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Detection of benzimidazole resistance in gastrointestinal nematodes of sheep and goats of sub-Himalyan region of northern India using different tests

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ABSTRACT

The present investigation was planned with the objective of studying the status of benzimidazole (BZ) resistance in gastrointestinal nematodes (GIN) of sheep and goats of different agro-climatic zones of sub-Himalyan region of northern India using *in vivo* faecal egg count reduction test (FECRT) and *in vitro* tests namely egg hatch assay (EHA) and larval development assay (LDA).

Out of fourteen flocks, FECRT detected resistance in eight flocks (two sheep flocks and six goat flocks) with FECR% ranging from 54.95 to 90.86. Pre treatment coproculture contained predominantly *Haemonchus contortus*, followed by *Trichostrongylus* spp., *Oesophagostomum* and *Strongyloides*, while post treatment coproculture results showed that only *H. contortus* survived fenbendazole (FBZ) (in FECRT) or thiabendazole (TBZ) (in LDA) treatment except in three flocks of Tarai region {one sheep flock (Us1), and two goat flocks (Ug1 and Ug5)} where BZ resistant *Trichostrongylus* were also detected. The GIN of those eight farms which were found resistant by FECRT were also detected resistant by EHA. Arithmetic mean and range of ED_{50} value of susceptible group was found to be 0.059 μg/ml and 0.037–0.096 μg/ml, respectively, and the same for the resistant group were found to be 0.119 μg/ml and 0.101–0.147 μg/ml, respectively. With LDA, the arithmetic mean and range of LC_{50} value of susceptible group was found 0.0030 μg/ml and 0.001–0.005 μg/ml, respectively, and those of resistant group was found 0.0105 μg/ml and 0.009–0.012 μg/ml, respectively. The values of Spearman rank correlation coefficient indicated that negative correlation was found between FECR% and ED_{50} and between FECR% and LC_{50} while positive correlation existed between ED_{50} and LC_{50} value and the *p*-values indicated that these correlations were statistically highly significant. In the present study, FECRT and EHA gave comparable results with regard to detection of BZ resistance in GIN in sheep and goats. Although with LDA, the threshold LC_{50} value could not be established as for EHA but LDA indicated the presence of low level of resistance in GIN of both sheep and goats. For effective worm control, regular monitoring for anthelmintic resistance is important to know the status of anthelmintic efficacy in a particular agro-climatic zone. The baseline information thus generated will enable timely management of benzimidazoles resistance in GIN.

1. Introduction

In small ruminants, gastrointestinal (G.I.) parasitism is one of the most important causes of production losses around the world (Molento, 2009). G.I. parasites are controlled using various anthelmintics in India although

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indiscriminate and frequent use of anthelmintics exerts selection pressure resulting in decline in their efficacy and hence emergence of anthelmintic resistance. High levels of anthelmintic resistance have already made it impossible to sustain economic sheep production in some farms (Sargison et al., 2005). Currently, goat industry also appears to be under great threat with widespread, multiple resistance reported throughout the world (Waller, 1997; Mortensen et al., 2003).

A variety of tests are available to monitor anthelmintic resistance including *in vivo* tests such as critical anthelmintic test (Hall and Foster, 1918), controlled anthelmintic test (Presidente, 1985), faecal egg count reduction test (Coles et al., 1992) and various *in vitro* tests such as egg hatch assay (Le Jambre, 1976), larval development assay (Hubert and Kerboeuf, 1992) *etc.* The faecal egg count reduction test (FECRT) is recommended by World Association for the Advancement of the Veterinary Parasitology (WAAVP) and is the test of choice especially in surveys for resistance, because it uses few resources, is easily performed and is applicable in the evaluation of the efficacy of any anthelmintic in many host species. The test also gives a direct measure of an anthelmintic efficacy, which is valuable to the farmer. However, FECRT is time consuming, expensive and inter-animal variation and the pharmacokinetics of the drug in host lead to poor quality of data. Therefore, increasing interest is paid to *in vitro* tests like egg hatch assay (EHA) and larval development assay (LDA) which are cheaper to perform, and therefore more suitable for large surveys (Ancheta et al., 2004). EHA has also been recommended by WAAVP and is capable of reliable evaluation of anthelmintic resistance. But the emerging problem of multiple resistance can not be diagnosed by this EHA which is suitable only for benzimidazoles (BZ). Here, *in vitro* larval development assay (LDA) proves to be a better substitute. However, standard operating procedure and reliable guidelines are yet to be produced for LDA. The ability to obtain comparable results when using any of the above tests and, therefore, declare with certainty the occurrence or absence of resistance is important.

