ORIGINAL RESEARCH PAPER

Veterinary Science

EVALUATION OF STATUS OF BENZIMIDAZOLE RESISTANCE IN NEMATODES OF GOATS BY IN VITRO METHOD IN THE PLAIN REGION CHHATTISGARH STATE

KEY WORDS: benzimadazole resistance, nematodes, EHT, thiabendazole, ED50

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ABSTRACT

The present study was carried out in three different farms and their adjoining fields of Chhattisgarh plain region to know the status of benzimidazole resistance in gastrointestinal (GI) nematodes of goats by in vitro Egg hatch test (EHT). Faecal samples of goats were collected from three Government farms namely Pakaria Goat Farm (Bilaspur), Kawrdha Goat farm and College Unit Goat farm, Durg. Faecal samples of field goats were also collected from the adjoining area of the above three farms. Faecal smaple from twenty goats from each farm as well as adjoining field area were collected and pooled together and were screened. About 10 to 20 gms of faecal samples were collected directly from the rectum from each goats. Above screening was done during monsoon season and post monsoon season in the Department of Veterinary Parasitology. Durg farm exhibit high degree of thiabendazole resistance and Bilaspur farm showed susceptibility to thiabendazole. In all the field area nematodes were susceptible to thiabendazole as ED50 is less than 0.1. There is no seasonal variation in the resistance pattern indicating no effect of season on the resistance status.

INTRODUCTION

Anthelmintic medications are the most effective tools for addressing the parasite threat to ruminants. Actually, anthelmintics continue to play a significant part in the parasite control program despite the introduction of alternative control methods such as worm vaccination, biological control by nematophagous fungi, etheno-veterinary medicines etc. One negative aspect of this situation is the rise of resistance to the main anthelmintic classes, which include benzimidazoles, salicylanilides, imidothiazole, and macrocyclic lactones (Gill, 1993; 1996). Resistance is an inevitable phenomenon but regular monitoring and surveillance of resistance status assists in timely implementation of resistance mitigation strategies in farms and field. Of many methods for the diagnosis Egg hatch assay or Egg hatch test is one of the easiest techniques for in vitro detection of benzimidazole resistance. Egg hatch Test (EHT) evaluates the ability of benzimidazoles to impede or prevent embryonation and hatching of nematode eggs and is the standard in vitro test for detection of benzimidazole resistance. Egg Hatch Test (EHT) was first applied by Le Jambre (1976) as a widespread tool to diagnose benzimidazole resistance. The Egg Hatch Assay describe over here is as per Coles et al (1992; 2006) and it was accepted by World Association for Advancement of veterinary Parasitology (WAAVP).

MATERIALS AND METHODS

Detection of Benzimidazole resistance by Egg Hatch Test (EHT)

Collection of Faecal Sample

About 10 to 20gm fresh faecal sample were collected per rectum from each animal and were pooled as per the place of collection. Half of the pooled sample was subjected to coproculture and half was kept for EHT. As laboratory was far away from collection site, faecal samples were maintained in anaerobic condition (Hunt and Taylor, 1989). For developing anaerobic condition pooled faecal samples were collected in sample container containing distilled water and glass beads. Sample container was screwed tight and shaked vigorously.

Preparation of Faecal Slurry

The pooled faecal sample maintained in anaerobic condition was taken in a large size beaker (2 lit) and water about 500ml to 1000 ml was added in the beaker. It was kept for 20-30 minutes so that faecal matter gets soaked and loosened. Faecal matter was thoroughly mixed with stirrer. After mixing faecal slurry was strained 2-3 times using tea strainer to remove the faecal debris.

Separation Of Eggs From Faecal Slurry

Strained faecal slurry was poured in centrifuge tubes and centrifuged at 2000 rpm for 5 minutes. After centrifugation supernatant were discarded from all centrifuge tubes. Sediment was well agitated and saturated sodium chloride solution was added up to top in each centrifuge tube for floatation of parasitic eggs. Microscopic cover slips were put on each centrifuge tube for adhering of eggs. After 10 minutes cover glasses were held with forcep and dipped in a beaker containing distilled water. Take out the cover slip and wash the eggs into a conical centrifuge tube. There should be 100–150 nematode eggs/ 100 µl.

