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BENZIMIDAZOLE RESISTANCE STATUS IN NEMATODES OF GOATS OF CHHATTISGARH PLAIN REGION

S. NATH*, S.PAL, S. MANDAL AND P.K. SANYAL
***Assistant Professor, Department of Veterinary Parasitology, College of Veterinary Science & Animal Husbandry, Jabalpur, India**

ABSTRACT: In the present study, prevalence of albendazole resistant nematodes in goats of Chhattisgarh Plain zone was carried out by *in vivo* method faecal egg count reduction test (FECRT). The prevalence study was carried out in three different farms and in their adjoining field area in pre and post monsoon season. Government farms namely Pakaria Goat Farm (Bilaspur), Kawrdha Goat farm and Durg College Unit Goat farm and their adjoining field area were taken for the said study. This paper aims to see the correlation between the seasons in the prevalence of benzimadazole resistance. The study taken in both farm and field area to know whether there is any effect of farm condition of resistance on field condition. Both College unit Goat farm, Durg and Kawardha Goat farm showed resistance to albendazole and thiabendazole in monsoon and post-monsoon season. Whereas Pakaria Goat farm, Bilaspur showed low resistance to albendazole and susceptibility to thiabendazole in both seasons. Values of FECR percentage in monsoon season for Durg farm, Kawardha Farm and Bialspur farm were 64%, 89% and 98% respectively and in post-monsoon season were 86%, 90% and 97% respectively. The status of resistance in farms and fields were unaffected by the change in season and the status of benzimidazole resistance do influence the status of resistance in field population of nematodes.

Key Words: Goats, nematodes, mansoon, resistance, population, farms.

Anthelmintic drugs are the predominant weapons available till date to combat parasite menace in ruminant livestock. In fact, even with the advent of alternative control measures like worm vaccination, biological control using nematophagous fungi, resistant hosts developement etc., anthelmintics are playing important role in control programme of parasites. The black side of this story is the emergence of resistance to major classes of anthelmintics, viz., benzimidazole, imidothiazole, salicylanilides and macrocyclic lactones (Gill, 1993; 1996). Goats were administered with albendazole (@7.5 mg/kg bwt orally) and faecal samples were collected on zero day and 10th day of treatment in both the seasons. About 10 to 20 gms of faecal samples were collected directly from the rectum from each goats in sterile polybags, labeled and brought to the Department of Veterinary Parasitology for further investigation.

MATERIALS AND METHODS

Collection of samples

The present study *i.e.* detection of prevalence of Benzamidazole (BZ) resistance was carried out in three different farms and their adjoining fields of Chhattisgarh. 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. Twenty goats from each farm as well as adjoining field area with a total of 120 (60 from farms and 60 from adjoining fields) were screened. Above screening was done during monsoon season and post monsoon season.

Detection of Benzimidazole Resistance by Faecal egg count reduction test (FECRT)

The faecal egg count reduction test (FECRT) was done according to the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for detection of anthelmintic resistance (Coles *et al.*, 1992). The maximum number of animals having uniformity in the nature and intensity of nematode infection, age, and weight from each flock were taken in the study. The

experimental animals were naturally parasitized with mixed species of gastrointestinal nematodes {having at least 150 eggs per gram of faeces (EPG)}. It was ascertained that the selected animals had not been treated in the previous 8-12 weeks. Faecal egg count reduction was the criterion for detection of resistance. About 10 to 20 grams of faecal samples were collected individually directly from rectum into sterile polybags from all animals before treatment and on day 10 of post-treatment. About 3 gms of the collected faecal material was used for FECRT and rest of the samples were pooled as per the place of collection. Faecal examination was conducted using standard procedures and EPG were counted by a modified McMaster method using McMaster slide (Plate 1); Skerman and Hillard, 1966).

Interpretation of Data: The faecal egg count data was analysed for faecal egg count reduction (%FECR) using RESO calculator (Martin and Wursthorn, 1991) and resistance to anthelmintic was considered if (a) the FECR is <95% and (b) the lower 95% confidence limit is <90%. If one of these two criteria was met then resistance was suspected (Coles *et al*., 1992; 2006).

RESULTS AND DISCUSSION

Albendazole resistance status in goats in Chhattisgarh

FECRT was carried out in monsoon and post monsoon season in three different farms and the adjoining field area for detection of albendazole resistance. 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.

