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Anthelmintic Resistance in Livestock Parasites: Indian Scenario

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A B S T R A C T

Parasitic diseases rank among the most prevalent infectious diseases. Chemotherapy forms the mainstay for control of parasites. Availability of antiparasitic drugs is limited due to high cost of development of newer drugs and rapid pace of development of resistance. Literature shows that resistance against all newly launched antiparasitic drugs is noticed within a decade time. Condition is severe and discouraging in tropical countries like India where animals carry substantial parasitic load and there is indiscriminate use of drugs. Anthelmintic resistance in helminthic parasites, particularly *Haemonchus contortus* are well reported from India. Molecular markers are identified for large scale screening of benzimidazole resistance. Chemotherapy being central to the control of parasites, drug resistance is likely to be a major issue in near future. Constant surveillance and monitoring of antiparasitic drug resistance is the need of the hour. The problem of resistance can be circumvented either by delaying its onset or use of alternate strategies in the form of integrated parasite management.

Introduction

Antiparasitic drug resistance is the genetic ability of parasites to survive treatment with a drug that was generally effective against those parasites in the past. It is due to the selection of a specific heritable trait in a population of parasites that results into significant increase in survivability of that parasite population to standard recommended dose of the drug. After an animal is treated with an antiparasitic drug, the susceptible parasites die and the resistant parasites survive to pass on resistance genes to their offspring. Antiparasitic resistance poses a significant threat to animal health and can result into substantial production losses. Antiparasitic resistance has been documented in livestock, both globally and within India.

Many factors contribute to antiparasitic resistance, including the biology of the parasite; the immune status of the host animal;

treatment practices; drug properties; and certain livestock management practices. The situation has recently become alarming in India particularly anthelmintic resistance in the nematode parasites of grazing animals and acaricide resistance in livestock ticks. To help combat this emerging problem, there is a need to develop an antiparasitic resistance management strategy that promotes sustainable use of approved antiparasitic drugs by slowing the development of antiparasitic resistance in livestock parasites in India. This paper will mainly discuss anthelmintic and acaricide resistance in addition to throwing some light on drug resistance against protozoan parasites of livestock and poultry.

Anthelmintic resistance

Anthelmintics are a group of antiparasitic drugs that kill or expel parasitic helminth worms from the body of the host. Since 1940s anthelmintics remain mainstay of worm control and treatment of parasitic infections in farm animals. However, resistant parasitic population to majority of the launched anthelmintics are recorded within two decades of their commercialisation, for instances, phenothiazine launched in 1940s as first anthelmintic and its resistance report came into record by 1957 in USA (Drudge *et al.,* 1957). Amongst livestock animals, sheep has witnessed maximum number of cases of anthelmintic resistance. In India, first report of anthelmintic resistance was published in 1976 from organised sheep farms of Uttarakhand region against phenothiazine and benzimidazole (thiabendazole) drugs (Varshney and Singh, 1976). Hitherto, the importance of anthelmintic resistance remained in dim light until 1990 when the condition become alarming and started reporting throughout India (Yadav, 1990). Globally, sheep was the main species of attention for the anthelmintic resistance until 1983, when the Kettle and co-workers reported first case of be benzimidazole resistance in goats (Kettle *et al.,* 1983). The wonder drugs of 1980s, ivermectin was found to be ineffective against gastrointestinal nematodes in South Africa (Carmichael *et al.,* 1987). Recently introduced anthelmintics such as derquantel and monepantel has also been reported to be ineffective in control of commonly found roundworm in small ruminants in certain parts of the world (Kaminsky *et al.,* 2011; Scott *et al.,* 2013). The emergence of anthelmintic resistance within a decade of their launch demonstrates the high vulnerability of these drugs. The following terms have been used while reporting resistance.

Cross resistance

Ability of the parasite strains to survive the recommended therapeutic doses of chemically unrelated drugs having different modes of action.

Side resistance

Parasites demonstrate resistance to a drug as a result of selection by another drug having similar mode of action.

