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Seasonal variation in efficacy of anthelmintics and prevalence of anthelmintic resistance in gastrointestinal nematodes of sheep in Rajasthan

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ABSTRACT

The objective of the study was to ascertain the extent of variation in the efficacy of anthelmintics in different seasons and its correlation with status of anthelmintic resistance in gastrointestinal nematodes of sheep in different agroclimatic regions of Rajasthan. Sheep flocks (141) distributed in different agroclimatic conditions were used for study. The efficacy of anthelmintics was determined by faecal egg count reduction test (FECRT). The seasonal analysis of data on % FECR exhibited that overall efficacy ranged from 56.80±3.99 (June-August) to 79.76±2.23 (December – February) and from 72.65±6.83 (March – May) to 91.46±2.07 (December – February) for benzimidazole and tetramisole, respectively. No seasonal variation was observed for efficacy of closantel against *Heamonchous contortus* in sheep and it remained > 99%. The anthelmintic treatment during climatic extreme (winter) when there is little or no translation of exogenous stage was found responsible for increased frequency of flocks possessing benzimidazole-resistant worms. The trend analysis exhibited positive skewness with platykurtic curve for benzimidazole while for tetramisole the skewness is moderately negative with leptokurtic curve. Our findings are suggestive that there is need for regular monitoring of drug efficacy and anthelmintic resistance, formulation of epidemiology based integrated worm management programme and in-depth study on role of refugia on drug efficacy.

Key words: Anthelmintics, Gastrointestinal nematodes, Resistance, Seasonal efficacy, Sheep

Helminth infections are an important cause of reduced productivity in livestock world-wide (Tembely *et al*. 1997). Owing to favourable climatic conditions for translation of exogenous stages and in the absence of alternate control strategies, control of parasitic gastroenteritis (PGE) in ruminants is primarily attempted by the frequent use of anthelmintics at short intervals which result in continuous decline in efficacy of anthelmintics and the emergence of high anthelmintic resistance (Singh and Swarnkar 2008). The demand for antiparasiticides differs from one geo-economic area to another. However, the key issue is that a moderate loss of efficacy (usually not perceived at field level) represents an enormous loss in terms of antiparasitic agent's cost-effectiveness and a significant step along the path of no return in terms of parasite resistance (Nari and Hansen 1999). Treatment of animals at frequent intervals for worm control resulted in dramatic productivity increases in the past but simultaneously led to serious anthelmintic resistant problems (Kaplan 2004). Farmers are now convinced of the benefit of worm control but they are to administer the right product at the right time to right category of animals (Keyyu *et al*. 2003).

Anthelmintic resistance is a major problem globally in nematode parasites of small ruminants (Waller *et* al. 2004, Coles 2005, Singh and Swarnkar 2008) but no reports of seasonal variation in efficacy of anthelmintics are available. As anthelmintic interventions are being undertaken in all the seasons and pharmacokinetics and bioavailability of pharmacologically active metabolites in the animal's body which are greatly influenced by host physiology, type of fodder ingested and route of administration, the initial objective of the study was to ascertain the extent of variation in the efficacy of anthelmintics in different seasons and its correlation with status of anthelmintic resistance in GINs of sheep in different agroclimatic regions of Rajasthan. This information is important to ensure that effective parasite control programmes are in place and to maintain the efficacy of anthelmintics and high proportion of *refugia,* thus reducing selection pressure for anthelmintic resistance.

MATERIALS AND METHODS

Sheep flocks (141) distributed in different agroclimatic conditions of Rajasthan were used to ascertain the seasonal variation in efficacy of anthelmintics. The flocks selected

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were having history of not using any anthelmintic for the last 3 months. All the flocks were managed under semi-intensive system by grazing during day hours and rest in corrals at night. The field flocks were exclusively managed on common property resources, top feeds and on stubbles after harvesting of fields.

Faecal egg count reduction test: The test was performed as per World Association for Advancement of Veterinary Parasitology (Coles *et al.* 1992). Depending on the flock, strength, animals with in a flock were randomly divided into 2 to 4 groups (12–16 animals/group). Within flock one group was kept as control (undrenched) for observing the natural changes in faecal egg counts during the test period. Animals of rest groups were drenched one of the anthelmintics. The anthelmintics put under test were fenbendazole (BZ) orally @ 5.0 mg, tetramisole (TEM) orally @ 15.0 mg kg^{-1} and closantel (CLS) orally @ 10.0 mg kg^{-1} BW. All the animals within flock were kept together and allowed to graze on similar pasture. Faecal samples were collected *per rectum* from each animal on the treatment day (0 day) as well as on 10–12 days post treatment. The intensity of infection was estimate by modified McMaster technique (MAFF 1986). The leftover faecal samples were pooled on both pre-treatment (flock-wise) and post-treatment (group-wise) occasions and subjected to coproculture for larval identification (Solusby 1965).

