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Research Article

Prevalence of gastrointestinal helminthoses in Garole sheep and selection of resistant and susceptible sheep using phenotypic indicator of gastrointestinal nematode resistance

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Abstract

The prevalence of naturally occurring gastrointestinal (GI) helminthoses in Garole sheep was determined in the present study with a view to select/identify resistant and susceptible Garole sheep. For the study, 45 Garole sheep of 4–12 months old of either sex were selected. Monthly per-rectal faecal samples were examined by both qualitative and quantitative methods, and coprocultural examination was done. The resistant and susceptible Garole sheep were selected on the basis of monthly faecal egg count (FEC) as an indicator of host resistance. The overall prevalence of GI helminthoses in Garole sheep was 63.70% with the highest prevalence of strongyle group of nematodes (57.40%). The prevalence (76.11%) as well as the intensity of infection (mean FEC = 432.22) was highest during the monsoon season. *Haemonchus contortus* (64.83%) was the predominant GI nematode species. The prevalence and intensity of GI helminth infection were higher in younger sheep compared to adult sheep. Seventeen (17) Garole sheep showed consistently low EPG ranging from 0 to 300 and those sheep were identified as resistant. About 45% of adult sheep and 32% of young sheep were identified as resistant based on their consistently low FEC (mean FEC<100). Rests of the 28 sheep were identified as susceptible as they exhibited a variable FEC ranging from 0 to 1200 EPG during the study period. The results of the present study could be exploited for strategic anthelmintic treatment in the farm. The resistance and susceptibility of Garole sheep, as observed in the present study, should be confirmed by further experimental studies.

Keywords: Garole sheep, Gastrointestinal helminths, Host resistance, Prevalence

Highlights

- Gastrointestinal helminth infection was a common occurrence in Garole sheep.
- *Haemonchus contortus* was the predominant GI nematode in Garole sheep.
- Garole sheep exhibited within-breed resistance to GI nematodes.
- A greater rate of host resistance was observed in adult sheep than in young sheep.

INTRODUCTION

Gastrointestinal helminth infection is an important constraint for optimum production in small ruminant livestock farming. Gastrointestinal helminths are widely prevalent all over the world (Khan *et al*., 2021; Tesfaye, 2021) including India (Jas *et al*., 2017a; Malathi *et al*., 2021). Out of different type of helminths parasite, nematodes are most prevalent in small ruminants in India (Allaie *et al*., 2018; Brahma *et al*., 2018). The habit and habitat of small ruminants including their rearing practice makes them highly susceptible to the GI helminth infection. Gastrointestinal parasitism is generally subclinical and causes huge economical losses to the farmers (Jas and Ghosh, 2009; Jas *et al*., 2017b).

Gastrointestinal helminths infections are mainly controlled by using anthelmintics. Indiscriminate use of anthelmintics among the livestock has resulted in the emergence of anthelmintic resistance all over the world (Peña-Espinoza *et al*., 2014; Mederos *et al*., 2018) including India (Rialch *et al*., 2013; Singh *et al*., 2017; Pawar *et al*., 2019). In recent years, research has been conducted to search for effective/sustainable control strategies against naturally occurring gastrointestinal helminthoses as an alternative to the anthelmintic treatment.

Use of vaccines (Smith, 2014), herbal anthelmintics (Liu *et al*., 2020), biological control by nematophagus fungi (Braga and de Araujo, 2014), grazing management (Maqbool *et al*., 2017), nutritional management (López-Leyva *et al*., 2022) and exploitation of host resistance (McRae *et al*., 2014; Emery *et al*., 2016) are the available alternative strategies to control GI nematode infection.

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Exploitation of host resistance is one of the most promising approaches for controlling GI helminths and, thereby, managing the problems of anthelmintic resistance (Andronicos *et al*., 2014; McRae *et al*., 2014; Brahma *et al*., 2022a). To develop the resistant animal population, first the resistant line of animals should be identified and selected based on the phenotypic indicators for resistance. Faecal egg count is the most important phenotypic indicator for host resistance against GI nematode (Karrow *et al*., 2014).

Garole sheep of West Bengal have been reported to have natural resistance against GI nematode infection (Ghosh *et al*., 2012; Brahma *et al*., 2022b). In the present study, faecal egg screening of GI nematode in Garole sheep maintained in semi-intensive system was done to determine prevalence and intensity of GI parasitic infection with a view to explore host resistance in Garole sheep and thereby selection of resistant and susceptible sheep population.