Multiple resistance

Parasites are resistant to two or more anthelmintic groups because of either selection by each group independently or by side resistance.

The current status of anthelmintic resistance in India

There are over 100 reports from various states of India documenting development of anthelmintic resistance in livestock parasites against commonly used anthelmintic drugs. The anthelmintic resistance reports in India have been reviewed by several researchers

(Singh *et al.,* 2002; Yadav and Garg 2004, 2005, 2007; Singh and Swarnkar, 2008, 2010). There is paucity of information on anthelmintic resistance from the north east states of India (Table 1).

The factors that have contributed to the development of anthelmintic resistance

Anthelmintics form the mainstay of worm control in Indian conditions. Intensive and indiscriminate use has accelerated the development of anthelmintic resistance. Rate of development of anthelmintic resistance is influenced by genetic, biological, operational or environmental factors. Major factors that seek considerable attention are worm biology (includes genetic makeup, fecundity and generation interval), host parasite relationship (presence or absence of hypobiosis), environmental conditions (climate, worms on host or off host), drench frequency, under dosing, time of drenching, continuous use of drug with similar mode of action, influx of pharmaceuticals, borrowing of resistance (shared pasture or animal purchase), worm survival strategy, physiology of animals and anthelmintic pharmacology.

The role of refugia in the management of anthelmintic resistance

Refugia are the proportion of worm population which escapes exposure to any sort of anthelmintic drugs. It is the most important factor contributing to selection for anthelmintic resistance in parasites (van Wyk, 2001). It is only the parasitic sub-population (the parasites within the host) that can be exposed to any anthelmintic treatment. Worms that are in the free-living sub-population (eggs, L_1, L_2, L_3) are not exposed to the anthelmintic and are said to be in refugia. Size of population in refugia at the time of anthelmintic treatment will determine the contribution of surviving susceptible worms to the subsequent generation. Worms in refugia provides a pool of genes susceptible to anthelmintics, thus diluting the frequency of resistance genes. As the relative size of refugia increases, the rate of evolution towards resistance decreases. The importance of refugia though varies between environment and seasons and while designing any deworming control one should consider the disadvantage of drenching an immune animal, drenching during dry season and drenching in situation of few or no worms in refugia. Nematodes in refugia come from three sources viz., pasture, untreated animals and inhibited stages surviving treatment in the host. Pasture contaminated with nematode larvae form an excellent source of nematodes in refugia so that continuously grazed pastures are less likely to permit the development of anthelmintic resistance provided that there is not heavy reliance on anthelmintic treatments (Coles, 2002).

Mechanism of anthelmintic resistance

Resistance in GI worms can arise due to target site insensitivity, metabolic detoxification or change in the drug transport (James *et al.,* 2009). In molecular studies, benzimidazole resistance in gastrointestinal nematodes is related with changes in the target site gene coding β-tubulin. Two distinct single nucleotide polymorphisms leading to changes in the amino acid sequence from phenylalanine (Phe, TTC) to tyrosine (Tyr, TAC) at position 167 (Silvestre and Cabaret, 2002), 198 (Ghisi *et al.,* 2007) and 200 (Kwa *et al.,* 1995) appears to play a major role in the mechanism of BZ resistance. Of these, F200Y mutation is common in *Haemonchus contortus* throughout the world. Although benzimidazole resistance appears in nematodes due to these mutations but the same mutation does not seem to cause triclabendazole resistance in liver flukes. In India, mutation at 200 position is most

commonly reported followed by 198 position, however, 167 mutation never detected in Indian isolates (Sankar, 2007; Garg and Yadav, 2009; Rialch *et al.*, 2014; Chandra *et al.*, 2014, 2015; Saini *et al.*, 2016). The mechanism of resistance to other anthelmintics has not been fully elucidated. Changes in P-glycoprotein and nicotine acetylcholinesterase receptor might be involved in ivermectin and levamisole resistance in gastrointestinal nematodes.

