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Detection of Anthelmintic Resistance in Goats of the North-Central Plateau and North-Eastern Coastal Plain of Odisha

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ABSTRACT

Gastrointestinal nematodes are an important impediment in goat husbandry and the efficient management of these parasites is hampered by the development of anthelmintic resistance. The present investigation was carried out to determine the prevalence and anthelmintic resistance in five districts of the two agroclimatic zones (ACZs*, viz.,* NCP (north-central plateau) and NECP (northeastern coastal plain) of Odisha during a period of 6 months (January 2019 to June 2019). From two ACZs, a total of 526 faecal samples of either sex, different age groups, and breeds were collected and examined qualitatively and quantitatively in the laboratory. A questionnaire survey was conducted on usage of anthelmintics. The status of resistance in gastrointestinal nematodes against Fenbendazole was carried out by *in vivo* faecal egg count reduction test (FECRT) and *in vitro* Egg Hatch Assay (EHA) test. Out of the 526 faecal samples examined, 377 samples were found positive leading to an overall prevalence of 71.77%. The prevalence pattern of different gastrointestinal nematodes revealed a higher presence of *Strongyle* sp (63.30%) over *Strongyloides* sp (20.64%) and *Trichuris* sp (16.05%). Single infection (57.82%) was more common than mixed infection (42.17%). The survey revealed dependence of benzimidazoles. The results of the anthelmintic resistance study indicated benzimidazole resistance in NCP and NECP agroclimatic zones.

HIGHLIGHTS

- Prevalence of gastrointestinal nematodes (>70%) in goats of agroclimatic zones of Odisha.
- Detection of anthelmintic resistance against fenbendazole in two districts.

Keywords: Anthelmintic resistance, Fenbendazole, Gastrointestinal nematodes, Goat

Goat rearing is an important economic activity especially for the small and marginalized farmers in our country (Kumar, 2007). The total goat population in India stands at 148.88 million accounting for 27.80 % of total livestock and in Odisha, the goat population is 6.39 million which is 35% of the total livestock population in the state (20th Livestock Census, 2019). The low productivity of goats in our country is influenced by several factors, among which parasitic diseases (internal and external) pose a major deterrent in small ruminant production across all agro-climatic zones. The gastrointestinal (GI) nematodes are a group of internal parasites that adversely cause economic loss, not due to mortality alone, but due to losses resulting from reduced milk/meat production, poor growth, cost of treatment, and prevention (Gwaze *et al.,* 2009).

The control of gastrointestinal nematodes in goat largely relies on the use of anthelmintic drugs at regular intervals. The benzimidazole group of drugs is the most extensively used anthelmintic against GI nematodes, which in turn has led to anthelmintic resistance and limitation of the efficacy of the drugs. The evaluation of anthelmintic resistance

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in GI nematodes of goat is essential for improving its management. Studies on the status on benzimidazole resistance in goats has been undertaken by several research workers in several states of the country (Godhara *et al*., 2011; Lata, 2018) as well as around the world (Zajac and Gipson, 2000).

As far as the state of Odisha is concerned, there have been few reports in limited districts of agro-climatic zones like East and South East coastal plain (Sahu *et al*., 2015) on prevalence and benzimidazole resistance against GI nematodes. However, systematic study concerning all districts of the agro-climatic zones in the state of Odisha is yet to be ascertained. This is essential for generating epidemiological data for better control and management of gastrointestinal parasites of goats in Odisha. The present study was undertaken to determine the prevalence of GI nematodes in goats of all districts of NCP (north-central plateau) and NECP (northeastern coastal plain) with special reference to benzimidazole resistant nematodes.

