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Worm control approaches and their impact on status of anthelmintic resistance at an organized sheep farm

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ABSTRACT

The aim of study was to ascertain efficiency of different worm management strategies in reversion to susceptibility to anthelmintics in sheep maintained at Sheep Breeding Farm, Fatehpur (Rajasthan). An increase in efficacy of benzimidazole (BZ) from nil to 14 and 75% was observed after 6 and 8 years of withdrawal, respectively. The restricted use of imidothiazoles enhanced efficacy from 25% in 1998 to 92% in 2007. From 2010, community dilution strategies through introduction of newly purchased sheep were implemented and evaluated for their impact on reversion to susceptibility after 5 years. A marginal increase in BZ efficacy (up to 43%) was observed after introduction of new animals possessing anthelmintic resistant/susceptible worms and rotational use of anthelmintic types. A moderate rise in BZ efficacy (up to 56%) was observed on community dilution through introduction of Nali sheep possessing susceptible *Haemonchus contortus* and use of BZs and closantel in rotation. A significant increase in BZ efficacy (up to 88%) was noticed on community dilution through introduction of Marwari sheep possessing susceptible *H. contortus* and application of targeted selective treatment (TST) using closantel. The efficacy of tetramisole (TEM) increased from 25% (1998) to 92% (2007) after 8 years of withdrawal TEM which further increases to 99% (2015) after introduction of TEM susceptible *H. contortus* through newer sheep coupled with rotation of anthelmintic classes. The results indicated that just withdrawal of ineffective anthelmintic is not sufficient enough to cause evident reversion to susceptibility. However, community dilution coupled with TST with newer class of anthelmintic will help in reversion to susceptibility in gastrointestinal nematodes at faster rate in sheep.

Key words: Anthelmintic resistance, Community dilution, Rotational use, Sheep, Targeted selective treatment

Gastrointestinal nematodes (GINs) are continued to be a threat to small ruminant farming worldwide (Bentounsi *et al*. 2012). Intensification of animal production system and thereby ever increasing use of anthelmintics to combat the losses caused by the GINs is one of the major reason for rampant emergence of anthelmintic resistance (Singh and Swarnkar 2008). At an organized sheep farm in arid Rajasthan higher mortality in sheep flocks occurred during April – May, 1998 due to varied reasons. However, majority of sheep showed heavy worm burden on necropsy. The sheep were treated several times with different classes of anthelmintics, but problem of haemonchosis remained uncontrolled. Study on anthelmintic resistance in *H. contortus* exhibited 0% and 25% efficacy for fenbendazole (FBZ) and levamisole (LEV), respectively on FECRT (Swarnkar *et al*. 1999b).

The withdrawal of the anthelmintic types has been suggested to manage the anthelmintic resistance (Jackson and Coop 2000) and accordingly both the drugs (BZ and LEV) were withdrawn and closantel (CLS) introduced in worm control programme in 1998. However, existence of resistance to BZ and LEV (with relatively increased efficacy) was observed even after their withdrawal for almost a decade (Maharshi *et al*. 2011b) suggesting limited role of withdrawal strategies in reversion to anthelmintic susceptibility in worms. The other strategies advocated to manage the anthelmintic resistance were– rotational use of anthelmintic (Geurden *et al*. 2014), simultaneous use of multiple actives with similar spectra of activity (Bartram *et al*. 2012), maximize the opportunities for retention of unselected genotypes by application of targeted selective treatment (TST) (van Wyk *et al*. 2001, Leathwick and Besier 2014), effective quarantine procedures to prevent the introduction of resistant genotypes (Swarnkar and Singh 2012, Leathwick and Besier 2014) and community dilution through introduction of susceptible genotypes (Bird *et al*. 2001). Following introduction of newly purchased animals possessing susceptible worms in 2010, the strategies like community dilution alone as well as coupled with TST were implemented to ascertain whether these were able to prevent resistance from becoming any worse or able to cause reversion to susceptibility to anthelmintics.

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MATERIALS AND METHODS

Study area: The study was conducted in sheep flocks maintained at Sheep Breeding Farm, Fatehpur (Rajasthan) located in arid agro-climate. The average monthly minimum and maximum temperature ranged from 7.9±0.3°C (January) to 28.1±0.2°C (June) and from 23.0±0.3°C (January) to 42.1±0.2°C (May). The average monthly total rainfall ranged from 2.8±0.7 mm (January) to 103.3±15.9 mm (August). The average relative humidity in atmosphere at 8.30 AM IST varied from 40.6±1.2% (April) to 76.9±0.9% (August).