To know the albendazole resistance in gastrointestinal nematodes of goats, faecal egg count reduction test (FECRT) was performed as per the guidelines of World Association for the Advancement of Veterinary Parasitology (Coles *et al*., 1992) in different farms and field in monsoon season of Chhattisgarh plain zone are presented in table 1. Gastrointestinal (GI) nematodes in the goats of College unit goat farm, Durg showed resistance with faecal egg count reduction percentage of 64% and lower 95% confidence limit of 14%. GI nematodes in goats of Pakaria goat farm in Bilaspur district showed faecal egg count reduction of 98% but the lower 95% confidence limit of 80% therefore the result indicated suspected resistance GI nematodes in goats of Kawardha goat farm showed FECR of 89% and value of lower 95% confidence limit was 57% revealing resistance to albendazole. The reason for supected resistant status in GI nematodes of Pakaria Goat farm is due to the managemental practices, which includes rotation of different anthelmintics during administration of drugs. They follow rotational grazing pattern which further confirmed the suspected resistance status for the GI nematodes of Pakaria Goat Farm.

Observations for FECRT in the adjoining field area are given in the table 1 to know whether the status of resistance in GI nematodes of different farms was spilled over to the GI nematodes harbouring goats in field area or not. In Durg field area the FECR was 96% and lower 95% confidence limit was 65% which revealed suspected resistance. In Bilaspur field region FECRT result showed EPG reduction of 97% with lower 95% confidence limit of 92% revealed susceptibility to benzimidazole. The results of FECRT in Kawardha fields showed 98% reduction in faecal egg count and lower 95% confidence limit was 88% exhibiting suspected resistance. From the above result it can be concluded that there was an impact of status of drenching effectiveness of the farm on the field area. GI nematodes of goats of both the adjoining field area of Durg and Kawardha farm showed status of suspected resistance for FECRT whereas GI nematodes of goats of Bilaspur field region had shown susceptibility for FECRT. As the animals of field area were in the close proximity to the farm goats chances of using the same grazing land was higher thereby getting resistant nematodes. Thus we can deduce that there is

dissemination of the phenomenon of resistance from farm to field.

In post-monsoon season FECRT was carried out in goats at the same farms and field area to see the effect of season on the status of resistance. Observations of FECRT of farms and adjoining field area in post-monsoon season have been depicted in table number 2. In College unit goat farm, Durg, FECR and lower 95% confidence limit were 86% and 60% respectively indicating resistance. Whereas in Pakaria goat farm, Bilaspur FECR was 97% and lower 95% confidence limit was 78% indicating suspected resistance. In Kawardha farm value of FECR was 90% and lower 95% confidence limit was 74%. The present data revealed that, there was no impact of season on the resistance status of GI nematodes of goats in the farms, as the results for drenching status was almost same in each farm in both monsoon and post-monsoon season. The reason being that if resistance in the parasites was eshtablished it is nearly impossible to reverse the situation, due to genetic mutation occurred in the parasites which continues from one generation to another generation.

Observations of field region were made in post-monsoon season from the same area as selected in monsoon season. *In vivo* FECRT result revealed suspected resistance in Durg and Kawardha. FECR and lower 95% confidence limit were 95% and 81% respectively for Durg field region. In Bilaspur field region FECR and lower 95% confidence limit were 99% and 90% respectively indicating susceptibility. In Kawardha field region FECR and lower 95% confidence limit was 97% and 87% respectively. 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.

The observations of FECRT in farms for monsoon and post-monsoon season have been depicted in table 3. In Durg farm, faecal egg count reduction percentage were 65% and 86% and lower 95% confidence limit were 14% and 60% in monsoon and post-monsoon season respectively indicating resistance against albendazole. FECR percentage in Bilaspur farm were 98% and 97% and lower 95% confidence limit were 80% and 78% in monsoon and post-monsoon season respectively demonstrating suspected resistance. Values of FECR percentage for Kawardha farm were 89% and 90% and lower 95% confidence limit were 57% and 74% in monsoon and post-monsoon season respectively indicating resistance to albendazole. In Durg field area FECR percentage were 96% and 95% and lower 95% confidence limit were 65% and 81% in monsoon and post-monsoon exhibiting suspected resistance (Table 4). The values of FECR percentage in Bilaspur field area were 97% and 99% and lower 95% confidence limit were 92% and 90% in monsoon and post-monsoon season, respectively suggesting susceptibility to albendazole (Table 4). In, Kawardha field area the values of FECR percentage were 98% and 97% and lower 95% confidence limit were 88% and 88% in monsoon and post-monsoon season, respectively indicating suspected resistance (Table 4). Thus, it could be concluded that status of resistance was not affected by the change in the season as the results of FECRT was 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 for FECRT. The present study also emphasized on implementation of targeted select treatment (TST) in the farms so that refugia can be maintained and spillage of resistance can be avoided.

