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Prevalence of *Haemonchus contortus* and other Gastrointestinal nematodes in different sheep breeds of Odisha

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Abstract
The present study aims to investigate the prevalence of GI nematodes in sheep breeds of Odisha with respect to different risk factors. A total of 701 faecal samples collected from different sheep breeds available in Odisha were examined for a period of one year (January to December, 2019) which revealed overall prevalence of gastrointestinal nematodes was 61.20% of which Strongyles were dominant (72.22%) followed by *Strongyloides* sp. (34.26%) and *Trichuris* sp. (16.08%). Prevalence of mixed infection was 22.61%. Coproculture revealed highest percentage of population of *Haemonchus contortus* larvae, followed by *Trichostrongylus* spp., *Oesophagostomum* spp. and *Strongyloides* spp.Higher prevalence of nematodes were recorded in rainy season followed by summer and least in winter. The variation in prevalence due to season was statistically significant (P<0.01). There was significant difference (P<0.01) among breeds of sheep with regard to frequency of nematode infestation which was highest in non-descript, followed by Balangir, Ganjam and Kendrapada breeds with 39.9%, 20.7%, 20.5% and 18.9%, respectively. Statistically significant (P<0.01) influence of other associated risk factors such as age (highest in 6-12 months and lowest in less than 6 months), sex (more in females) and faecal consistency on rate of prevalence of GI nematodes were recorded.

Keywords: Prevalence, gastrointestinal nematodes, sheep

Introduction
The contribution of livestock sector to Agricultural GDP is about 33 per cent. The livestock sector is one of the fastest growing agricultural subsectors in developing countries like India. The growth in demand for various livestock products has been showing an increasing trend in tune with population growth, urbanization and increasing income in developing countries.

Sheep husbandry has been traditionally associated with many communities particularly belonging to small and marginal farmers in arid, semi-arid and mountainous area of the country due to its multi-faecet utility for wool, meat, skin and manure. It is easy to manage sheep along with cattle under semi-intensive system of rearing to their grazing capability that saves labour for which sheep rearing is preferred to goats in many parts of our country. Sheep husbandry provides a dependable source of income to the farmers through sale of wool and mutton. They play a significant role in the livelihood of small and marginal farmers and landless laborers engaged in sheep rearing.

Like other grazing animals, sheep are also prone to parasitism with many ecto and endoparasites. The nematode class is one of the main causative worm parasites that causes economic losses in sheep production (Coop and Angus, 1981) [1]. Amongst the different nematodes infecting sheep, nematodes of gastrointestinal tract are a major hindrance to production performance of sheep throughout the world including India (Kumar *et al*., 2008) [2]. Gastrointestinal nematodes in general and *Haemonchus contortus* in particular poses a serious threat to sheep husbandry throughout the world. Haemonchosis is more a health problem in tropics and subtropics. Epidemiology of any parasitic infections which is variable with respect to breed, age, sex, geographical location, topography and climate plays an important role to understand the seasonality of parasitism and prepare region specific control measures. This is the reason why number of epidemiological studies have been carried out in different parts of India (Mamatha and D'Souza, 2007; Singh *et al*., 2015; Singh *et al*., 2017) [3-5].

The clinical diagnosis of gastrointestinal nematodes is difficult, since the signs are not pathognomonic. However, diagnosis of the infections plays a major role in investigating epidemiology of the parasite. The ante mortem diagnosis of nematode infections in livestock, primarily, is based on the detection of nematode eggs or larvae in the faeces while quantifying of the egg per gram of feces is the best way of estimating parasite loads (Roeber *et al*., 2013) [6]. The study under report was carried out to investigate the clinical and subclinical infections with GI nematodes in different sheep breeds of Odisha with respect to different risk factors.

Materials and Methods

The State of Odisha is situated between 17.49'N and 22.34'N latitudes and meridians of 81.27'E and 87.29'E in the eastern region of India. It covers a geographical area of 1,55,707 sq.km. and has a coast line of about 480 kms on the Bay of Bengal. The State is broadly divided into 4 Physiographic Zones namely Coastal Plains, Central Table Land, Northern Plateau and Eastern Ghats which are further differentiated into ten Agroclimatic Zones. The state has a tropical climate marked by high temperature, high humidity, medium to high rainfall and short and mild winter. The normal annual rainfall is 1451.2 mm.

