



# Within Breed Resistance to Naturally Occurring Gastrointestinal Nematodes in Garole Sheep of West Bengal, India

Anupam Brahma<sup>1</sup>, Trina De<sup>1</sup>, Ruma Jas, Surajit Baidya<sup>1</sup>, Soumitra Pandit<sup>1</sup>, Subhas Chandra Mandal<sup>1</sup>, Dhananjay Kumar<sup>1</sup>, Saroj Rai<sup>2</sup>

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## ABSTRACT

**Background:** Gastrointestinal (g.i.) nematodes is the main constrain in sheep husbandry and control of g.i. nematodes is challenging due to anthelmintic resistance. Exploitation of host resistance could be an alternative approach to nematodes.

**Methods:** Within breed resistance of Garole sheep to g.i. nematodes was determined in terms of monthly faecal egg count (FEC), Hb concentration and PCV% in 4- 6-months-old Garole sheep (n=80) in South 24 Parganas district from April, 2018 to March, 2019. On the basis of 6 months' FEC data sheep were divided into resistant and susceptible group and Hb and PCV were measured in 15 animals from each group at monthly interval.

**Result:** The prevalence of g.i. nematodes was 70 per cent and *Haemonchus contortus* was the predominant species. The overall FEC in Garole sheep was 360.67 in terms of eggs per gram (EPG) of faeces. Among the 80 Garole sheep 28 sheep showed persistent low FEC. The mean EPG of resistant group were 97.62 whereas, mean EPG of susceptible group were 502.31. In resistant sheep Hb and PCV did not vary whereas, in susceptible Hb and PCV reduced significantly ( $P<0.05$ ) than the resistant sheep. Within breed resistance was present in Garole sheep and that could be utilized in selection of resistant breed.

**Key words:** Faecal egg count, Garole sheep, Gastrointestinal nematodes, Resistance.

## INTRODUCTION

Sheep rearing forms an integral part of rural livelihood for the marginal and landless farmers whose only source of income is from agriculture-based activity (Ruto et al., 2008). They can be both resilient and productive at the same time but this trait is most often severely jeopardised by parasitic gastroenteritis caused due to g.i. nematodes exhibited by morbidity, mortality and loss of body weight due to reduced growth rate resulting in great economic losses (Jas and Ghosh 2009; Jas et al., 2007, 2017a) along with increased cost of treatment (Miller and Horohov, 2006). Sheep in Indian field conditions mostly acquire mixed g. i. nematodal infections including *Haemonchus contortus*, *Oesophagostomum*, *Trichostrongylus*, *Gaigeria*, *Bunostomum*, *Strongyloides*, *Moniezia*, *Fasciola* and Paramphistomes (Jas and Ghosh, 2007; Brahma et al., 2015, 2018; Jas et al., 2017b).

Although application of anthelmintics has always been a thumb rule intervention for dealing with the insidious g. i. parasites, the ubiquitous increase in number of resistant worms (Saddiqi et al., 2006; Sargison et al., 2010; Kamaraj et al., 2011; Singh et al., 2017b) arising out of selection pressure by anthelmintics has jeopardised the pharmaceutical industry (Pawar et al., 2019; Singh et al., 2019). Currently the most extensively used approach and crucial alternative to manage g.i. nematodes is the utilization of enhanced disease resistant trait in sheep breeds (Gamble and Zajac, 1992; Stear and Murray, 1994), dietary protein supplementation (Coop and Holmes, 1996; Datta et al., 1999) and biological control by the use of nematophagous fungi (Sanyal, 2000).

Department of Veterinary Parasitology, West Bengal University of Animal and Fishery Sciences, Kolkata-700 037, West Bengal, India.

<sup>1</sup>Krishi Vigyan Kendra, Nawada-805 106, Bihar, India.

<sup>2</sup>National Dairy Research Institute-Eastern Regional Station, Nadia, Kalyani-741 235, West Bengal, India.