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 Durg and claimed low resistance with FECR and lower 95% confidence limit were 97% and 68% respectively against albendazole. 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. FECR test evidenced fenbendazole resistance by partial elimination (24.90%) copro-egg counts in the treated group of animals vis-à-vis controls with a lower confidence interval of 26%.

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. FECR value after treatment with albendazole and levamisole confined from 69-80% and from 70-82% respectively in the three farms examined. 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.

CONCLUSIONS

Anthelmintic resistance is now accepted as inevitable phenomenon and the genes or alleles conferring resistance to anthelmintics are believed to be in existence in unselected worm population. Consequently, for all anthelmintics that have been invented to date, it appears that the development of anthelmintic resistance is an inescapable consequence of their use but its development can be delayed. Worms that are in free-living subpopulation (eggs and larval stages) are not exposed to the anthelmintics and are said to be in *refugia* and are being viewed as an important tool to slowdown the anthelmintic resistance. The suitable measures like targeted selective treatment by applying FAMACHA need to be implemented urgently to minimise spreading of anthelmintic resistance.

Table- 1: Status of benzimidazole resistance in gastrointestinal nematodes of farm and field goats in Chhattisgarh plain during monsoon sesson

Farm Goats						
Parameters	**Durg**		**Bilaspur**		**Kwardha**	
	Farm	**Feild**	**Farm**	**Feild**	**Farm**	**Feild**
***In vivo* FECRT**						
Number of Animals	10	10	10	10	10	10

Percentage Reduction	64	96	98	97	89	98
Variance (Reduction)	0.17	1.02	1.01	0.28	0.42	0.58
Upper 95% Confidence Limit	85	99	100	99	97	100
Lower 95% Confidence Limit	14	65	80	92	57	88
Drench Effectiveness	**Resistant**	**Suspected Resistance**	**Suspected Resistance**	**Susceptible**	**Resistant**	**Suspected Resistance**

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

Farm Goats						
Parameters	**Durg**		**Bilaspur**		**Kawardha**	
	Farm	**Feild**	**Farm**	**Feild**	**Farm**	**Feild**
***In vivo* FECRT**						
Number of Animals	10	10	10	10	10	10
Percentage Reduction	86	95	97	99	90	97
Variance (Reduction)	0.25	0.45	1.00	1.00	0.19	0.96
Upper 95% Confidence Limit	95	99	100	100	96	99
Lower 95% Confidence Limit	60	81	78	90	74	87
Drench Effectiveness	**Resistant**	**Suspected Resistance**	**Suspected Resistance**	**Susceptible**	**Resistant**	**Suspected Resistance**

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

Farm Goats						
Parameters	**Durg**		**Bilaspur**		**Kawardha**	
	Monsoon	**Post-**	**Monsoon**	**Post-**	**Monsoon**	**Post-monsoon**

		monsoon		**monsoon**		
***In vivo* FECRT**						
Number of Animals	10	10	10	10	10	10
Percentage Reduction	**64**	**86**	**98**	**97**	**89**	**90**
Lower 95% Confidence Limit	**14**	**60**	**80**	**78**	**57**	**74**
Drench Effectiveness	**Resistant**	**Resistant**	**Suspected Resistance**	**Suspected Resistance**	**Resistant**	**Resistant**

Table- 4: Status of benzimidazole resistance in gastrointestinal nematodes of field goats in Chhattisgarh plain during monsoon and post-monsoon season

Field Goats						
Parameters	**Durg**		**Bilaspur**		**Kwardha**	
	Monsoon	**Post-monsoon**	**Monsoon**	**Post-monsoon**	**Monsoon**	**Post-monsoon**
***In vivo* FECRT**						
Number of Animals	10	10	10	10	10	10
Percentage Reduction	**96**	**95**	**97**	**99**	**98**	**97**
Lower 95% Confidence Limit	**65**	**81**	**92**	**90**	**88**	**87**
Drench Effectiveness	**Suspected Resistance**	**Suspected Resistance**	**Susceptible**	**Susceptible**	**Suspected Resistance**	**Suspected Resistance**

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