

EFFECTIVE USES AND RESISTANCE OF DEWORMING DRUGS AGAINST GASTROINTESTINAL NEMATODES IN SMALL RUMINANTS

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ABSTRACT

Gastrointestinal nematode parasitism is one of the major factors limiting sheep production worldwide because they cause heavy economic losses in meat and wool production. Gastrointestinal parasitism is the major cause for morbidity and mortality in ruminants. Severe anemia, reduction in functional gastric gland mass, severe damage to gastric mucosa and villous atrophy caused by these worms are responsible for the death due to parasitic gastroenteritis. In India, the common nematode species encountered is *Haemonchus contortus* in small ruminants. This review throws light on the different mechanisms and contributory factors affecting the development of anthelmintic resistance, the diagnosis and means to prevent the resistance at field level. The emergence of multi-resistant nematode has shown that the previously used control strategies is no longer a chemically success method. The interpretation of fecal egg count reduction test has been modified and suggestions are made on its use with persistent anthelmintics.

Keywords: Gastrointestinal nematode parasitism, small ruminants, anthelmintic resistance.

INTRODUCTION

According to AHDF report (2012) there are 65.1 million sheep and 135.2 million goats in India with a large genetic diversity as reflected by 40 species of sheep and 20 species of goats which account for 0.5 to 5 per cent of the value of total output of the livestock sector (Singh, 1995). Indian goats have adapted to the variety of climatic and agro-economic situations prevalent in different parts of the country and cost little to maintain as they feed on harvested or fallow fields, canal banks or overgrazed commons. Sheep in India are kept by people who have traditionally taken to sheep farming and are reared in the migratory system, which is dependent on season and availability of pasture to graze. The sheep breeding regions of India are classified as (1) The northern plains, (2) The semi-arid western region, (3) The southern humid region and (4) The temperate and sub-temperate mountains. Both sedentary and migratory system of sheep production are common in India.

Gastro-intestinal parasitism constitutes a major challenge to the health, welfare and productivity of sheep worldwide (Andrea et al. 2011). Sheep are important source of income particularly for marginal farmers and landless labourers in India and contribute a lot towards their economy. Out of the various diseases affecting sheep, parasitic gastroenteritis caused by

nematodes is important in sheep. In India, *Haemonchus contortus* is the species responsible for high mortality and morbidity (Yadav, 1997). Gastrointestinal parasitism also causes morbidity, in certain cases mortality, while subclinical infections reduce significantly the overall income of sheep farmers (Jackson et al. 2009; Mavrogianni et al. 2011; Varadharajan and Vijayalakshmi 2015). Use of anthelmintics is the 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 gastrointestinal nematodes, due to constant use and improper use of some anthelmintics (Satyavir Singh and Gupta, 2010). Intensive use of anthelmintics to control gastrointestinal nematodes selects for anthelmintic resistance (AR), which has become an important issue in most sheep-rearing countries, due to its clinical and financial significance (Von Samson-Himmelstjerna 2006; Cudekova et al. 2010 and Varadharajan and Vijayalakshmi 2015).

Anthelmintics

An anthelmintic is compounds which destroy or remove helminths from the hosts. Many modern anthelmintics are effective against both adults and larval stages and an increasing number are efficacious against arrested or dormant larvae. They should not be used indiscriminately. The ideal anthelmintic has the following properties: (a) A broad spectrum activity against adult and larval helminth parasites. (b) A rapid metabolism in the body and short-lived presence at low levels in the milk and/or tissues. (c) A low toxicity in the target species. The ratio of the therapeutic dose to the maximum tolerated dose should be as large as possible. (d) No unpleasant side-effects to the animal or to the operator. (e) Suitable for practical and economical integration into various management systems.

Administration of anthelmintics: It is important to first identify the nature of the parasitic problem in order to select the appropriate drug to treat the infection. The optimal time and mode of administration of the drug should then be considered.