MATERIALS AND METHODS

The two agroclimatic zones, NCP (districts Mayurbhanj and Keonjhar) and NECP (Balasore, Bhadrak, Jajpur) were chosen for our study with a goat population of 1.67 lakhs and 6 lakhs, respectively. The region lies in between 22.01º N, 86.4 ºE and 21.63 º N,85.58 ºE having a moist sub-humid climate. The goats in these regions are reared mostly by the semi-intensive system and allowed to graze in the common pastureland. A total of 526 faecal samples from goats of either sex, different age groups, and breeds were collected from different villages in the blocks selected during a period of 6 months (January to June 2019). The samples were examined by direct smear and concentration (floatation) methods in the laboratory. The eggs of nematodes were identified based on their morphological characters (Soulsby, 1982) and the samples found positive for nematodes were then selected for quantitative examination by McMaster egg counting technique.

Investigation on the development of anthelmintic resistance in the nematodes was undertaken by *in vivo* faecal egg count reduction test (FECRT) and *in vitro* egg hatch assay (EHA) methods recommended by Coles *et al*. (1992). Twenty animals from each of the five districts which were not dewormed atleast within the last 2- 3 months were identified and divided into two groups of ten each as treatment and control groups for anthelmintic resistance study and were administered with Fenbendazole @ 7.5 mg/kg b.wt.

For EHA, a portion of the sample collected from treated and control group animals was pooled and stored immediately in the anaerobic condition by a tight screwed capped container containing 8-10 glass beads. About 50 gm of faeces were collected and mixed with water to fill up the plastic container, and shaked vigorously to make the sample anaerobic (Coles *et al.,* 2006). The eggs were collected by centrifugation in floatation solution and the concentration of these eggs was adjusted to 150 eggs per 1 mL by adding the required volume of distilled water. The stock solution was prepared as described by Himmelstjerna *et al*. (2009).

$$\text{EHA } \% = \frac{\text{No. of hatched larvae counted}}{\text{Total no of eggs and larvae}} \times 100$$

Effective $Dose_{50}$ (ED_{50}) value was calculated for the eggs by log probit analysis. Eggs having ED_{50} value exceeding 0.1 µg BZ anthelmintic per mL was indicative of resistance against Benzimidazoles (Coles *et al.* 2006).

For the coproculture study fresh fecal pellets were triturated with pestle and mortar, were incubated at 27 °C and after 7 days, the larva was collected by Baerman's technique and cleaned by centrifugation (448 g for 4 min). The identification of L3 larvae was done based on characteristics of the tail, head, oeosophagus, and intestinal cell numbers (Van Wyk and Mayhew, 2013).

A questionnaire was prepared and information regarding the goat flocks, their housing, management, and anthelmintic practices were asked to the goat owners of both agro-climatic zones. The question regarding anthelmintic practices (time, dosage, frequency) was also asked to the local veterinarians.

The data generated were statistically analyzed by SPSS version 22 to test the association among attributes such as age, sex, breed, and prevalence. A log probit model was used to estimate the ED_{50} of the concentration of the drug based on the hatching percentage in EHA.

RESULTS AND DISCUSSION

Out of 526 faecal samples examined, 377 *s*amples were found positive for the presence of gastrointestinal parasitic ova leading to an overall prevalence of 71.77%. The maximum prevalence of GI nematodes was recorded in the NCP zone over the NECP zone although with no statistical significance. Single infection was more common than mixed infection. A perusal of Table 1 reveals that in both the zones the most prevalent GI parasite was *Strongyle sp* followed by *Strongyloides sp* and *Trichuris sp*.

The prevalence of GI nematodes in the present study was quite similar to the findings of Brahma *et al*. (2015) in West Bengal. However, a prevalence of more than 80% has been recorded from Punjab (Singh *et al*., 2017), while a much lower prevalence ranging from 25% to 50% has also been reported from north east India (Das *et al*., 2017). The discrepancy in prevalence rates could be due to different agro-climatic zones and managemental practices. Amongst the GI nematode ova recovered, *Strongyle* sp was the dominant nematode over other nematodes. These findings were in accordance with the report of Verma *et al.* (2018). The high prevalence of Strongyle could be due to its high prolificacy and lesser time required for its development to infective larva.