Faecal samples were collected from 701 sheep belonging to different recognized breeds (Ganjam, Kendrapada and Balangir) and non-descript breeds available in Odisha, for a period of 12 months from January,2019 to December, 2019 comprising of all three seasons. Different area in the district of Ganjam, Jagatsinghpur, Khorda, Puri, Kendrapada, Sonepur and Balangir were selected for random collection of dung samples.

Samples collected in plastic containers (Himedia)®were brought to the Department of Veterinary Parasitology and subjected to qualitative (Flotation and Sedimentation techniques) and quantitative (Mc Master technique) examination. Dung samples collected from same farm or herd which were detected positive for strongyle nematode ova were pooled and cultured following the standard technique for fecal culture (Soulsby, 1982) [7]. Larvae (L_3) of strongyle nematodes harvested by Baerman's method were identified to generic level based on morphological characters (Soulsby, 1982) [7].

In order to know the correlation between the rate of prevalence and different risk factors such as age, breed, sex & season, Chi-square test was used. Correlations with $P<0.05$ were considered significant and $P<0.01$ were considered highly significant. All statistical analysis was done with SPSS-25 Software.

Results and Discussion

Overall prevalence

In the present study, a total of 701 fecal samples of different sheep breeds were examined, out of which 429 (61.20%) were found positive for gastrointestinal nematodes (Table 1). Strongyles were dominant (72.22%) followed by *Strongyloides* sp. (34.26%) and *Trichuris* sp. (16.08%). Prevalence of mixed infection was 22.61%. A higher rate of overall prevalence (61.20%) recorded in the population of sheep under study corroborated with the observations of earlier authors (Velusamy *et al*., 2015; Raza *et al*., 2014; Jena *et al*., 2018) [8-10]. Studies from different regions of India such as Karnataka (Singh *et al*., 2015) [4], Marathwada (Dappawar *et al*., 2018) [11], semi-arid zone of Rajasthan (Swarnakar *et al*., 1996) [12], Haryana (Gupta *et al*., 1987) [13] and Uttar Pradesh (Singh *et al*., 2013) [14] also revealed high incidence of GI nematodoses in sheep. An overall prevalence of 65.11% in Bonpala sheep with specific prevalence of *Haemonchus* sp. (57.63%), *Trichuris* sp. (40.07%), *Oesophagostomum* sp. (21.48%), and *Trichostrongylus* sp. (11.04%) has been reported by Molla and Bandyopadhyay (2016) [16]. Prevalence of Trichostrongylid eggs (72.85%) followed by *Nematodirus* from Kashmir Valley has been observed (Allaie *et al*., 2018) [17]. However, high incidence of Strongyle was reported from Haryana (Priyanka *et al*., 2020) [18].

Season wise Prevalence

The present study revealed the seasonality in rate of prevalence of various GI nematodes parasites (Table 1). There was significant difference among seasons with regard to frequency of nematode infestation revealing the highest percentage of occurrence in rainy season, followed by summer and winter season with 47.6, 35.4 and 17.0%, respectively. All the species of GI nematodes showed higher infestation during rainy season, followed by summer and winter season (Table-2) and the variation was statistically significant ($P< 0.01$). Strongyle infection recorded the highest frequency followed by *Strongyloides* spp., mixed and *Trichuris* spp. in all the seasons. The present findings agree with reports from Haryana which recorded highest strongyle infection during monsoon and lowest during winter (Priyanka *et al*., 2020) [18]. A higher prevalence was recorded in rainy seasons than dry seasons (Nginyi *et al*., 2001; Vlassoff *et al*., 2001) [19, 20]. During rainy season conditions suitable for the development of larvae prevails and its transmission on pasture is favoured by optimal moisture (Agyei *et al*., 1991) [21]. A significant higher prevalence ($P<0.01$) of GI helminths was also recorded in rainy season followed by winter and summer in sheep of Jharkhand state (Jena *et al*., 2018) [10]. However, the higher GIN prevalence in sheep in summer and lowest in winter were recorded from Kashmir valley (Nasreen *et al*., 2005) [22]. These variations in the observation may be due to varying climatic conditions and management practices.