Statistical analysis: The observations were grouped for different seasons and analyzed for % faecal egg count reduction (% FECR) by RESO calculator (Martin and Wursthorn 1991) for combined species of strongyle worms (against BZ and TEM) and only for *H. contortus* (against closantel). Resistance to anthelmintic was considered present if (i) the % FECR was less than 95, and (ii) the lower 95% confidence limit was < 90. If only 1 of these 2 criteria was met, resistance was suspected and grouped into susceptible category. The trend and frequency distribution of flocks as per anthelmintic efficacy were assessed by determining the skewness and kurtosis using SPSS programme, ver. 15.0.

RESULTS AND DISCUSSION

Seasonal efficacy of anthelmintics: In present state-wide study, the overall mean efficacy for BZ and TEM anthelmintics against strongyle worms (predominantly *H. contortus*) in sheep flocks was 66.51±2.26 and 85.77±1.71%, respectively, while for CLS against *H. contortus* was 99.43±0.16% (Table 1). The seasonal analysis of data on % FECR exhibited that efficacy of BZ anthelmintics ranged from 56.80±3.99 in monsoon (June-August) to 79.76±2.23 in winter (December – February) and the observed variation could be attributed to type/quality of fodder available in different seasons as well as quantum of *refugia* in population and climatic extremes. The availability of only lush and highly succulent type of grasses for grazing on common property resources during monsoon resulted in increased gastric motility, reduced absorption and bioavailability of pharmacologically active metabolites and decreased drug – parasite contact with ultimate reduction in efficacy compared to other seasons. With the maturation of grasses in grazing area during post-monsoon (Sept – November) and practice of offering dry fodder through allowing the flock to graze on stubbles after harvesting of crop during winter (December – February), there is increased retention time and prolonged absorption of anthelmintic metabolites resulted in higher efficacy of BZ anthelmintics during these seasons. The frequency distribution of flocks exhibited that only ~ 20.0% of the flocks tested had > 95% efficacy for BZ (Fig. 1) indicating extent of spread of BZ resistance in sheep flocks. Similar to BZ, seasonal variation in efficacy was also noticed for TEM against strongyle worms from a minimum of 72.65±6.83% (March – May) to maximum of 91.46±2.07% (December – February). Lower efficacy of TEM during the period from March – May (summer) indicated that in host

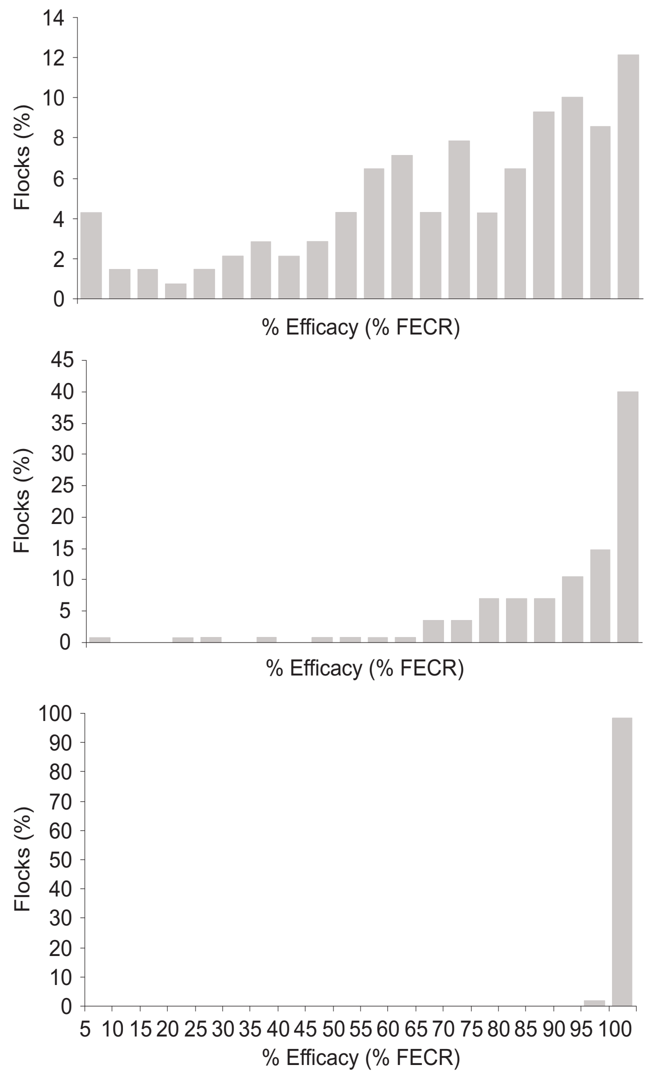

Fig. 1. Frequency distribution of sheep flocks for anthelmintic efficacy against strongyle worms.