A questionnaire survey was conducted in the five districts of the two ACZs, which revealed fenbendazole to be the most commonly used anthelmintic by goat owners/ farmers for deworming. Deworming percentage ranged from 71-96%. The dominance of benzimidazole as the choice for treatment/deworming is in agreement with the reports from India and around the globe (Zanzani *et al*., 2014; Singh *et al*., 2017). This could be due to the cheap cost and easy availability with ease of administration of these anthelmintics.

Our study revealed a frequency of four treatments annually, while a higher frequency has been depicted elsewhere (Lata, 2018). Treatment frequency as low as once annually has also been reported (Zanzani *et al*., 2014). There was a

Table 1: Coproprevalence study of gastrointestinal nematodes in goats of ACZs (NCP and NECP)

Agro-climatic Zones	Total sample collected	Sample found positive	Positive for mixed infection	Positive for single infection	Positive for single infection		
					Strongyle	Strongyloides	Trichuris
NCP	261	190 (72.79%)	75 (39.47%)	115 (60.52%	76 (66.08%)	28 (24.34%)	11 (9.56%)
NECP	265	187 (70.56%)	84 (44.91%)	103 (55.08%)	62 (60.19%)	17 (16.50%)	24 (23.30%)
Total	526	377 (71.77%)	159 (42.17%)	218 (57.82%)	138 (63.30%)	45 (20.64%)	35 (16.05%)

$\chi 2$ (p value) = 0.57.

Table 2: Efficacy of Fenbendazole against naturally occurring gastrointestinal nematodes in goats of five districts of the ACZs (NCP and NECP) of Odisha

District	Pre-treatment EPG	Post-treatment EPG	FECR %	Confidence interval		Remarks
				Lower	Higher	
Mayurbhanj	725±50.14	60±22.11	91.62%	84	99	Resistant
	740±55.68	795 +63.44	—	—	—	Control
Keonjhar	815±63.71	10±10.00	98.73%	97	—	Susceptible
	890 ±59.54	925±63.66	—	—	—	Control
Balasore	775±68.82	40±22.11	94.57%	89	99	Resistant
	875±42.33	900±42.82	—	—	—	Control
Bhadrak	810±102.96	30±15.28	96.05%	91	100	Susceptible
	840±45.22	850±41.5	—	—	—	Control
Jajpur	895±91.42	40±16.33	95.53%	90	99	Susceptible
	795±69.30	835±70.73	—	—	—	Control

Table 3: Gastrointestinal larval composition (%) in pre- and post-treatment fecal culture

District	Nematode larva	Pre-treatment (%)	Post-treatment by Fenbendazole (%)	Control (%)
Mayurbhanj	*Haemonchus* sp	64.00	80.00	68.00
	Oesophagostomum sp	8.00	4.44	8.00
	*Trichostrongylus*sp	22.00	15.56	19.00
	Strongyloides sp	6.00	0.00	5.00
Keojhar	*Haemonchus* sp	62.67	78.00	66.00
	Oesophagostomum sp	8.00	6.00	12.00
	*Trichostrongylus*sp	22.67	16.00	16.00
	Strongyloides sp	6.66	0.00	6.00
Balasore	*Haemonchus* sp	59.00	64.00	61.67
	Oesophagostomum sp	15.00	8.00	11.67
	Trichostrongylus sp	20.00	28.00	18.33
	Strongyloides sp	6.00	0.00	8.33
Bhadrak	*Haemonchus* sp	60.00	77.50	55.71
	Oesophagostomum sp	12.86	5.00	8.57
	Trichostrongylus sp	21.43	17.50	27.15
	Strongyloides sp	5.71	0.00	8.57
Jajpur	*Haemonchus* sp	52.50	70.00	54.67
	Oesophagostomum sp	17.50	6.67	14.66
	Trichostrongylus sp	17.50	23.33	22.67
	Strongyloides sp	12.50	0.00	8.00

positive association between the frequency of anthelmintic treatments on a farm and the presence of benzimidazole resistance (Mohammadsalliah *et al*., 2019). The variation in treatment frequency could be attributed to factors like the type of animal owners, their awareness levels as well as the efficiency of the animal health delivery system.

