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Evidence of benzimidazole resistance in *Haemonchus contortus* of sheep in north-eastern zone of Tamil Nadu

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ABSTRACT

Gastrointestinal nematode population in four unorganized sheep flocks of north-eastern zone of Tamil Nadu were monitored for benzimidazole resistance using faecal egg count reduction test (FECRT), following oral administration of fenbendazole. The faecal egg count reduction obtained was 99, 70, 98 and 67% with a 95% lower confidence limit of 96, 50, 93 and 47 in the flocks of Tiruvallur, Vellore, Kancheepuram and Thiruvannamalai districts, respectively. Vellore and Kancheepuram flocks showed resistance whereas Tiruvallur and Thiruvannamalai flocks were susceptible to benzimidazole. *Haemonchus contortus* was the predominant nematode species present in coproculture.

Keywords: **Benzimidazole resistance, FECRT, *Haemonchus contortus*, Fenbendazole, Sheep.**

Parasitic gastroenteritis (PGE) caused by gastrointestinal nematodes (GINs) represents a real threat to sheep industry causing heavy economic losses in terms of reduced meat and wool production as well as increased mortality in young animals (Gordon, 1974). Among the GINs the important strongyles responsible for PGE are *Haemonchus contortus, Ostertagia* spp. and *Trichostrongylus* spp. (Bal *et al*., 2007). Benzimidazoles (BZ) have been the mainstay broad-spectrum anthelmintic for the last five decades to control GINs in small ruminants. This has resulted in the co-evolution of resistant parasites across the globe including India (Garg and Yadav, 2009). Reports of benzimidazole resistance in Tamil Nadu are increasing at an alarming rate (Manikkavasagan *et al*., 2016). Thus, regular monitoring of efficacy of anthelmintics is essential to detect the emergence of resistance and its subsequent management. The present study was undertaken to detect the status of benzimidazole resistance in unorganized sheep flocks in North-eastern Tamil Nadu.

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Materials and Methods

Faecal egg count reduction test (FECRT) was conducted in one selected sheep flock each from organized farms of four districts each as per World Association for the Advancement of Veterinary Parasitology (WAAVP) protocol (Coles *et al.,* 1992). Animals of around six months of age which were not dewormed before 12 weeks of trial and having an EPG greater than 150 from four flocks belonging to Pennalurpet of Uthukkottai taluk of Thiruvallur, Kattupakkam of Uttiramerur taluk of Kancheepuram, Kilpakkam in Arakkonam taluk of Vellore and Beemanandhal of Chengam taluk of Thiruvannamalai districts were selected randomly for FECRT. From each flock, 15 animals were kept as infected control group and another 15 were taken as treatment group. About 5 g of faecal sample was collected from each animal, kept in individual zip lock cover, sealed and labelled. The samples were further processed to find the eggs per gram (EPG) count using modified McMaster technique (Coles *et al*., 1992). The animals in the treatment group were dewormed orally

using Fenbendazole (Panacur™, Intervet India) at the rate of 5 mg/kg. body wt. Post-treatment samples were collected after 14 days to estimate EPG. The resistance status of each flock was analysed by RESO calculator (Martin and Wursthorn, 1991).

Coprocultures were made from pre and post-treatment samples of individual flocks. The L_3 larvae were recovered and identified under microscope based on their morphological characters (van Wyk and Mayhew, 2013). Data were analyzed using chi-square test.

Results and Discussion

The reduction in faecal egg counts in Tiruvallur, Vellore, Kancheepuram and Thiruvannamalai districts were 99, 70, 98 and 67%, respectively and the lower 95% confidence limits were 96, 50, 93 and 47%, respectively (Table 1). According to WAAVP protocol, if the reduction in EPG is more than 95%, the worms are susceptible to benzimidazole. If the reduction in EPG is below 95% and the lower 95% confidence limit is above 90% or *vice versa*, the flock is considered as 'suspected resistance'. If EPG reduction is less than 95% and the lower 95% confidence limit is below 90%, then the worms are resistant to benzimidazole (Coles et al., 1992). Two flocks, one in Kancheepuram and one in Tiruvallur were detected by FECRT to have susceptible populations of strongyles to benzimidazole and two flocks in Vellore and Thiruvannamalai had resistant populations. Resistance to the commonly used anthelmintics like albendazole and levamisole in organized farms/unorganized sheep flocks is well documented in Tamil Nadu by FECRT (Vijayasarathi *et al*., 2016). Singh *et al*.(1999) opined that the chances of refugia and greater availability of a mixture of dry and green fodder during late monsoon increased bioavailability of anthelmintic metabolites which resulted in higher efficacy in field flocks. In the present study these chances were ruled out owing to the fact that Tamil Nadu was deficient in rainfall during 2016-2017. The variation of resistance status might be due to the fact that these drugs were less frequently used by the farmers.

The larvae collected from pre and post treatment coprocultures were predominantly *H. contortus*. The nematode appeared susceptible to fenbendazole in the flocks of Tiruvallur and Kancheepuram districts and were resistant in the flocks of selected from Vellore and Thiruvannamalai district. The percentage reduction in faecal egg counts in between districts was statistically significant (Table 1).

Table 1. Mean EPG reduction in sheep after fenbendazole treatment in FECRT

		Tiruvallur	Vellore	Kanchipuram	Thiruvannamalai
Mean EPG (Control)	0 day	738	652	570	362
	14th day	708	700	590	420
Mean EPG (Treatment)	0 day	518	774	788	348
	14th day	6	210	10	140
95% upper confidence limit		100	82	100	79
95% lower confidence limit		96	50	93	47
FECR (%)		99	70	98	67
Status of resistance		Susceptible	Resistant	Susceptible	Resistant
Chi square value			65.69**		

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Faecal egg count reduction test (FECRT) is a widely applied procedure to assess anthelmintic resistance (Coles *et al.,* 1992; Wood *et al.,* 1995) status of sheep flocks. Amarente (2016) reported that significant association was found between worm burden and EPG, which indicates that fecal egg count in most of the situations correlated with the level of infection by gastrointestinal nematodes in sheep.

Therefore, it can be concluded that benzimidazole resistance as well as susceptibility is evident in certain regions of North-eastern zone of Tamil Nadu. So benzimidazole drugs in those areas should be avoided.

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