Table 1. Seasonal variation in efficacy and extent of anthelmintic resistance in strongyle worms of sheep in Rajasthan

	Seasons (Periods)				Overall
	Mar - May	June – Aug	Sep – Nov	Dec - Feb	
		Benzimidazole			
No. of flocks	20	54	29	37	140
Mean (±SE) efficacy (%)	60.65±6.52	56.80±3.99	72.00±4.69	79.76±2.23	66.51±2.26
BZ-resistant (%)	90.0	94.4	75.9	81.1	86.4
H. contortus (%) on culture	86.06±4.92	96.53±0.97	94.47±2.03	79.37±5.29	90.05±1.76
		Tetramisole			
No. of flocks	17	44	26	28	115
Mean (±SE) efficacy (%)	72.65±6.83	90.39±1.53	80.38±4.39	91.46±2.07	
TEM-resistant (%)	70.6	54.5	57.7	46.4	55.7
H. contortus (%) on culture	88.06±4.76	96.42±1.16	97.70±0.83	75.01±6.68	90.26±2.01
		Closantel			
No. of flocks	4	22	10	22	58
Mean (±SE) efficacy (%)	100.00	99.55±0.28	99.90±0.10	99.00±0.29	99.43±0.16
CLS-resistant (%)	0.0	0.0	0.0	0.0	0.0
H. contortus (%) on culture	90.20±7.02	97.06±0.84	97.98±1.31	72.00±8.19	87.24±3.50

more number of resistance worms were present due to practice of drenching the flock in October, shifting of locks on post-harvested fields and might be resumption of hypobiosis. The effect of type of fodder was not evident on the efficacy of TEM. Around 55.0% of the flocks showed > 95% efficacy for TEM (Fig. 1) revealing possibility of limiting the spread of TEM resistance in field if suitable monitoring and migration policy will be adopted. In the present study, no seasonal variation was observed for efficacy of CLS against *H. contortus* in sheep and higher efficacy (> 99.0%) could be attributed to non-exposure of parasites to CLS in field flocks.

Status of anthelmintic resistance: Based on the values for % FECR with 95% confidence limits, the overall prevalence of BZ resistance in strongyle worms (predominantly *H. contortus*) was 86.4% (Table 1). The treatment with BZ anthelmintics during extreme summer (March – May) when there were little or no *refugia* on pasture resulted in increased selection pressure for anthelmintic resistance as reflected in presented study where proportion of flocks possessing BZ resistant strongyle worms increases in monsoon just after summer drenching mainly due to propagation and predominance of resistant genotypes in population. However, with the repetition of life cycles of worms, an increase in susceptible genotypes in environment and host (community dilution of worms) in subsequent post-monsoon season could be the possible factor for decrease in proportion of flocks (75.9%) harbouring BZ-resistant strongyle worms. The anthelmintic treatment during climatic extreme (winter season), when there is little or no translation of exogenous stage found to be responsible for increased frequency of flocks possessing BZ resistant worms (81.1%). The overall prevalence of TEM resistance in strongyle worms in sheep of Rajasthan was 55.7% and seasonally varied from 46.4% (winter) to 70.6% (summer). Unlike BZ resistance, the lower prevalence of TEM resistance and generic composition of strongyle larvae during winter suggested that *Trichostrongylus* species are susceptible to TEM.

Trends of anthelmintic efficacy in field flocks: The skewness and kurtosis for efficacy of all 3 anthelmintics was measured and compared with standard when drug is first launched by assuming > 99.0% efficacy for anthelmintic when no resistance was present. At the time of introduction of drug, the trend in anthelmintic efficacy in population is highly skewed (Fig. 2) to negative and a highly leptokurtic curve was obtained indicating that drug in question will cover maximum population in short range. In the present study the trend analysis for BZ exhibited skewness towards positive with a platykurtic curve suggested that a large range is required to cover maximum population to attain satisfactory efficacy of BZ. The skewness for TEM is moderately negative and leptokurtic nature of curve indicated that in a short range maximum population will be covered. In the present study almost similar trend was observed for CLS as observed for standard (Fig. 2).