Preparation of Thiabendazole Stock Solution

Ten mg of thiabendazole (TBZ) was weighed using precision monopan balance. Thiabendazole was put in a glass vial. 5ml of di-methyl-sulphoxide (DMSO) was added to dissolve the drug. This solution was used as stock solution. By using stock solution five different doses of thiabendazole was prepared in a two-fold serial dilution method as depicted in the table 1.

Charging and Incubation of 24 Multiwell Plate

A twenty four multiwell plate was used in this technique. Ten l of prepared dilutions of TBZ: 0.125, 0.25, 0.5, 1.0 and 2.0 g/ml was added to five different wells in duplicate. 10 l DMSO was added to two wells and kept as control wells. 1.89 ml distilled water (DW) was added to each well in a 24 multiwell plate. Egg suspension (100 l) was placed in each well containing TBZ or DMSO. Plates were sealed to prevent drying out and incubated in a BOD incubator at 28°C for 24-48 hr.

Counting of eggs and larvae

After 24 hrs of incubation of the multiwell plate, embryonation was checked from control wells using 10x objective of compound microscope. When larvae were found in control well, then further processing was carried out. Solution from different wells were pipetted out and poured in separate centrifuge tubes. A drop of Lugol's iodine was added in each tube. These tubes were centrifuged at 2000 rpm for 5 min. Supernatant was discarded very carefully. Aliquot of sediment was observed for counting of eggs and larvae under 10x objective of compound microscope.

Interpretation Of Data

The data was analysed by Probit Analysis to obtain ED_{50} value for egg hatch. ED_{50} value in excess of 0.1µg TBZ ml^{-1} was considered as emergence of TBZ resistance (Coles et al 1992; 2006).

RESULTS

The present study was carried to check whether there was any impact of status of benzimidazole resistance in nematodes of goats from farms on field condition and also to compare the effect of seasons on resistance status at farms and fields.

Gastrointestinal (GI) nematodes in the goats of College unit goat farm, Durg showed resistance where EHT reveled ED_{50} value of 0.594 indicating high degree of resistance. GI nematodes in goats of Pakaria goat farm in Bilaspur district showed ED_{50} value of 0.051 exhibiting susceptibility for thiabendazole. GI nematodes in goats of Kawardha goat farm have ED_{50} value of 0.121 indicating resistance. The reason for susceptibility in Pakaria Goat farm is due to the managemental practices, which includes rotation of different anthelmintics during administration of drugs and rotational grazing pattern. The ED_{50} values for Durg, Bilaspur and Kawardha farms during post-monsoon season were 0.231, 0.014 and 0.125 respectively indicating resistance in Durg farm and Kawardha farm and susceptibility to thiabendazole in Bialspur farm (Table 2).

Observations EHT in the adjoining field area in monsoon and post-monsoon season was given in the table 3. During monsoon season in Durg field area the ED_{50} value was 0.287 during monsoon indicating resistance. In Bilaspur and Kawardha field region and ED_{50} value was 0.033 and 0.057 respectively during monsoon season indicating thiabendazole susceptibility. The result of EHT in fields showed susceptibility for thiabendazole as the animals of field area generally not being frequently treated.

In post-monsoon season EHT were carried out in goats at the same field area to see the effect of season on the status of resistance. As per the table 3, the ED_{50} values for Durg, Bilaspur and Kawardha field area were 0.033, 0.015 and 0.025 respectively indicating susceptibility to thiabendazole. Thus, it could be concluded that in field condition where there is minimal use of anthelmintics and maintenance of refugia the GI nematodes of goats showed susceptibility to anthelmintics, that would be maintained for longer period.

Thus, it could be concluded that status of resistance was not affected by the change in the season as the results of and EHT were similar in farms and field area in both monsoon and post monsoon season. As resistance is mainly due to genetic mutation in the nematodes and is rarely affected by the change in the season therefore no variation was recorded.