Introduction of animals and status of anthelmintic resistance: In the year 2010, new animals belonging to native breeds like Marwari, Nali and Chokla were purchased and stationed as under:

Breed	Source of purchase	Status of anthelmintic resistance at source of purchase	Stationed at location
Nali	Farmer's flocks	Not known	Site no. 4
Marwari	Arid Region Campus (ARC), Bikaner	Susceptible to benzimidazole and imidothiazole (Swarnkar *et al.* 1999a)	Site no. 5
Chokla	Arid Region Campus (ARC), Bikaner	Susceptible to benzimidazole and imidothiazole (Swarnkar *et al.* 1999a)	Site no. 6
	ICAR-Central Sheep and Wool Research Institute, Avikanagar	Resistance to benzimidazole (Swarnkar *et al.* 2001, Maharshi *et al.* 2011)	

Evaluation of strategies for anthelmintic resistance management: The strategies implemented and evaluated consisted of withdrawal / restricted use anthelmintic types (up to 2010); community dilution (since 2010) through either introduction of Chokla sheep possessing anthelmintic resistance / susceptible worms coupled with annual rotational use of anthelmintic types (BZ, TEM and CLS) or introduction of Nali sheep possessing susceptible *H. contortus* with use of BZ and CLS anthelmintics in rotation; and introduction of Marwari sheep possessing susceptible *H. contortus,* with application of TST (based on eye colour chart, Singh and Swarnkar 2012) using CLS. The susceptibility of *H. contortus* in newly purchased Marwari and Nali sheep was confirmed by *in-vivo* faecal egg count reduction test (FECRT) (with efficacy of 96 and 98% FBZ and TEM, respectively) and *in-vitro* egg hatch assay (EHA) (ED_{50} value of 0.057 µg TBZ/ml). The status of anthelmintic resistance in worm population was estimated on different occasions by using FECRT for different types of anthelmintics and EHA for benzimidazole (Coles *et al*. 1992). The data on FECRT and EHA were analysed through RESO programme (Martin and Wursthorn 1991) and probit analysis, respectively.

RESULTS AND DISCUSSION

The values of % faecal egg count reduction and ED_{50} for thiabendazole on different occasions are presented in Table 1. The data on effect of withdrawal of type of anthelmintics exhibited that efficacy of benzimidazoles against *H. contortus* of sheep increased from nil (1998) to 14% after 6 years of withdrawal and to 75% after 8 years of withdrawal. Like-wise, restricted use of imidothiazoles in worm management programme enhanced efficacy from 25% in 1998 to 92% in 2007. Following detection of resistance to particular anthelmintic class and its withdrawal from use in worm management programme may lead to decreased selection pressure in worm population and allow establishment of susceptible population of worms leading to increased efficacy over the time. Complete reversion to susceptibility to levamisole at faster rate (3 and 1 year in sheep and goat, respectively) was reported after withdrawal of drug and adoption of rotational grazing (Zajac and Gipson 2000). In India, Singh and Rayulu (2011), reported partial reversion of susceptibility to morantel resistant strain of *H. contortus* in an organized sheep farm after withdrawal of drug for 10 years. Similarly, Singh and Gupta (2009) observed partial reversion to susceptibility to fenbendazole and levamisole resistant strains of *H. contortus* in an organized sheep farm after switching over to ivermectin and closantel for 12 years. In a goat farm at Hisar (Haryana, India) after withdrawal of morantel since 1995, complete reversion to susceptibility in *H. contortus* resistant to morantel was observed by Singh and Poonia (2011). By exclusive use of combination anthelmintics and integration with other resistance management strategies, Leathwick *et al* (2015) observed a significant improvement in the effectiveness of both levamisole and ivermectin against *T. circumcincta*, and a positive but nonsignificant trend in efficacy of albendazole, i.e. reported evidence for reversion towards susceptibility.

The result of FECRT obtained after implementation of different community dilution strategies showed a marginal increase in BZ efficacy (up to 43%) after introduction of new animals possessing both the types of (anthelmintic resistant / susceptible) worms and rotational use of BZ, TEM and CLS for 5 years. A moderate rise in BZ efficacy (up to 56%) was observed after 5 years of community dilution through introduction of Nali sheep possessing susceptible *H. contortus* and use of BZ and CLS anthelmintics in rotation. A significant increase in BZ efficacy (up to 88%) was noticed after 5 years of community dilution through introduction of Marwari sheep possessing susceptible *H. contortus* and application of TST using CLS only since last 5 years (Table 1). The efficacy of TEM increased from 25% (1998) to 92% (2007) after 8 years of withdrawal of TEM from worm management programme which further increases to 99% (2015) after introduction of TEM susceptible *H. contortus* through introduction of newer sheep coupled with rotation of anthelmintic classes. The efficacy of CLS against *H. contortus* was maintained at 100% from 1998 to 2015 through adoption of practice of rotational anthelmintic use in worm management programme.