The epidemiological studies from Rajasthan revealed monthly incidence of GIN to the tune of 10.0 to 84.0% with predominance of *H. contortus*. Peak of intensity of infection occurred only during monsoon and favourable climate for translation of larvae exist from June to September. There was no appreciable periparturient rise in FECs. The farmer's practices exhibited use of anthelmintics for worm control at the frequency ranging from 2.31 to 2.67/sheep/year. The timings of anthelmintic treatment are not based on epidemiology of GIN but usually coincide with availability of money with them after shearing and type of anthelmintic is mainly selected on the choice of local pharmaceuticals. In addition there are also misconceptions like that top performance of flock is obtained with high and regular anthelmintic input, deworming is the only way of controlling

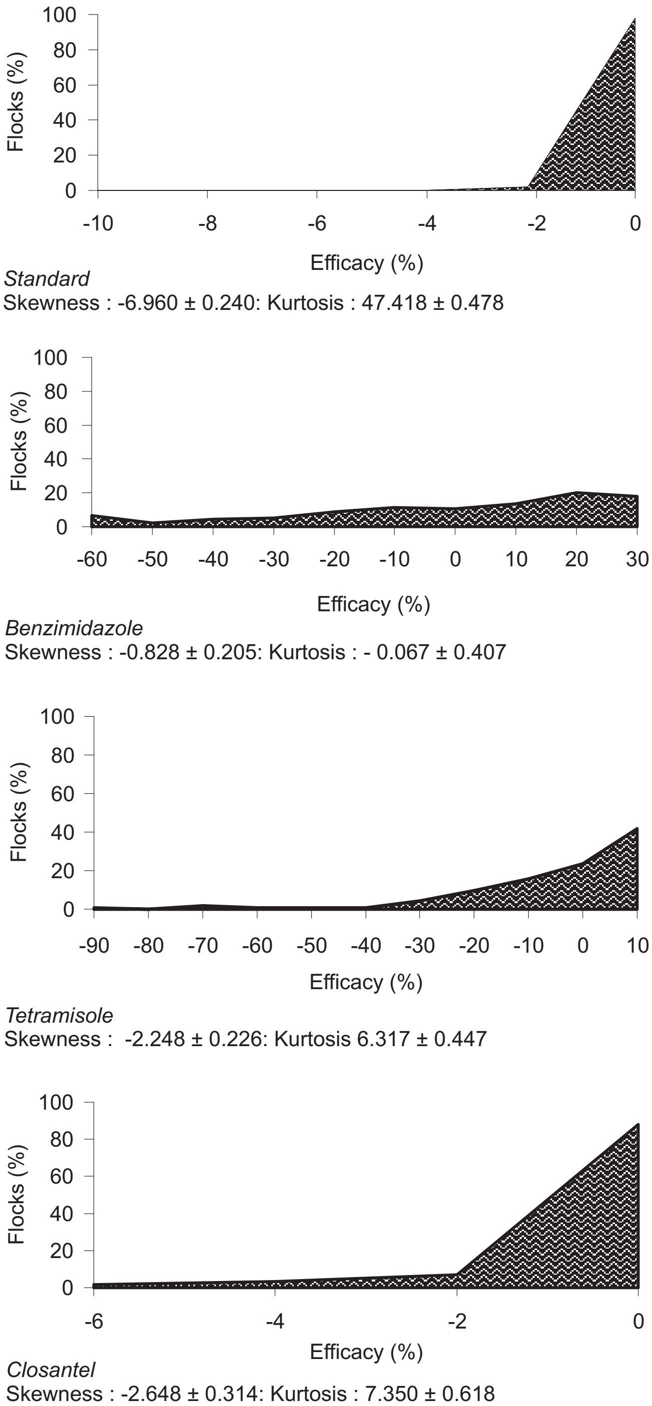

Fig. 2. Trends of anthelmintic efficacy against strongyle worms in sheep flocks of Rajasthan.

worms, enteritis, anorexia and debility, and that anthelmintics are the only medicines that cure all ailments in sheep flock (Swarnkar *et al*. 2008). At the market level, it has been observed in most of the advertisements that reach farmers refer to use of anthelmintics with a catch "regularly treat your flock against worms", "optimize production by the use of anthelmintics" as well as growing attitude of different institutions/agencies and livestock owners towards adoption of liberalization, globalization and privatization (LPG) policy resulted in a remarkable increase in veterinary pharmacies in towns, drug shops in rural and remote areas, influx of several brands of veterinary drugs and a multitude of para-veterinarians and quacks. Further at infrastructural/ technological level it has been observed that there is very poor interaction between technocrats and sheep breeder as well as insufficient liaison exists between researchers and extension personnel. Thus, under above mentioned scenario a lot of anthelmintics are being pushed down the gullet of the animal (frequent and indiscriminate use) with the resultant rise in selection pressure for anthelmintic resistance.