**Corresponding Author:** Ruma Jas, Department of Veterinary Parasitology, West Bengal University of Animal and Fishery Sciences, Kolkata-700 037, West Bengal, India.  
Email: rumajas@gmail.com

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Resistance to parasitic diseases is a genetically variable characteristic of animals (Gray et al., 1995). The animals resistant or susceptible to g. i. nematodes can be identified by parasitological indicators of resistance such as faecal egg count (FEC) and for haematophagous parasites haemoglobin (Hb) and packed cell volume (PCV) (Kaur et al., 2018). Faecal egg count is considered as the important phenotypic indicator for g. i. nematode resistance (Karrow et al., 2014). Nematode resistance assessed by using FEC has a low to high heritability in small ruminants ranging from 0.01 to 0.65, which means there is a good potential for obtaining genetic gain through selection (Lobo et al., 2009).

Among many economically valuable sheep breeds in India Garole sheep of West Bengal well known for its unique

characteristics like biannual lambing, high prolificacy, grazing nature on aquatic weeds and grass and its ability to withstand against diseases is the most popular sheep breed of eastern India (Banerjee *et al.*, 2010). The saline belt of the Sunderban Delta of South 24 Parganas district, some parts of North 24 Parganas and some saline zones of Midnapore district in West Bengal are the hometracts as well as breeding tracts of the Garole sheep.

Resistance and/or resilience in Garole sheep to naturally occurring nematodes was reported by Ghosh *et al.* (2012) and that study was conducted 10 years back also from different location in West Bengal. Therefore, the present study was designed to validate the within breed resistance of Garole sheep against naturally occurring g.i. nematodes on the basis of FEC, Hb and PCV, the indicator of parasite resistance.

## MATERIALS AND METHODS

### Study area and animals

The present study was carried out in two villages near Sundarban Delta of South 24 Parganas of West Bengal, the native tract of Garole sheep. In those villages Garole sheep were reared by the small and marginal farmers. Semi-intensive system of sheep rearing was practiced and routine deworming was not followed in the study area. Garole sheep were reared only by grazing and giving them kitchen waste and no concentrate food was given to them. Eighty Garole sheep of either sex and in the age group of 4-6 months were selected on the basis of phenotypic characteristic. Animals were properly identified by body colour, owner name and also colouring their wools.

### Collection and examination of faecal sample

Per rectal faecal samples or freshly voided faecal samples were collected from all the sheep at monthly interval from April, 2018 to March, 2019. About 5 grams of faecal samples were collected in plastic vials (Tarsons, India) containing 10% formalin and then brought to the laboratory for further processing.

A part of individual faecal sample was examined qualitatively for the presence of nematode eggs by standard salt floatation technique (Soulsby, 1982) and the remaining part of the sample was subjected to quantitative faecal examination to determine the intensity of g.i. nematode infection by modified Mc. Master technique (Soulsby, 1982).

### Coprological examination

Pooled faecal samples comprising of faecal samples of all selected sheep were collected in polythene bags without any preservatives for coprocultural examination to determine the species composition of nematode larvae by the method described by Soulsby (1982) with slight modification. After completion of coproculture, the infective third stage larvae were harvested and the species composition of nematodes in the larval samples was determined following guidelines of Anonymous (1971).

### Collection of blood samples and estimation of haematological parameters

On the basis of faecal egg count (EPG) data collected for six months, the Garole sheep were divided into comparative resistant and susceptible group. Blood samples were collected from representative number of animals (n=15) under each of the group from seventh month for 6 occasions at monthly interval for estimation of Hb and PCV. Two milliliter of blood were drawn out aseptically by jugular vein-puncture with the help of disposable syringe and were collected in sterilized screw capped vial containing EDTA @ 1 mg/ml of blood as anticoagulant. Blood samples were brought to the laboratory by maintaining cold chain carriage. Haemoglobin (gm/dl) concentration was estimated by Sahlis' method and PCV (%) was estimated by Wintrobe's haematocrit method as described by Schalm *et al.* (1986).

### Statistical analysis

The significance (P-value) was recorded at 5% (P<0.05) level and 1% (P<0.01) level by separate analysis of parameters (between post-infection days) using Duncan method (One-way-ANOVA). The complete statistical analyses were done with the help of Statistical Package for Social Scientist (SPSS), Windows Version 20.0.