MATERIALS AND METHODS

Study area, duration and animal: The present study was conducted on Garole sheep in the farm of Germplasm Centre at West Bengal University of Animal and Fishery Sciences. Garole sheep of either sex and in the age group of 4-12 months were selected for the present study. The sheep in the said farm were maintained under the semi-intensive system and selected animals were identified by their neck tags. The Garole sheep in the age group of 4-6 months were considered as young animal (n = 25), and sheep from 7-12 months of age were considered as adult (n = 20). To determine the prevalence and intensity of naturally occurring GI helminth infection, the routine deworming schedule of the farm was withheld for the selected sheep, and the farm authority was assured to provide treatment if any emergent situation occurred during the study period. The present work was continued for one year covering all the 3 seasons (summer, monsoon, and winter) from March 2022 to February 2023.

Collection and examination of faecal samples: Per-rectal or freshly voided faecal samples were collected from all the selected Garole sheep at monthly intervals. About 3 g of faecal samples from individual Garole sheep were collected in collecting vials containing 10% formalin. Pooled samples consisting of faecal samples of all the Garole sheep were also collected at monthly intervals in polythene zipper bags for coproculture studies.

A part of the faecal sample was examined qualitatively using standard sedimentation and salt floatation techniques (Soulsby, 1982) to determine the presence or absence of helminthic eggs. Intensity of GI nematodes, particularly of strongyle group of nematodes, was determined by modified McMaster technique (Soulsby, 1982) in terms of eggs per gram of faeces (EPG).

Coproculture of pooled faecal samples was done at monthly intervals following the standard method (Soulsby, 1982) in a Biological Oxygen Demand (BOD) incubator at 27±1°C temperature and relative humidity ≥80% for 10 days and finally third stage infective larvae of strongyle group of nematodes were harvested. Harvested larvae were then identified using the following keys, as described by MAFF (1971). About 200 larvae of strongyle group of nematodes were counted, and percentage of different types of nematodes were determined.

RESULTS

In the present study, monthly prevalence of different gastrointestinal helminth parasites was recorded in Garole sheep maintained in semi-intensive system, and the overall prevalence was 63.70% (Table 1). All three types of helminth parasites; nematodes, trematodes and cestodes were recorded. The prevalence of strongyle group of nematodes was highest (57.40%). Paramphistome (5.37%) and *Moniezia* (9.09%) were the only trematode and cestode species respectively, recorded in the present study (Table 1).

Table 1. Monthly and seasonal prevalence of naturally occurring gastrointestinal helminthoses in Garole sheep

Months	Total examined	Total positive (%)	Strongyle (%)	*Strongyloides* sp. (%)	*Trichuris* sp. (%)	Paramphistome (%)	*Moniezia* sp. (%)
March	45	57.78	51.11	2.22	6.67	4.44	11.11
April	45	48.89	42.22	4.44	6.67	2.22	6.67
May	45	51.11	44.44	4.44	4.44	2.22	6.67
June	45	66.67	57.78	6.67	8.89	4.44	8.89
Summer	**180**	**56.11**	**48.89**	**4.44**	**6.67**	**3.33**	**8.33**
July	45	73.33	66.67	6.67	11.11	6.67	11.11
August	45	77.78	71.11	4.44	6.67	8.89	8.89

Cont. Table 1.

Table 1., Cont. ...

Months	Total examined	Total positive (%)	Strongyle (%)	*Strongyloides* sp. (%)	*Trichuris* sp. (%)	Paramphistome (%)	*Moniezia* sp. (%)
September	45	80.00	73.33	4.44	6.67	8.89	11.11
October	45	73.33	68.89	4.44	4.44	8.89	8.89
Monsoon	**180**	**76.11**	**70.00**	**5.00**	**7.22**	**8.33**	**10.00**
November	45	71.11	66.67	4.44	4.44	6.67	6.67
December	45	60.00	51.11	6.67	8.89	6.67	8.89
January	45	53.33	48.89	6.67	6.67	2.22	11.11
February	45	51.11	46.67	4.44	4.44	2.22	8.89
Winter	**180**	**58.89**	**53.33**	**5.56**	**5.56**	**4.44**	**8.89**
Overall	**540**	**63.70**	**57.40**	**5.00**	**6.48**	**5.37**	**9.09**

During monsoon season, the overall prevalence (76.11%) as well as the prevalence of strongyle group of nematodes (70.00%), Paramphistomes (8.33%), and *Moniezia* (10.00%) was highest followed by winter and lowest in summer (Table 1). As the prevalence of Strongyle group of nematodes was highest, the species composition of larvae of gastrointestinal nematodes was performed in the present study. About seven types of GI nematodes *viz.*, *Haemonchus* (64.83%), *Oesophagostomum* (15.58%), *Trichostrongylus* (8.63%), *Bunostomum* (3.21%), *Oestertagia* (2.92%), *Nematodirrus* (1.08%) and *Strongyloides* (3.71%) and out of these nematodes *Haemonchus contortus* was the predominant species infecting the Garole sheep throughout the year (Fig. 1). Out of 45 Garole sheep, 20 nos. were adult, and 25 sheep were young animals. The overall prevalence of naturally occurring GI helminths was comparatively higher in younger sheep (68.67%) than the adult sheep (57.50%). The strongyle group of nematodes was 60% in young Garole sheep, and it was 54.16% in adult sheep during the study period (Fig. 2).