Methods for detection of anthelmintic resistance

The growing importance of anthelmintic resistance has led to an increased need for reliable and standardised detection method. Most of the methods described have drawbacks either in terms of cost, applicability and interpretation or reproducibility of findings (Varady and Corba, 1999).

The most widely used method for detecting and monitoring the presence of anthelmintic resistance in nematodes is the faecal egg count reduction test (FECRT), which is suitable for all types of anthelmintics including those that undergo metabolism in the host. In addition, a number of *in vitro* assays that measure the effects of anthelmintics on development, growth or movement of nematode stages have been developed as alternative methods of detection.Anthelmintic resistance can be detected by *in vivo* as well as *in vitro* techniques (Taylor *et al.*, 2002).

The following *in vivo* tests are being used to detect anthelmintic resistance

Controlled test

This test is the most reliable method of assessing anthelmintic efficacy but also costly in terms of labour requirements and animal usage and is now rarely used. In an attempt to reduce the costs and time taken, laboratory animal models have been used (Taylor *et al.*, 2002).To characterise the sensitivity of a field isolate, groups of worm free animals should be inoculated with infective larvae and the anthelmintic tested at 0.5, 1 and 2 times the recommended dose rate. Inclusion in the test of a known susceptible strain has been recommended. Resistance is generally confirmed when the reduction in geometric mean worm counts is less than 90%.

Faecal Egg Count Reduction Test (FECRT)

The FECRT provides an estimation of anthelmintic efficacy by comparing faecal egg counts of animals before and after treatment. A good correlation has been found between faecal egg counts and worm counts for *Haemonchus contortus*.

Various *in vitro* assays used for surveillance of anthelmintics resistance are as follows

Egg Hatch Assay (EHA)

Benzimidazole anthelmintics prevent embryonation and hatching of nematode eggs. A number of egg hatch/embryonation assays have been developed for the detection of resistance to this group of anthelmintics.

Larval Feeding Inhibition Assay

A larval feeding assay devised for detection of macrocyclic lactones and imidazothiazoles resistance in gastrointestinal nematodes (Alvarez-Sánchez *et al.*, 2005). It is based upon the principle that the concentration of anthelmintic required to inhibit larval feeding in 50% of L_1's juvenile of nematode is higher in parasites resistant to either macrocyclic lactones or imidazothiazoles than those of susceptible isolates indicating development of resistance against these anthelmintics.

Table.1 State wise reports of anthelmintic resistance in livestock parasites in India

State	Year	Animal Species	Anthelmintic	References
Jammu and Kashmir	2012	Sheep	Benzimidazole, Levamisole, Ivermectin	Itoo and Shahardar, 2012
	2012	Sheep	Tetramisole	Itoo *et al.*, 2012
	2015	Goat	Benzimidazole	Sharma *et al.*, 2015
Uttarakhand	1976	Sheep	Phenothiazine, Triclabendazole	Varshney and Singh (1976)
	1999	Goat	Benzimidazole, Ivermectin	Laha *et al.*, 1999
	2000	Sheep	Benzimidazole	Yadav *et al.*, 2000
	2002	Equines	Benzimidazole	Pal, 2002
	2004	Goat	Albendazole, Rafoxenide	Hira Ram *et al.*, 2004
	2007	Sheep	Benzimidazole, Imidathiazole, Salicylanilide	Garg *et al.*, 2007
	2012	Sheep	Benzimidazoles	Kumar *et al.*, 2012
	2013	Sheep	Benzimidazoles	Rialch *et al.*, 2013
	2014	Equines	Benzimidazole, Ivermectin	Kumar and Vatsya, 2014
Haryana	1990	Sheep	Benzimidazoles	Yadav, 1990
	1992	Goats	Levamisole	Yadav and Uppal, 1992
		Goats	Tetrahydropyrimidines, Levamisole, Morantel, Benzimidazole	Uppal *et al.*, 1992
	1993	Goat	Benzimidazole	Yadav and Uppal, 1993
	1993	Sheep	Benzimidazole, Morantel	Yadav *et al.*, 1993
	1995	Sheep	Benzimidazole, Levamisole, Morantel	Yadav *et al.*, 1995
	1997	Sheep	Benzimidazole,	Chaudhari *et al.*, 1997
	1997	Sheep and Goat	Benzimidazole, Morantel	Singh and Yadav, 1997
	1997	Cattle	Morantel	Yadav and Verma, 1997
	2000	Sheep	Oxyclozanide and Tetramisole hydrochloride	Chaudhry, 2000
	2003	Sheep	Closantel	Gupta *et al.*, 2003
	2004	Sheep	Benzimidazole,	Yadav and Garg,