## RESULTS AND DISCUSSION

### Parasitological indicators for resistance

Gastrointestinal nematodes comprising different types of nematode parasites were of common occurrence in the selected Garole sheep during the study period. Three types of gastrointestinal nematode parasites; *Strongyle*, *Strongyloides* and *Trichuris* species were recorded in Garole sheep. The overall prevalence of naturally occurring g.i. nematodes was 70% (Table 1) and *Strongyle* group of nematodes was predominant (66.77%). Highest prevalence of g.i. nematodes was observed during monsoon (80.62%) followed by winter (65.94%) and lowest (63.44%) in summer. As the *Strongyle* group of nematodes are pathogenic and predominant too, therefore, the intensity of *Strongyle* group of nematode infection was considered for FEC.

The mean FEC of selected sheep was varied from 200 to 613.75 eggs per gram of faeces (EPG) with an overall mean EPG of 360.67 (Table 2). Like prevalence, intensity of infection (FEC) was also found to be highest in monsoon (EPG = 557.00). Out of 80 sheep selected in the study area 28 sheep showed persistent low FEC and comparatively higher mean FEC were recorded in rest of the 52 sheep during the study period. The overall mean EPG of resistant group of Garole sheep were of 97.62 whereas, mean EPG of susceptible group of sheep were recorded as 502.31 (Table 2). The sheep of the resistant group showed persistent low EPG ranging from 42.86 to 150 during the study period, on the other hand sheep of susceptible group showed a variable EPG ranging from 253.85 to 875 (Table 2). Per cent prevalence of *Strongyle* larvae as determined by coprocultural examination revealed that five species of

**Table 1:** Overall prevalence of naturally occurring g.i. nematodes in selected Garole sheep.

Months	Total examined	Overall prevalence (%)	Strongyle (%)	<i>Strongyloides</i> (%)	<i>Trichuris</i> (%)
March	80	66.25	62.50	5.00	6.25
April	80	61.25	60.00	2.50	1.25
May	80	58.75	57.50	2.50	2.50
June	80	67.50	62.50	3.75	2.50
<b>Summer</b>	<b>320</b>	<b>63.44</b>	<b>60.62</b>	<b>3.44</b>	<b>3.12</b>
July	80	75.00	71.25	5.00	3.75
August	80	86.25	81.25	3.75	3.75
September	80	88.75	86.25	3.75	2.5
October	80	72.50	70.00	2.5	5.00
<b>Monsoon</b>	<b>320</b>	<b>80.62</b>	<b>77.18</b>	<b>3.75</b>	<b>3.75</b>
November	80	71.25	66.25	2.50	5.00
December	80	63.75	60.00	5.00	3.75
January	80	65.00	62.50	5.00	6.25
February	80	63.75	61.25	3.75	3.75
<b>Winter</b>	<b>320</b>	<b>65.94</b>	<b>62.50</b>	<b>4.68</b>	<b>5.00</b>
Overall	960	70.00	66.77	3.96	3.96

**Table 2:** Monthly mean faecal egg count of comparative resistant and susceptible Garole sheep naturally infected with g. i. nematodes.

Months	Resistant sheep (EPG)	Susceptible sheep (EPG)	Overall (EPG)
March	78.57	300.00	222.50
April	92.85	265.38	205.00
May	42.86	253.85	180.00
June	121.43	601.92	433.75
<b>Summer</b>	<b>83.93</b>	<b>355.89</b>	<b>260.31</b>
July	135.71	765.38	545.00
August	150.00	843.08	600.50
September	128.57	875.00	613.75
October	71.43	682.92	468.75
<b>Monsoon</b>	<b>121.43</b>	<b>791.60</b>	<b>557.00</b>
November	64.29	537.08	395.00
December	92.86	340.38	253.75
January	92.85	273.08	210.00
February	100.00	253.85	200.00
<b>Winter</b>	<b>87.5</b>	<b>351.10</b>	<b>260.19</b>
Overall	97.62	502.31	360.67

Strongyle nematodes namely *Haemonchus*, *Oesophagostomum*, *Trichostrongylus* and hookworm (*Bunostomum/Gaigeria*) and also *Strongyloides* were prevalent (Table 3). *Haemonchus contortus*, the most pathogenic nematode was the predominant species (64.45%) infecting the Garole sheep of the present study.