(a) **Dosing by mouth :** Majority of anthelmintics are given orally as liquid preparations, pastes, boluses and tablets. Several devices such as syringes, bottles and drenching guns can be used for delivering the dose. It is important to keep the drenching equipment clean after use. The dose to be delivered should be checked before-and several times during-dosing to ensure that the correct dose is given to all animals. Pastes are relatively easy to administer if a proper dispenser is available. Boluses and tablets can be placed deep in the mouth of the animal by using a dosing gun or a pair of long-handled forceps. Bolus and tablet formulations have the advantage that if the dose is rejected, it is usually the total dose and a replacement can then be administered.

Dosing by injection : A number of anthelmintics are available in the form of injection. The size of needles should be appropriate for the formulation and the site of injection. In order to avoid local reactions (such as abscess formation at the injection site) the highest possible hygienic standards should be maintained.

Dosing by external application : Several dewormers are now available in a formulation for external application, termed “pour-on” preparations. The active ingredient of the drug is absorbed through the skin reaching its target via the circulatory system.

Anthelmintic resistance

The ability of worms to survive treatments that are generally effective at recommended dose rate is considered a major threat to the future control of worm parasites of small ruminants.

Use of anthelmintics is the mainstay to reduce the adverse effects of these nematode parasites but their usefulness is constrained by the emergence of AR (Saddiqi et al. 2011). The clinical definition of resistance is 95% or less reduction in a "Faecal Egg Count" test. Treatment with an anthelmintic drug kills worms whose phenotype renders them susceptible to the drug. Worms that are resistant survive and pass on their "resistance" genes. Resistant worms accumulate and finally treatment failure occurs. Increased productivity in ruminants through the control of helminth parasites will depend upon the availability of low cost, effective anthelmintics (Maharshi et al. 2011). It is therefore of great concern that the regular use of anthelmintics has led to the selection of drug-resistant helminths. This has become a serious problem in many countries and resistance of parasites to one or more anthelmintics is now widespread, particularly in sheep. In India, the first report of AR 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 poured in especially from organized farms (Yadav and Uppal, 1992; Singh and Yadav, 1997; MeenaDas and Satyavir Singh, 2010 and Sudharani et al. 2013).

AR is often first suspected in cases of apparent anthelmintic failure, but it should be kept in mind that several other factors can be responsible for the lack of efficiency of a drug. These include:

Under dosing: Most farmers usually estimate the weight of their animals and many surveys have shown that such estimates are often considerably below the actual weight. This automatically results in under dosing.

Rapid reinfection: If animals are grazed on heavily contaminated pastures, reinfection occurs immediately and this may give the impression of drug failure. This is particularly relevant where *Haemonchus contortus* is dominant, as it develops rapidly and is very pathogenic.

Inefficiency against arrested or dormant larvae: Arrested larvae which are unaffected by the anthelmintic being used may continue development immediately after treatment.

Presence of drug resistant parasites: Frequent regular treatments using the same anthelmintic given at low dosages over a prolonged period of time, will predispose to the development of drug resistance.

Factors affecting AR development: The development rate of AR appears to be slow at first, but once a certain level of resistance genes has been established, the subsequent treatments result in an exponential increase of these resistance genes to a level where treatment failure occurs (Barnes et al., 1995; Sangster, 1999). The more intensively parasites are controlled with drugs; the more likely resistance will develop. Once resistance is present in a parasite population, there is no evidence of reversion or loss of resistance (Andronicos et al. 2010). The dynamics of the selection for AR of parasites in sheep have been well studied

(Leathwick et al. 2009). These factors act either independently or in an additive fashion and may be associated with the parasite species, the infected host, drug treatment, on-farm control management or the environment.