For maximizing long-term profitability in flock, parasite management programs should involve either delaying resistance through drench conservation or exploiting the anthelmintic resource. The adoption of best practice management strategies may extend the useful life of anthelmintics even after diagnosis of resistance. Though, there were sporadic reports on occurrence of resistance to multiple anthelmintic classes on rotational use of anthelmintics (Torres-Acosta *et al*. 2012, Geurden *et al*. 2014) but the modeling studies (Leathwick *et al*. 2012) showed that simultaneously using multiple actives had the potential to dramatically slow the development of resistance.

It was found that the size of the worm population in *refugia* was also important, with resistance being delayed more and reversion occurring at lower fitness costs when a larger proportion of the worm population was not exposed to treatment (Maingi *et al*. 1990). Scott and Armour (1991) also found that the proportion of viable eggs being produced by resistant isolates of *H. contortus* was much less than that produced by susceptible isolates. *Refugia* slows the development of resistance by allowing the conservation of susceptible individuals to dilute the progeny of resistant parasites that survive treatment (van Wyk *et al*. 2006). In an initial FEC reduction test conducted in 1995, the efficacy of albendazole, rafoxanide, ivermectin and levamisole varied from 0–76%. However, using the technique of genetic dilution, the population was largely reverted to susceptibility by artificial introduction of susceptible *H. contortus* infective larvae, to the extent that the variation after the dilution varied from 75.1% for albendazole to 97.9% for levamisole in lambs and from 88.0% for ivermectin to 97.9% for levamisole in ewes (van Wyk *et al*. 2001). Further, this level of efficacy apparently being maintained over the ensuing 8 years with the use of TST, based primarily on the FAMACHA© system (van Wyk 2008). On the application of TST in sheep farms, Valcarcel *et al.* (2015) observed significant reduction in number of anthelmintic treatment with maintain the productivity in low challenge environment. In commercial sheep farm in Ohio having confirmed resistance to LEV in *Haemonchus*, *Telodorsagia* and *Trichostrongylus* spp and suspected resistance to ALB in *Trichostrongylus* spp, Bird *et al.* (2001) successfully introduced a new community (more

Table 1. Periodical status of anthelmintic resistance in gastrointestinal nematodes at Sheep Breeding Farm, Fatehpur

Strategy	Period	FECR	EHA (ED_{50})	Remarks
Frequent use of anthelmintics since long	Nov 1998	BZ – 0% TEM – 25% RFX – 96% CLS – 100%	0.213 µg TBZ/ml 0.265 µg TBZ/ml	First time detection of anthelmintic resistance
Withdrawal / restricted use of the anthelmintic types	Aug 2005	FBZ – 14%	-	Six years after withdrawal of benzimidazole
	Jul 2007	FBZ – 75% TEM – 92% MOX – 100%	0.404 µg TBZ/ml	Eight years after withdrawal of benzimidazole
	Jul 2008	CLS – 100%	-	Maintained efficacy since 2004-05
Community dilution strategies since 2010				
(i) Introduction of Chokla sheep possessing anthelmintic resistant / susceptible worms coupled with annual rotational use of anthelmintic types (BZ, TEM and CLS)	Jun 2015	FBZ – 43% TEM – 99% CLS – 100%	-	Marginal increase in BZ efficacy after
(ii) Introduction of Nali sheep possessing susceptible *H. contortus* with use of BZ and CLS anthelmintics in rotation		FBZ – 56% TEM – 99% CLS – 100%	-	Moderate increase in BZ efficacy
(iii) Introduction of Marwari sheep possessing susceptible *H. contortus,* with application of TST using CLS		FBZ – 88% TEM – 99% CLS – 100%	-	Significant increase in BZ efficacy

susceptible) of endoparasites under normal flock management conditions with reversion to susceptibility. The results of present study indicated that just withdrawal of ineffective anthelmintics from worm management programme is not sufficient enough to cause evident reversion to susceptibility. However, community dilution coupled with targeted selective treatment with newer class of anthelmintic will help in reversion to susceptibility in gastrointestinal nematodes at faster rate in sheep flocks at farm.

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