causes gastroenteritis are mainly *Haemonchus contortus*, *Trichostrongylus axei*, *Nematodirus* spp. and *Strongyloides papillosus*. These parasite are highly pathogenic and major hurdle in sheep farming, in these parasite *H. contortus*, is most pathogenic, widely prevalent and important worm in sheep in India responsible for high mortality and morbidity (Yadav, 1997). To control these parasite many anthelmintics are used and mainstay to reduce the adverse effects of these nematode parasites but their usefulness is constrained by the emergence of anthelmintic resistance (Ancheta *et al.*, 2004). Resistance to various anthelmintics has been observed in ruminants infected with GINs, due to constant and improper use of these drugs (Singh and Gupta, 2010). Anthelmintic resistance from different parts of India have been reported like Buttar *et al.*, 2012; Meenakshisundaram *et al.*, 2014) as well as from other countries (Verissimo *et al.*, 2012; Balmer *et al.*, 2015). Therefore, for maintaining the efficacy of the available drugs, regular monitoring of the status of anthelmintic resistance is required, at least once in two years (Rialch *et al.*, 2013). Hence, this study was designed to detect the efficacy of fenbendazole, closantel and ivermectin against GINs of unorganized sheep farms

from dry sub-humid zone of Haryana.

MATERIALS AND METHODS

The present study was conducted on unorganized sheep farms from dry sub-humid zone of Haryana. For this two unorganized sheep farms from Samlehri village, Ambala (SVA) and Philkani village, Ambala (PVA) were selected to determine the efficacy of anthelmintics against gastrointestinal nematodes using FECRT as described by the World Association for the Advancement of Veterinary Parasitology (WAAVP) (Coles *et al.*, 1992). Sixty animals from each village naturally infected with GINs and having egg per gram (EPG) of ≥ 150 counts prior to treatment were selected. The selected animals had not been administered any anthelmintics during the previous two months. These animals were identified, weighed and their EPG was estimated. Four groups of 15 sheep (S1, S2, S3 and S4) of SVA and (B1, B2, B3 and B4) of PVA were created. Group S1 and B1 were treated with fenbendazole (@ 5 mg/kg b.wt. orally, Panacur®, MSD), group S2 and B2 with closantel (10 mg/kg b.wt. orally, Zycloz®, Zydus), group S3 and B3 with ivermectin (0.2 mg/kg b.wt. subcutaneous injection, Zenvet®, INTAS) and group S4 and B4 served as untreated control. The faecal egg count of each animal was ascertained on zero and 14th day post-treatment (PT) by modified McMaster technique to an accuracy of one egg counted representing 50 EPG. Pooled faecal cultures were kept at $27 \pm 2^\circ\text{C}$ for 7 days to recover infective third stage larvae from each group. The infective larvae were

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Table 1. Response to various anthelmintics in sheep naturally infected with gastrointestinal nematodes at Samlehri and Philkani village, Ambala

| Group | Anthelmintic | Dose (mg/kg) | No. of sheep treated | Route of Administration | Faecal egg counts on days (Mean ± S.E.) | | Faecal egg counts reduction on day 14 post-treatment | | Confidence limits at 95% | |
|-------|--------------|--------------|----------------------|-------------------------|---|-----------------------------|--|----------|--------------------------|-------|
| | | | | | 0 | 14 | % | Variance | Upper | Lower |
| S1 | Fenbendazole | 5 | 15 | Oral | 680 ^a ±105.19 | 260 ^b ±318.02 | 62.85 | 0.11 | 86.57 | 11.19 |
| S2 | Closantel | 10 | 15 | Oral | 653.33 ^a ±104.13 | 146.66 ^b ±241.62 | 79.04 | 0.20 | 92.84 | 39.50 |
| S3 | Ivermectin | 0.2 | 15 | S/C | 686.66 ^a ±115.41 | 220 ^b ±278.82 | 68.57 | 0.12 | 88.92 | 18.40 |
| S4 | Control | — | 15 | — | 633.33 ^a ±492.67 | 700±374.16 | 0 | — | — | — |
| B1 | Fenbendazole | 5 | 15 | Oral | 653.33 ^a ±90.95 | 226.66 ^b ±65.80 | 66.66 | 0.11 | 83.75 | 31.61 |
| B2 | Closantel | 10 | 15 | Oral | 646.66 ^a ±94.54 | 100 ^b ±54.33 | 85.29 | 0.32 | 95.58 | 51.04 |
| B3 | Ivermectin | 0.2 | 15 | S/C | 640 ^a ±94.01 | 160 ^b ±66.04 | 76.47 | 0.20 | 90.86 | 39.36 |
| B4 | Control | — | 15 | — | 640 ^a ±132.08 | 680 ^a ±123.13 | 0 | — | — | — |

Means with same superscripts in column are not significantly different (p<0.05)

identified as per the 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 (Coles *et al.*, 1992) using arithmetic mean egg counts. The drug was considered fully effective when it reduced the egg counts by more than 95% and lower confidence limits were higher than 90%. The drug was considered moderately resistant when they reduced the egg counts between 60% to 95% and considered severely resistant when the reduction in egg counts was below 60% along with lower confidence limits below 90% (Wood *et al.*, 1995). All the recorded data was statistically analyzed by one way ANOVA test using SPSS software version 27.0.