Gastrointestinal nematode parasites were recorded in the selected Garole sheep all-round the year. Prevalence and as well as intensity of naturally occurring g.i. nematode infection as revealed in the present study was in agreement with earlier reports (Ghosh *et al.*, 2012; Brahma *et al.*, 2018). The prevalence and overall intensity of infection was highest in monsoon (80.62%; EPG=557) and comparatively lower prevalence and intensity were observed in winter (65.94%; EPG=260.19) and summer (63.44%; EPG= 260.31). The

agro-climatic condition of South 24 Parganas and also the natural environment such as presence of small ponds, water-logging area and also the availability of ample green grasses during monsoon favours the survival of free-living stages of g.i. nematodes and also increases the chances of transmission to the animal (Jas *et al.*, 2017b). In India including West Bengal *Haemonchus contortus* has been recorded as the predominant g. i. nematode species (Singh *et al.*, 2017a; Jas *et al.*, 2017b) and in the present study too it has been recorded as the predominant g.i. nematode species.

On the basis of FEC, the selected Garole sheep were identified as comparative resistant and susceptible sheep and the overall FEC of comparative resistant sheep (EPG = 97.62) was significantly ( $P < 0.01$ ) lower compared to

**Table 3:** Monthly species composition of infective larvae of gastrointestinal nematodes of sheep under study.

Months	<i>Haemonchus</i> (%)	<i>Oesophagostomum</i> (%)	<i>Trichostrongylus</i> (%)	Hookworm (%)	<i>Strongyloides</i> (%)
March	63.70	15.50	9.56	6.24	5.00
April	64.00	16.00	9.45	5.50	5.05
May	62.50	15.50	9.70	6.70	5.80
June	64.75	15.75	9.67	6.50	3.33
July	65.33	15.67	9.33	5.00	4.67
August	65.67	16.33	9.67	5.00	3.33
September	65.00	16.00	10.67	4.67	3.67
October	65.67	17.00	9.00	4.67	3.67
November	64.67	17.67	10.00	4.00	3.67
December	63.00	17.33	10.67	5.00	4.00
January	65.33	14.00	11.00	4.00	5.67
February	64.00	14.00	10.00	6.33	5.67
Overall	64.45	15.90	9.89	5.30	4.46

**Table 4:** Haemoglobin concentration and packed cell volume (%) in comparative resistant and susceptible Garole sheep naturally infected with g.i. nematodes.

Occasions	Haemoglobin concentration (gm/dl)			PCV (%)		
	Resistant sheep	Susceptible sheep	P value	Resistant sheep	Susceptible sheep	P value
1 <sup>st</sup>	10.95±0.26	10.66 <sup>a</sup> ±0.17	0.365	34.40±0.40	32.50 <sup>a</sup> ± 0.87	0.063
2 <sup>nd</sup>	11.00±0.22	10.53 <sup>a</sup> ±0.17	0.110	33.55±0.65	31.60 <sup>ab</sup> ± 1.04	0.131
3 <sup>rd</sup>	11.08±0.19	9.65 <sup>by</sup> ±0.17	0.000	33.12±0.67	29.95 <sup>by</sup> ± 0.65	0.003
4 <sup>th</sup>	11.14±0.36	10.48 <sup>a</sup> ±0.18	0.118	32.85±0.77	30.80 <sup>ab</sup> ± 0.68	0.062
5 <sup>th</sup>	10.93±0.21	10.83 <sup>a</sup> ±0.22	0.746	33.70±0.86	32.70 <sup>a</sup> ± 0.49	0.315
6 <sup>th</sup>	10.86±0.22	11.00 <sup>a</sup> ±0.08	0.557	33.90 ± 0.31	32.80 <sup>a</sup> ± 0.59	0.118
P value	0.974	0.000	N=15	0.582	0.047	N=15