In India, the first report of anthelmintic resistance was documented against phenothiazene and thiabendazole in *Haemonchus contortus* at State Sheep and Wool Research Station, Pashulok, Rishikesh (Varshney and Singh, 1976). Since then, BZ resistance has been commonly reported in sheep population of India (Kumar and Yadav, 1994; Garg and Yadav, 2009; Pandey, 2011) including different agro-climatic zones of northern India (Pandey and Vatsya, 2013). However, no systematic studies have been undertaken to determine the status of BZ resistance against GIN in sheep and goats of sub-Himalyan region of northern India. Moreover, the suitability of different *in vitro* tests in detecting BZ resistance has also not been studied earlier in this part of India.

Therefore, the present investigation has been planned with the objective to know the status of BZ resistance in GIN of both sheep and goat flocks of sub-Himalyan region of northern India using different tests and to examine the feasibility of using the results of one of these tests to predict the results of another.

2. Materials and methods

2.1. Animal flocks

The sheep and goat flocks selected for present study were located in two geographical locations: (i) Tarai region of foothills of the Himalyas with a sub-tropical climate {including the towns of Gadarpur (550 m above sea level), Pantnagar and Jawahrnagar (243.8 m above sea level), Rudrapur (208 m above sea level), Kiccha (293 m above sea level), Sitarganj (284 m above sea level)} and (ii) Hill region or high altitude locations with temperate climate {including the towns of Ramnagar (1729 m above sea level), Nainital (2084 m above sea level)} of northern India situated between 28°43′ N to 31°27′ N latitude and 77°34′ E to 81°02′ E longitudes. The sheep and goat flocks of Tarai region were designated as Us and Ug, respectively and goat flocks of Hill region as Ng. The animal flocks were kept under either intensive (Us1, Us4, Ug1), semi-intensive (Ug2, Ug3, Ug5, Ug6, Ug7) or extensive (Us2, Us3, Ug4, Ug8, Ng1, Ng2) managemental systems.

2.2. Faecal egg count reduction test (FECRT)

The status of BZ resistance was studied in a total of fourteen flocks (four sheep flocks and ten goat flocks) across the study regions. Animals selected from each flock were screened for the presence of GIN. FECRT was carried out as described by Coles et al. (1992). Twenty animals from each flock were selected and randomly allocated into two groups (Treatment and Control group) of ten animals each with arithmetic mean epg of both groups almost equal. Selected animals were not treated with any kind of anthelmintic 8–12 weeks before the commencement of the test and their faecal examination revealed that eggs per gram (epg) of host faeces was above 150.

On the first day of treatment (Day zero), animals of treatment group were weighed individually and treated with fenbendazole (Panacur, Intervet, India Pvt. Ltd., Pune) @ 5 mg/kg body weight in sheep and @ 10 mg/kg body weight in goat, orally. Rectal faecal samples were again collected 10 days post treatment (D_{10}) from both Treatment and Control groups and epg was assessed. For species identification of resistant and susceptible GIN, faecal culture of samples from selected animals of treated and control group was done separately.

2.3. Egg hatch assay (EHA)

For EHA, pooled faecal samples were collected from each flock. These samples were either used within 3 h of collection or were stored anaerobically as described by Hunt and Taylor (1989) and were used within 7 days of collection. Nematode eggs were collected by sieving through a 0.15 mm aperture, 20 cm diameter sieve and then finally recovered by using saturated salt solution (MAFF, 1986). Eggs were re-suspended in deionised water with 100–150 eggs/100 μl of water. EHA was carried out as described by Le Jambre (1976) with slight modifications (Taylor et al., 2002; Coles et al., 2006). Briefly, 10 μl of prepared dilutions of TBZ: 0.01, 0.025, 0.05, 0.1, 0.2, 0.3, 0.5 μg/ml (prepared