Breed wise prevalence

Significantly higher percentage was observed on infection with all types of nematodes species among non-descripts than registered breeds of sheep in the present study. However, Strongyle was found to be major species of nematode followed by *Strongyloides Sp*, mixed and *Trichuris Sp.* in all breeds of sheep. There was significant difference ($P<0.01$) among breeds of sheep with regard to frequency of nematode infestation revealing highest percentage in non-descript, followed by Balangir, Ganjam and Kendrapada breeds with 39.9%, 20.7%, 20.5% and 18.9%, respectively (Table 1 and 2). The present observations on breed wise prevalence akin to the previous observations who recorded the significant influence of breed on faecal egg count and they recorded few sheep breeds were resistant to gastrointestinal nematodes infection (Bahirathan *et al*, 1996; Li *et al*., 2001; Matika *et al*., 2003) [23-25]. These findings by above workers could be due to genetic factors associated with relative susceptibility of different sheep breeds to GI nematodoses coupled with managemental practices.

Age wise prevalence

There was highly significant difference ($P<0.01$) among age groups with regard to frequency of GI nematode infection that

revealed highest percentage of prevalence in age group (6-12 months) , followed by age group (>12 months) and age group (< 6 months), with 42.2, 38.2 and 19.6%, respectively. All the species of nematodes showed significantly higher infestation in age group (6-12 months), followed by age group (>12 months) and least in age group (<6 months), Strongyle infection recorded the highest frequency followed by *Strongyloide*s, mixed and *Trichuris* in all the age groups. (Table 2). Results of present study on prevalence as per age are in conformity with earlier studies that depicted significantly (p<0.01) higher prevalence in adults as compared to young sheep (Priyanka *et al*., 2020) [18]. Similar results were also recorded from Bangladesh (Rahman *et al*., 2017) [26] and Ethiopia (Dabasa *et al*., 2017) [27]. Higher rate of prevalence observed in adults might be due to frequent exposure to infective larval stage during grazing and physiological stress caused in adults during periparturient period and lactation rendering the adults more susceptible to patent infections.

Sex wise prevalence

Sex wise prevalence revealed that females were more susceptible than their male counterparts for every species of GI nematode parasites in the present study. There was significant difference (P<0.01) among sex with regard to frequency of GI nematode infection that revealed highest percentage of prevalence in females (72.3%) than in males (27.7%). The present results are in general agreement with reports from Chottanagpuri sheep in Ranchi that showed significantly higher (P<0.01) prevalence in female over male (Jena *et al*., 2018) [10]. Similarly a significantly higher prevalence in females (83.6%) than males (64.7%) was observed at Mymensingh, Bangladesh (Islam *et al*., 2017) [28]. Higher parasitism observed among females might be due to low immunity and stress condition during pregnancy, post parturition and also during the lactational period (Dabasa *et al*., 2017) [27]. However, contradictory results were also recorded from Pakistan (Asif *et al*., 2007) [29].

Association of Feacal Consistency with Prevalence of nematodes

Intensity of gastrointestinal nematode infection was significantly different (P<0.01) with regard to faecal consistency. Highest prevalence of nematodes was recorded in sheep that defecated normal pellet followed by semisolid and diarrheic with 45.7%, 41.7% and 12.6% respectively. Present study on association of feacal consistency with intensity of GI nematode infection results partially agrees with previous reports who recorded the inverse relationship between gastrointestinal nematode FECs and faecal consistency (Seyoum *et al*., 2018) [30]. Alteration of faecal consistency is mostly due to irritation caused by immature parasites during their tissue invasion and migration in intestine resulting in diarrhea (Soulsby, 1982) [7] where presence of nematode ova gets diluted. The presence of mucous and blood in dung are indicatives of gastroenteritis which might be due to heavy load of adult parasites dwelling in the GI tract.

Larval Composition in Coproculture

Coproculture of pooled dung samples of sheep were done to assess the generic composition of strongyle nematodes (Figure 1-4), which revealed that highest percentage composition was with larvae of *Haemonchus contortus*, followed by *Trichostrongylus* spp., *Oesophagostomum* spp. and *Strongyloides* spp. (Table.3). Predominance of infective larvae of *Haemonchus contortus* among above four species were recorded throughout the study period in different seasons, as well as in different breeds of sheep. Coproculture studies from previous surveys also recorded the dominance of *Haemonchus* spp. and *Trichostrongylus* spp. during the whole year in sheep and goat in eastern Haryana (Gupta *et al*., 1987) [13] and the predominance of *Haemonchus* spp has been observed from other regions of India (Priyanka *et al*., 2020; Varadharajan and Vijayalakshmi, 2015; Rajarajan *et al*., 2017; Vohra *et al*., 2018) [18, 31-33].