N.B. Values bearing superscripts x, y... in a row and a, b...in a column varies significantly (P<0.05).

susceptible Garole sheep (EPG = 502.31). Thus, within breed resistance of Garole sheep to naturally occurring g.i. nematodes as observed in the present study was also reported earlier by Ghosh *et al.* (2012). Within breed resistance to g. i. nematode infection has also been reported by many workers around the world (Pena *et al.*, 2004). Australian Merino sheep (Andronicos *et al.*, 2010) and Coast Native line (Gruner *et al.*, 2004) showed within breed resistance against *H. contortus* and *T. colubriformis* and Scottish Black Face line showed against *Teladorsagia circumcincta* (Stear and Murray, 1994), Gulf Coast Native sheep also exhibited within breed resistance against *H. contortus* (Pena *et al.*, 2004).

**Haemoglobin concentration and PCV (%) of selected resistant and susceptible sheep**

The selected Garole sheep were preliminarily grouped as resistant and susceptible based on monthly FEC record for six months. From the 7<sup>th</sup> month of field study Hb concentration and PCV (%) were measured in randomly selected 15 sheep each from the resistant and susceptible group at monthly interval for 6 occasions. The Hb concentration and PCV (%) of resistant group of sheep were higher than the susceptible group on all the 6 occasions and these differences were not statistically significant

(P>0.05) except on 3<sup>rd</sup> occasion *i.e.* in the month of September when the Hb concentration and PCV (%) was significantly (P<0.05) lower in the susceptible sheep compared to the resistant group (Table 4). Within the resistant group, the Hb concentration and PCV (%) did not differ significantly (P>0.05) during the various occasions of blood collection. Whereas in susceptible group Hb concentration was decreased significantly (P<0.05) on 3<sup>rd</sup> occasion (in the month of September) than the other occasions (Table 4).

Within breed resistance in Garole sheep as observed in the present study was also substantiated with the findings of Hb concentration and PCV (%). Haemoglobin concentration and PCV (%) of both the selected resistant and susceptible sheep were recorded during monsoon (July to October) and two months of winter season (November and December). In resistant group of Garole sheep the Hb concentration and PCV (%) did not vary significantly (P>0.05) on different occasions due to naturally occurring g. i. nematode infection. Whereas, in susceptible sheep the Hb concentration and PCV (%) decreased significantly (P<0.05) in the month of September compared to other months as well as compared to the resistant group of Garole sheep under field condition.

Anaemia due to reduced Hb concentration and PCV (%) is a common occurrence in animals infected with



naturally occurring g.i. nematodes (Jas *et al.*, 2008). In susceptible sheep reduced Hb concentration and PCV (%) were recorded in the month of September when there was highest intensity of Strongyle group of nematodes (EPG= 857.14). Packed cell volume is generally considered as the marker for resistance to g. i. nematodes predominating with blood sucking nematode like *H. contortus* (Baker *et al.*, 2001) and in the present case *H. contortus* was the predominant species. In the resistant Garole sheep selected on the basis of consistently low FEC (mean EPG= 98.41) showed more or less stable PCV during the present study under natural exposure to g.i. nematodes and this finding was in agreement with other workers (Miller *et al.*, 1998; Bricarello *et al.*, 2002) who reported a stable PCV in sheep breed showing resistance to *H. contortus*. Therefore, the evidence of low FEC and a more or less stable PCV as recorded in some Garole sheep infected with naturally occurring g.i. nematodes, were sufficient to establish within breed resistance in Garole sheep (Ghosh *et al.*, 2012).

## CONCLUSION

Garole sheep, the most popular breed of West Bengal, exhibited within breed resistance to g.i. nematode infections in terms of FEC and PCV, the phenotypic indicator for resistance. Resistant line of Garole sheep could be utilized in the breeding programme for selection of parasite resistance after detail study on molecular and immunological mechanism responsible for such resistance in Garole sheep.

**Conflict of interest:** None.

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