			Rafoxanide, Morantel	2004
	2005	Sheep and Goat	Benzimidazole, Morantel	Das and Singh, 2005
	2007	Sheep	Benzimidazole, Tetramisole, Morantel	Chaudhari *et al.*, 2007
	2010	Sheep	Morantel, Ivermectin	Singh and Gupta, 2010
	2012	Goat	Benzimidazole	Singh *et al.*, 2012
	2012	Goat	Ivermectin	Singh and Poonia, 2012
	2014	Goats	Benzimidazole, Ivermectin, Closantel	Vohra *et al.*, 2014
	2015	Sheep	Benzimidazole, Levamisole, Ivermectin	Sharma *et al.*, 2015
	2015	Sheep	Benzimidazole	Singh *et al.*, 2015
	2016	Sheep	Benzimidazole	Saini *et al.*, 2016
	2016	Sheep	Fenbendazole, Levamisole, Morantel, Ivermectin	Kumar and Singh, 2016
	2017	Cattle	Fenbendazole, Ivermectin, Morantel, Levamisole	Singh, 2017
Punjab	2012	Sheep	Fenbendazole, Ivermectin, Morantel, Levamisole	Buttar *et al.*, 2012
	2017	Sheep and Goat	Benimidazole	Singh *et al.*, 2017
Himachal Pradesh	1992	Sheep	Benimidazole	Singh *et al.*, 1992
Rajasthan	1993	Sheep	Benzimidazole	Swarnkar *et al.*, 1993
	1995	Sheep	Benzimidazole	Singh *et al.*, 1995
	1996	Sheep	Rafoxenide,	Singh *et al.*, 1996
	1996	Sheep	Benzimidazole, Levamisole	Gill, 1996
	1999	Sheep	Benzimidazole, Levamisole, Rafoxenide,	Swarnkar *et al.*, 1999
	2001	Sheep	Benzimidazole, Tetramisole, Rafoxenide,	Swarnkar *et al.*, 2001
	2004	Sheep	Benzimidazole, Tetramisole	Swarnkar *et al.*, 2004
	2012	Sheep	Tetramisole	Swarnkar and Singh, 2012
Uttar Pradesh	1995	Sheep	Tetramisole, Benzimidazole	Srivastava *et al.*, 1995