Parasitic factor: Due to their genetic diversity, parasites in a population do not respond uniformly to treatment (Vercruyssen and Rew, 2002). It is presumed that resistance alleles already exist within the parasite population, prior to the first introduction of a drug (Wolstenholme et al. 2004). However, an alternative hypothesis suggests multiple origins of resistance by spontaneous and recurrent mutations (Skuce et al. 2010). Although the genetics of resistance are still poorly understood, resistance develops more quickly if only one gene is involved than when multiple genes are involved. Moreover, resistance develops faster if the genes are dominant rather than recessive: both heterozygote and homozygote worms will survive the treatment and contribute to the next generation (Le Jambre et al. 2000 and Coles, 2004). Furthermore, some parasites have biological characteristics that favour resistance alleles to build up faster in the population, such as their direct life cycles, a short generation time and high fecundity. It is assumed that, if resistant parasites have enhanced fitness or if resistance is linked to other fitness genes, the spread of resistance in the population will also increase. Fitness includes all properties that enable more worms to complete their life cycles, such as the egg-laying rate, the persistence of worms in the host survival on the pasture, the ability to migrate on herbage and their infectivity when ingested (Coles, 2005).

Management systems: It has high influence on the epidemiology of gastrointestinal nematode. High stocking density increases the contamination of the environment with nematode eggs and thus makes the infective stages to be more accessible to susceptible animals. Low stocking rate and extensive management systems in the traditional husbandry systems preclude a built-up of high worm burdens. The frequency of anthelmintic treatment and the extent of under dosing are mainly responsible for inducing AR (Van Wyk, 2001). To decrease the selection pressure, it is of major importance that treatment and pasture management are fulfilled. Pasture contamination derives from worms surviving short interval treatments, which creates a selection pressure on AR. Farmers should be aware that summer drought is a variable factor that clears out the free-living stages on pasture (Saeed et al. 2010). Additionally, bought in new entry of the herd animals should be effectively quarantine drenched before they are placed on pasture in order to dilute out the progeny of survivors of the quarantine treatment (Pomroy, 2006).

Prevention of the development of AR:

There is an urgent need for the development and adoption of strategies to prevent the spread of AR, particularly in nematodes of sheep and prevent it from becoming a problem. The following practical measures can be taken to delay the occurrence.

Use the correct dose: Maintain drenching equipment. A common cause for incorrect dosing is faulty dosing equipment. It is very important that equipment is tested for accuracy before the start of dosing.

Reduce dosing frequency: It is important to establish the epidemiology of the helminth infections and introduce strategic deworming program based on a few well-timed treatments given when it is most advantageous.

Establish treatment and quarantine for all animals introduced to the farm. It is advisable to keep the newly introduced animals isolated for 72 hours after arrival and treatment.

Alternate anthelmintics: Present information recommends continued use of an anthelmintic for at least a whole season (one year for many tropical and sub-tropical countries) provided it is effective. When changing the anthelmintic, a drug from a different class should be selected.

In addition farmers should consider establishing grazing management practices which reduce parasite burden and subsequently the need for treatment (Makawana and Veer Singh, 2009).

IN-VIVO method of diagnosing the AR

With the development and spread of AR in nematodes of livestock, the need for methods to detect resistance has evolved simultaneously. A wide range of tests has been developed to detect AR for research and diagnostic purposes (Presidente, 1985). The growing importance of AR has led to an increased need for reliable and standardized detection methods (Coles et al., 1992) some of which have been previously described and reviewed (Taylor et al. 2002). Most of the methods described have drawbacks either in terms of cost, applicability and interpretation or reproducibility of findings (Varady and Corba, 1999). Since the publication of the World Association for the Advancement of Veterinary Parasitology (WAAVP) methods for the detection of AR in 1992, AR was considered to be present if the percentage reduction in faecal egg counts was less than 95% and the lower limit of the 95% confidence interval was less than 90% (Coles et al., 1992). If only one of these criteria is met, AR is suspected. Based on this criterion, the FECR percentage and the lower confidence limit obtained from Dale district smallholder sheep and goat production system revealed the absence of a significant level of GIN resistance to tetramisole and ivermectin (Levecke and Vercruyse, 2012; AmitKumar et al. 2013), the importance of resistance to the three groups of broad spectrum anthelmintics has increased dramatically in nematodes of sheep and goats in many parts of the world (Wolstenholme et al., 2004). The most accepted methods are two in-vivo methods: the fecal egg count reduction test (FECRT) and the controlled efficacy test (CET). Although the CET is the most reliable method, it is not feasible in commercial farm settings (Satyavir Singh and Gupta, 2010).