The studies showed anthelmintic resistance development on small ruminant farms where treatment frequency was less than 2 drench a year, as if a high selection pressure by anthelmintic treatments was neither necessary, nor sufficient to promote anthelmintic resistance development in real life (Coles *et al*. 1995). This led to propose the hypothesis that anthelmintic resistance may develop under an efficient treatment (at the right time) rather than under apparent (number of treatments) selection pressure (Silvestre *et al*. 2002). A selection pressure is efficient when the resistant worms that survived the treatment can contribute for a large part to the subsequent worm generation. So, anthelmintic resistance development is a complex phenomenon, which implicates other factors than treatment frequency in itself (Jackson 1993). If drugs do not effectively kill worms on a premise, it does little good to continue their use as the drug becomes even less effective. Evaluation of the efficacy of anthelmintics on each farm/locality is essential to any on going control programme.

For most anthelmintics, efficacy is related directly to the duration of contact between drug and parasite. To ensure sufficient drug-parasite contact time, it is important that the full dose is lodged in the rumen where the drug then binds to rumen particulate matter and slowly released as digesta passes down the digestive tract. Once in the rumen, duration of drug availability as it flows to more distal sites of absorption is largely dependent on the flow-rate of the digesta (Hennessy 1997). Differences have been reported in the plasma levels of anthelmintics depending on the dietary intake of dry feed or grazing (Ali and Chick 1992), restricting feed intake for 24 h before treatment slow digesta flow and increase drug availability and efficacy. Fasting animals overnight before drenching may increase the efficacy of benzimidazole anthelmintics but not ivermectin or levamisole (Ali and Hennessy 1995, Escudero *et al*. 1997, Charlier *et al*. 1997). Sissay *et al.* (2006) observed high level of efficacy for anthelmintics from 3 different action families when used after withholding (12 h) the animals from feed.

Preservation of anthelmintic susceptibility amongst the nematode parasite population of the sheep flock compared to goat flock is likely to attribute to difference *in refugia*

(Sissay *et al*. 2006). Ample field evidences have now shown that the combination of anthelmintic treatment to all animals plus extreme weather conditions on pasture (unfavourable for development, survival and dissemination of the free-living stages of nematode parasites) imposes potent selection for the development of anthelmintic resistance (Besier 1996, Papadopoulos *et al*. 2001). Change in management also resulted in changes in efficacy of anthelmintics as demonstrated by Sissay *et al.*(2006) where they prevented livestock grazing on a paddock for 3 months, followed by harvesting of forage and feeding of hay for further 3 months and finally grazing the area for another 3 months and with sort of management the degree of efficiency of anthelmintics increased to 95–98% from earlier level of 57–70%. Thus reversion back to high level anthelmintic efficacy had occurred by exploiting natural *refugia* in the parasite population of small ruminants in Ethiopia.

The present study indicated that there is high prevalence of BZ and TEM resistance in *H. contortus* from sheep flocks of Rajasthan. There is a declining trend in efficacy of both BZ and TEM in population with much faster rate in BZ anthelmintics. The findings of the study are suggestive that at research level there is need for regular monitoring of drug efficacy and anthelmintic resistance, formulation of epidemiology based integrated worm management programme and detail study on role of refugia on drug efficacy. At field level involving both veterinarians and sheep breeders, it is need of hour to increase awareness among them about rotational and minimum use of anthelmintics through increasing the interaction among them by regular refresher/ training programme. Like-wise at corporate level, as introduction of newer class of anthelmintic is uncertain, it is call of day to frame a policy for planned influx of type of anthelmintics in a particular region with changes in extension materials with message like “how a farmer can get better result using their product”. The available resources of anthelmintics could be used judiciously by exploiting the knowledge of epidemiology, host physiology and pharmacokinetics of anthelmintics. It is essential to give the drug the best chance to work by taking actions which would prolong the effective lives of valuable anthelmintics (Sanyal 2009). While care must be exercised to adhere to the basic elements of parasite control like, knowing the status of anthelmintic resistance (Swarnkar *et al.* 2001), selection of anthelmintic type (Sanyal 1998) and epidemiologically based strategic treatment programmes (Singh *et al.* 1997), there are a number of practical options based on understanding of pharmacokinetics of anthelmintics, physiology of the hosts and their interaction (Swarnkar *et al*. 1998, Singh *et al*. 1999, Sanyal *et al*. 2003) that can be implemented to maximise anthelmintic efficacy.

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