Table 1: Frequency of nematode infestation across different risk factor

Factors	Sub factors	Frequency	Percentage (%)	Chi-square (χ^2)
Season	Rainy	204	47.6	60.853**
	Winter	73	17.0	
	Summer	152	35.4	
Breed	Kendrapada	81	18.9	50.879**
	Ganjam	88	20.5	
	Bolangir	89	20.7	
	Non-descript	171	39.9	
Age	<6m	84	19.6	37.524**
	6-12m	181	42.2	
	>12m	164	38.2	
Sex	Male	119	27.7	85.037**
	Female	310	72.3	
Feacal Consistency	Diric	54	12.6	84.098**
	Semisolid	179	41.7	
	Normal	196	45.7	
Feacal Colour	Normal	350	81.6	171.20**
	Abnormal	79	18.4	
Presence of Mucous/Blood	Yes	252	58.7	13.112**
	No	177	41.3	
Total		429	100.0	

**p<0.01

Table 2: Frequency of species of nematodes across different risk factors

			Species				
			Strongyl	**Strongyloid**	**Trichuris**	**Mixed**	**Chi-square (χ^2)**
Season	Rainy	Count	147	75	33	51	170.056**
		% within species	47.4%	51.0%	47.8%	52.6%	
	Winter	Count	60	22	8	19	
		% within species	19.4%	15.0%	11.6%	19.6%	
	Summer	Count	103	50	28	27	
		% within species	33.2%	34.0%	40.6%	27.8%	
Breed	Kendrapada	Count	58	26	14	16	35.533**
		% within species	18.7%	17.7%	20.3%	16.5%	
	Ganjam	Count	66	29	15	22	
		% within species	21.3%	19.7%	21.7%	22.7%	
	Bolangir	Count	65	34	15	25	
		% within species	21.0%	23.1%	21.7%	25.8%	
	Nondescript	Count	121	58	25	34	
		% within species	39.0%	39.5%	36.2%	35.1%	
Age	< 6m	Count	65	25	9	17	405.232**
		% within species	21.0%	17.0%	13.0%	17.5%	
	6-12m	Count	124	70	36	48	
		% within species	40.1%	47.6%	52.2%	49.5%	
	> 12m	Count	120	52	24	32	
		% within species	38.8%	35.4%	34.8%	33.0%	
Sex	Male	Count	93	39	14	27	155.573**
		% within species	30.0%	26.5%	20.3%	27.8%	
	Female	Count	217	108	55	70	
		% within species	70.0%	73.5%	79.7%	72.2%	
Total		Count	310	147	69	97	
		% within species	100.0%	100.0%	100.0%	100.0%	

**$p<0.01$

Table 3: Gastrointestinal larval population (%) in coproculture of different sheep breeds of Odisha

Breeds	Larva	Summer (%)	Monsoon (%)	Winter (%)
Kendrapada	*Haemonchus*sp	53	55.5	51
	*Oesophagostomum*sp	19	17	8
	*Trichostrongylus*sp	21	23	18
	*Strongyloides*sp	7	4.5	23
Ganjam	*Haemonchus*sp	55.67	58.0	53
	*Oesophagostomum*sp	15	18.0	9
	*Trichostrongylus*sp	22.67	20.0	20
	*Strongyloides*sp	6.67	4	18
Balangir	*Haemonchus*sp	60	65.7	58.67
	*Oesophagostomum*sp	15.0	13	5. 67
	*Trichostrongylus*sp	17.33	18.3	19.33
	*Strongyloides*sp	7.67	3	16.33
Non-descript	*Haemonchus*sp	65.30	69.5	64.71
	*Oesophagostomum*sp	13.86	12.0	6.57
	*Trichostrongylus*sp	15.43	15	16.4
	*Strongyloides*sp	5.41	3.5	12.32

Conclusion

The current study revealed considerably higher prevalence of gastrointestinal nematode infection in sheep breeds of Odisha. Strongyles were found to be the most prevalent nematode followed by *Strongyloides* sp. and *Trichuris* sp. Further, *Haemonchus contortus* was the most predominant strongyle nematode prevalent in all breeds and age groups round the year. There was statistically significant difference in rate of prevalence with respect to all risk factors such as age, sex, breed, season and consistency of dung considered in the study.

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