RESULTS AND DISCUSSION

The faecal egg counts (Mean±S.E.) on 0 and 14th day PT, percent reduction in faecal egg counts (FECR%), variance, upper and lower confidence limits (95%) for fenbendazole, closantel and ivermectin in sheep naturally infected with gastrointestinal nematodes at SVA and PVA are given in table 1, respectively. Results revealed that FBZ, CLS and IVM reduced the faecal egg counts by 62.85%, 79.04% and 68.57% in SVA and 66.66%, 85.29% and 76.47% in PVA, respectively indicating moderate resistance (Wood *et al.*, 1995) against all anthelmintics in unorganized sheep farms from semi-arid zones of Haryana.

The fenbendazole @ 5 mg/kg b. wt. reduced faecal egg count by 62.85% and 66.66% in SVA and PVA, respectively indicating resistance. The reason for resistance may be the continual use of fenbendazole with other anthelmintics, as per availability and convenience. Also, the farmers in this area have a practice of treating the animals with fenbendazole without knowing the appropriate bodyweight of animals which lead to over-underdosing.

The resistance to fenbendazole in gastrointestinal nematodes of sheep has been reported in India by Sharma *et al.* (2015) in Hisar and Rialch *et al.* (2013) in sub-Himalyan region of northern India as well as aboard by Maciel *et al.* (1996) in America and Melo *et al.* (2009) in Brazil.

The closantel @ 10 mg/kg b. wt. reduced faecal egg count by 79.04% and 85.29% in SVA and PVA, respectively indicating resistance. As we know closantel is narrow-spectrum anthelmintic with activity specifically against blood-feeding helminths, they are of particular importance for the control of *H. contortus* and *Fasciola hepatica* which may be the reason for resistance. A similar observation was reported given Gupta *et al.* (2003) in sheep of western Haryana, Flavia da Silva *et al.* (2018) in Brazil and Parmar *et al.* (2020) in Uttar Pradesh.

The ivermectin @ 0.2 mg/kg b.wt. reduced faecal egg count by 68.57% and 76.47% in SVA and PVA, respectively indicating resistance. Ivermectin belongs to avermectin group of drugs which is effective against gastrointestinal nematodes as well as ectoparasites therefore termed endectocite (El-Saber Batiha *et al.*, 2020). History revealed that sheep were treated with ivermectin by farmers for a prolonged time. Thus, continuous use of ivermectin for a prolonged time could be the reason for development of resistance. Resistant, against gastrointestinal nematodes, has been also reported by Das and Singh, (2005), Singh and Gupta (2010) and Ploegera and Everts (2018). So, there is a need to be vigilant while using anthelmintic against GINs of sheep.

The pooled faecal cultures of infective third stage larvae in different groups and untreated control on day zero and 14 PT are depicted. A total of 100 infective larvae in each group (S1, S2, S3 and S4) of SVA and (B1, B2, B3 and

B4) of PVA were counted. The result showed different genera of gastrointestinal nematodes of sheep with the predominance of *H. contortus* (77 to 88%) followed by *Trichostrongylus* spp. (2-11%), *Oesophagostomum* spp. (1-9%) and *Strongyloides* spp. (5-11%) larvae in all the treated and untreated control groups on day zero in both villages. After 14th day of treatment, there was predominance of *H. contortus* larvae in fenbendazole, closantel and ivermectin treated animals. The presence of *H. contortus* as predominant larvae after treatment with fenbendazole, closantel and ivermectin was also reported by Sarika, (2012) and Sharma *et al.* (2015).

CONCLUSION

Two unorganized sheep farms (SPV and PVA) included in the study were showing moderate resistance against fenbendazole, closantel and ivermectin anthelmintics. So, the present work is first study in unorganized sheep farms from dry sub-humid zone of Haryana which reports fenbendazole, closantel and ivermectin were not effective against *H. contortus*. It can be concluded that the choice of anthelmintic in a flock should be based on the status of anthelmintic resistance and anthelmintic should be administered as per the body weight, selection of drug should be based on the previous history of use of drug and frequency of use of the drug in the farm.

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