Fecal egg count of naturally infected goats of the two zones was recorded pretreatment and post-treatment and compared with the control group. The pretreatment fecal EPG ranged from 500 to 1200. As per the fecal egg count reduction percentage detailed in Table 2, the efficacy of the anthelmintic in the five districts ranged from 91.62% to 98.73%. The results obtained by using FECRT in 5 districts showed low level of resistance of GI nematodes to Fenbendazole in 2 districts (Mayurbhanj and Balasore). The other three districts had >95% FECR%. Similar observation has been reported by Jaiswal *et al*. (2013) in goats of Mathura.

From the same state, there has been a report of FECR% of 90-97 in goat farms from Khurda district (Sahu *et al.,* 2015). Higher resistance to Fenbendazole has been reported by Godhara *et al*. (2011) in Jamunapari goats with FECR% as low as 23.66, and by Dixit *et al*. (2017) in goats of Jabalpur (24.9). Gelot *et al*. (2016) in Gujarat observed FECR 53-76%. Benzimidazole resistance has also been well documented abroad with efficacy ranging from 25% to 90% (Zajac and Gipson, 2000; Mohammadsalliah *et al*., 2019). The higher efficacy of fenbendazole during our study might be due to less number of treatments with the right dosage as well as good managemental practices.

The percentage of gastrointestinal larval composition in pre- and post-treatment samples (Table 3) clearly showed the dominance of *Haemonchus sp* over *Trichostrongyloides* sp, *Oesophagostomum* sp, and *Strongyloides* sp in all the five districts of the two zones studied. However, post-treatment fecal culture revealed the absence of *Strongyloides* sp.

The predominance of *Haemonchus sp* corroborates with findings from different parts of the India and world (Rialch *et al*., 2013; Crook *et al*., 2016) as well as Odisha (Sahu *et al*., 2015). The higher presence of *Haemonchus* sp indicates that resistance to the benzimidazole group is

mostly associated with *Haemonchus* sp. This might be due to the high biotic potential of *Haemonchus sp,* which produces new generations within a short period, this also enhances their selection for resistance.

The ED_{50} values for fenbendazole in the *in vitro* egg hatch assay of naturally occurring GI nematodes in goats of the five districts, *viz.,* Mayurbhanj, Keonjhar, Balasore, Bhadrak and Jajpur were 0.1638, 0.0634, 0.1203, 0.0223 and 0.0217 µg/mL, respectively. As per the results of regression, there was evidence of anthelmintic resistance in the goats of Mayurbhanj and Balasore districts, while the other three districts did not show evidence of anthelmintic resistance.

Our findings from the EHA are somewhat similar to the observations by Rialch *et al*. (2013), who found the ED_{50} value of the susceptible group to be 0.037-0.096 µg/mL, while for the resistant group the values were 0.101-0.147 µg/mL in small ruminants of North India. Similarly. Deepa (2005) observed ED_{50} value (µg/mL) to be 0.211556 against benzimidazole anthelmintics in the goat farms of Mannuthy.

The presence of anthelmintic resistance may be due to the selection of resistant genotypes within the parasitic population or reselection of resistant individuals already present in the population at a lower frequency. The use of anthelmintics increases the frequency of the resistant worms, so efforts should be aimed at reducing the further multiplication of these resistant worms.

CONCLUSION

The study indicated considerable presence of gastrointestinal nematodes in goats of both the agroclimatic zones (NCP, NECP) of Odisha and there was detection of anthelmintic resistance against fenbendazole in two districts. Further studies involving the detection of resistance in anthelmintics by utilizing sensitive molecular techniques are necessary to know the status of resistance in small ruminants in the region.

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