	2009	Goat	Benzimidazole, Morantel	Sharma and Rout, 2009
	2012	Sheep	Benzimidazole	Kumar *et al.*, 2012
	2013	Goat	Ivermectin	Jaiswal *et al.*, 2013
	2014	Sheep	Benzimidazole	Chandra *et al.*, 2014
	2016	Equines	Benzimidazole	Kumar *et al.*, 2016
Madhya Pradesh	2012	Goat	Benzimidazole	Agrawal *et al.*, 2012
	2012	Goat	Benzimidazole	Dongre *et al.*, 2012
Chattishgarh	2012	Goat	Levamisole	Kerketta *et al.*, 2012
	2014	Goat	Benzimidazole, Levamisole, Ivermectin	Kumar *et al.*, 2014
Maharashtra	2012	Sheep	Benzimidazole	Ghalsasi *et al.*, 2012
Bihar	2015	Goat	Benzimidazole, Levamisole, Ivermectin	Kumar and Kumar, 2015
Andhra Pradesh	1996	Sheep	Benzimidazole, Levamisole	Gill, 1996
Karnataka	2003	Sheep	Benzimidazole, Rafoxenide	Dhanlakshmi *et al.*, 2003
	2012	Sheep	Benzimidazole	Kumar *et al.*, 2012
	2016	Sheep	Benzimidazole,	Amulya *et al.*, 2016
Tamil nadu	1996	Sheep	Benzimidazole, Levamisole	Gill, 1996
	2005	Sheep	Benzimidazole, Morantel	Arunachalam *et al.*, 2005
	2005	Sheep	Levamisole	Sundaram *et al.*, 2005
	2009	Sheep	Benzimidazole, Levamisole, Ivermectin	Easwaran *et al.*, 2009
	2012	Goats	Benzimidazole, Levamisole, Ivermectin	Manikkavasagan *et al.*, 2012
	2013	Sheep/ Goat	Benzimidazole, Levamisole, Ivermectin	Arunachalam *et al.*, 2013
	2014	Sheep	Benzimidazole, Levamisole	Arunachalam *et al.*, 2014
	2014	Sheep	Benzimidazole	Meenakshisundaram *et al.*, 2014
	2015	Goat	Benzimidazole, Levamisole	Varadharajan and Vijayalakshmi, 2015
Gujarat	1996	Sheep	Benzimidazole	Gill, 1996

	2009	Sheep	Benzimidazole, Tetramisole, Ivermectin	Mekwana and Singh, 2009
	2016	Goat	Levamisole, Benimidazole, Ivermectin	Gelot *et al.*, 2016
Kerala	2006	Goat	Benzimidazole, Morantel, Ivermectin	Deepa and Devada, 2006
	2011	Goat	Benzimidazole	Deepa and Devada, 2011
	2013	Goat	Benzimidazole	Rajagopal *et al.*, 2013

Larva Development Test (LDT)

Most anthelmintics affect the metabolism of a parasite in some way that affects parasite growth. In LDT, L_1 are cultured to L_3 in the presence of heat treated lyophilised *Escherichia coli*, as a food source, and the anthelmintic under test (Coles *et al.*, 1988). Suitable controls are also run without the presence of anthelmintic. Further a dose response curve is generated to determine LD_{50} values.

Larval paralysis test

A larval paralysis test has been developed for the detection of levamisole and morantel resistance (Martin and Le Jambre, 1979). In the assay, infective third stage larvae are incubated for 24h in serial dilutions of the anthelmintic. After this time the percentage of paralysed larvae is determined at each concentration and a dose-response line plotted and compared to known reference strains (Alvarez Sanchez *et al.*, 2005).

Larva migration inhibition assay

L_3 obtained from larval cultures are isolated and stored in ventilated cell culture flasks at 6–10 °C in a fridge for a maximum of 3 months prior to use. LMIAs were performed before and (in case of a positive EPG) after treatment. L_3 are subjected to different concentrations of ivermectin and then migrated and non-migrated larvae are counted under a stereo microscope and the percentage of non-migrated larvae to the total amount of larvae are calculated (Demeler *et al.*, 2010).

Ways to maximize the effectiveness of anthelmintics

The main recommendation is to reduce the selection pressure for anthelmintic resistance in worm population by reduction in drench frequency, appropriate doses, alteration of anthelmintic classes and preferred use of narrow spectrum anthelmintics when possible depending on parasitic species. Another recommended approach is to follow target selective treatment (TST) instead of systematic ones. Diluting the alleles of resistance in worm populations could slow down the rate of selection of resistance. Goal of current research should be to translate the targeted treatment concept into practical.

Constant surveillance and monitoring of antiparasitic drug resistance in various common parasites of livestock and poultry is the need of the hour. The problem of resistance can be circumvented either by delaying its onset or use of alternate strategies

in the form of integrated parasite management.

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