Similar results were obtained by Das et al. (2015) in their experiment where they found resistance against fenbendazole and levamisole at recommended dose to the goats of organized farm in Jabalpur, Madhya Pradesh. The present result was congruent with the results of Sanyal et al. (2014) where resistance was recorded against albendazole in Chhattisgarh plain zone in nematode parasite of goats. But, in the present study GI nematodes of goats of Pakaria Goat Farm, Bilaspur showed supected resistance which may be due to anthelmintic management programme mainly anthelmintic rotation regimen. Kumbhkar (2015) conducted experiments at the same farm of Durg and claimed low resistance with FECR and lower 95% con dence limit were 97% and 68% respectively against albendazole. The ED_{50} for egg hatch was 0.196 indicating resistance to benzimidazole. Whereas our result showed higher intensity of resistance at the same farm which may be due to same drug regimen for controlling of nematode causing intensive selection of resistant allele.

The present study was also in accordance with Dixit (2016) where the status of benzimidazole resistance against caprine nematodes at Amanala goat farm, Jabalpur was investigated. EHA revealed ED_{50} value of 0.335 g of thiabendazole/ml, confirming benzimidazole resistance in the animals of that farm.

Similar type of outcome was seen in three institutional sheep farms in Tamil Nadu, India by Easwaran et al. (2009). They investigated the occurrence of multiple anthelmintic resistance by applying Faecal Egg Count Reduction Test (FECRT), Egg Hatch Assay (EHA) and Larval Migration Inhibition Assay (LMIA) for both benzimidazoles and levamisole. The ED_{50} values for thiabendazole resistance in EHA for isolates from three farms were 0.627, 0.678 and 0.388 g/ml of TBZ. The results of the survey indicated multiple resistance in H. contortus and Ostertagia sp. to benzimidazoles and levamisole in farm I, simultaneous resistance in Ostertagia sp. to benzimidazoles and levamisole in farm II and resistance in H. contortus to benzimidazoles and levamisole in farm III.

There are several reports on the high prevalence of anthelmintic resistance in organized farms or intensively managed farms in India. In the study conducted by Swarnakar et al. (2001) in sheep flocks maintained at Central Sheep and Wool Research Institute (CSWRI), Avikanagar, Rajasthan. They reported that after eight years of administration of Benzimidazole, resistance had developed for this compound. It is known that selection pressure generated by continuous use of anthelmintics is responsible for generation of resistance. The frequent use of dewormer at farm might be one of the reason for generation and continuous increase of resistant strains in the farm.

Table 1. Concentration of TBZ in different tubes with two-fold dilution

Tube no.	1	2	3	4	5
Volume of stock solution (ml)	0.5	-	-	-	-
Volume of D.W. (ml)	4.5	1.0	1.0	1.0	1.0
Volume taken from tube 1 (ml)	-	1.0	-	-	-
Volume taken from tube 2 (ml)	-	-	1.0	-	-
Volume taken from tube 3 (ml)	-	-	-	1.0	-
Volume taken from tube 4 (ml)	-	-	-	-	1.0
Total Volume (ml)	5.0	2.0	2.0	2.0	2.0
Conc.TBZ(g TBZ/10 l)	2.0	1.0	0.5	0.25	0.125

Table 2. Status of benzimidazole resistance in gastrointestinal nematodes of farm goats in Chhattisgarh plain during monsoon and post-monsoon season

Parameter	Durg		Bilaspur		Kawardha	
	Monsoon	Post-monsoon	Monsoon	Post-monsoon	Monsoon	Post-monsoon
ED_{50}	0.594	0.231	0.051	0.014	0.121	0.125
Result	Resistant	Resistant	Susceptible	Susceptible	Resistant	Resistant

Table 3. Status of benzimidazole resistance in gastrointestinal nematodes of field goats in Chhattisgarh plain during monsoon and post-monsoon season

Parameters	Durg		Bilaspur		Kwardha	
	Monsoon	Post-monsoon	Monsoon	Post-monsoon	Monsoon	Post-monsoon
ED_{50}	0.287	0.033	0.033	0.015	0.057	0.025
Result	Resistant	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible

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