Faecal egg count reduction test (FECRT) :

The FECRT has been the most recommended method so far (Waller, 1986; Jackson and Coop, 2000), being broadly utilized either for field or research studies (Wood et al., 1995). This test is easy to perform, suitable for ruminants, horses and pigs as well as for all types of anthelmintic. In addition, it can be carried out on any species of nematodes in which eggs are shed in the faeces. This test estimates the efficacy and resistance by comparing egg counts before and after the treatment (Gill et al. 1998). The untreated group which serves as a control is used to monitor any changes that occur in nematode egg counts during the test period. Egg counts for *Nematodirus* spp. are generally low and bear little relationship to actual worm burdens (Chalmers, 1985; Atle et al. 2012). A good correlation has been found between faecal egg counts and worm counts for *H. contortus*, but not for *T. colubriformis* (Bartley et al. 2009) or *T. circumcincta* (Atle et al. 2012). Later, Dhanalakshmi et al., (2003)

recorded benzimidazole resistant against *H. contortus* in 9 out of 10 farms in Karnataka in Southern India and Jeyathilakan et al., (2003) recorded the prevalence of benzimidazole resistant *H. contortus* in two institutional farms in northern Tamil Nadu in South India. Quarantine importance to prevent the spread of AR among sheep breeds was done by FECRT method. Thus simply following quarantine measures multiple AR in the sheep farm was successfully managed (Swarnkar and Singh, 2012). Multiple AR due to the need in the measures to control resistance in two genera of nematode was emphasized in south India (Chinnakarupiah et al. 2009).

Palcy et al. (2010) concluded that the FECRT is widely used method of assessing anthelmintic efficacy, has a low sensitivity for the detection of AR in one nematode species in the context of multi-species nematode infections. This test is particularly suitable for field surveys and it has the advantage that the number of groups can be increased if appropriate, to test the efficacy (Lespine et al. 2012) of a range of broad or narrow spectrum anthelmintics at one time. The interpretation of the FECRT is affected by a complex interplay of various factors, including the detection limit of the FEC method, the number of animals per treatment group and the level of excretion and aggregation of FEC's (Desie Sheferaw, 2013). Recently, Torgerson et al. (2005) has demonstrated that negative binomial distribution, which is a mathematical distribution model, can detect evidence of AR with a FECRT that otherwise might require a slaughter trial to demonstrate. In addition, the simulated data sets confirm that there is a significant probability of failure to detect the low anthelmintic efficacy with commonly used mathematical techniques (Jabbar et al. 2006).

The access to efficient drugs and the ease with which they could be applied, combined with the immense progress made in establishing the epidemiology of the gastro-intestinal nematodes of ruminants, led to a period of relative success in the control of worms, particularly in the livestock production systems of the industrialized countries. However, the false assumption that worm control is easy and can be accomplished by using broad spectrum drugs without an epidemiological database was also being promoted, preventing or delaying the epidemiological studies that are a prerequisite for effective control. Further complicating the situation today and for future parasite control program is the fact that all the economically important parasite species of sheep and goats have developed resistance to all four groups of anthelmintics. FECRT provides the better mean to detect AR in field trials and more scientific tools are required to increase the accuracy and efficiency in egg counting, in Belgium the commercial FECPAK kit is available which has a detection limit of 10 EPG to test for nematode egg. A regular insight monitoring of AR in field is required and has to be conducted by the owners of the herd, the farmers. Other requirements to avoid sub-optimal treatment dosage, variance in pharmacokinetics of drug and lack of sensitive counting must be met with modern ideologies. Further efficacy of drug after in-vivo detection can found out by in-vitro and molecular detection techniques which provide results more accurately. There must be a rotation in drug use within the broad and narrow spectrum which can decrease the resistance among species.

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