The intensity of strongyle group of nematodes was measured in terms of EPG at monthly interval in all sheep. The overall intensity of infection was highest in the month of September (mean EPG = 501.11) and lowest in the month of May (mean EPG = 108.89) irrespective of the age of sheep (Fig. 3). The intensity of infection was highest during the monsoon season (mean EPG = 432.22) followed by winter (mean EPG = 183.61) and lowest in summer (mean EPG = 152.22) with an overall EPG of 256.02 (Fig. 3). In the adult sheep the mean FEC ranged from EPG = 75 (May) to 490 (September) with an overall mean EPG of 235.21 during the study period (Fig. 3).

Out of 20 adult sheep coprologically screened monthly, 9 Garole sheep showed consistently low or no faecal egg count (FEC) during the one year of study period, whereas, rest 11 sheep showed a variable FEC ranging from EPG = 0 to EPG = 1200. In 9 adult sheep, the mean FEC ranged from EPG = 33.33 to EPG = 88.89, whereas the mean EPG in the rest of the 11 adult sheep varied from 109.09 (May) to 818.18 (September). Therefore, those adult sheep (9) showing persistently low EPG were considered as resistant based on faecal egg count.

In the young sheep, the FEC was highest in the month of October (EPG = 566) and lowest at the month of January (EPG = 110) with an overall FEC of 272.67 (Fig. 4). Out of 25 young sheep coprologically screened at monthly intervals for one year, 8 Garole sheep showed consistently low EPG during the study period. The mean FEC of those 8 sheep ranged from EPG = 25 to EPG = 175, whereas the mean FEC of the 17 young sheep ranged from EPG = 135.29 to EPG = 794.12 (Fig. 4). Hence, those young sheep (8) were identified as resistant sheep based on their monthly FEC.

A total of 17 Garole sheep (young = 8; adult = 9) out of 45 sheep showed consistently low EPG with a mean FEC of 67.64 and the mean EPG ranged from 35.29 to 120.58 during the one year of study period. The FEC of those 17 Garole sheep ranged from 0 to 300 during the study period and even during the monsoon when the pasture contamination used to be very high. Therefore, those Garole sheep showing very low EPG in every month were identified as resistant based on FEC, the most important phenotypic marker of host resistance against GI nematodes. Rest 28 Garole sheep showed variable FEC ranging from mean EPG = 150 to 746.43 with an overall mean EPG of 371.57 (Fig. 3). Those sheep (n=28) showed low EPG during summer (mean EPG = 213.83) and winter (mean EPG = 265.18) but very high EPG during monsoon (mean EPG = 635.72). Therefore, 28 Garole sheep were identified as susceptible sheep based on FEC, the

Selection of GI nematode resistant Garole sheep using faecal egg count

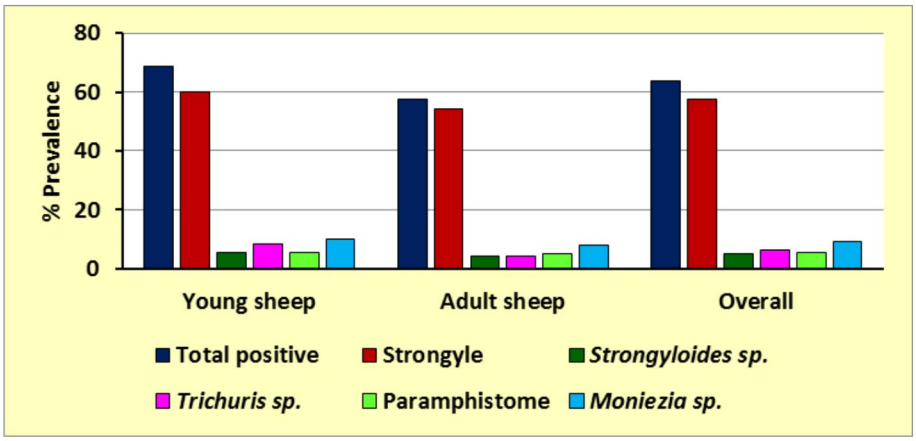

Fig. 1. Prevalence of naturally occurring gastrointestinal helminths in young and adult Garole sheep

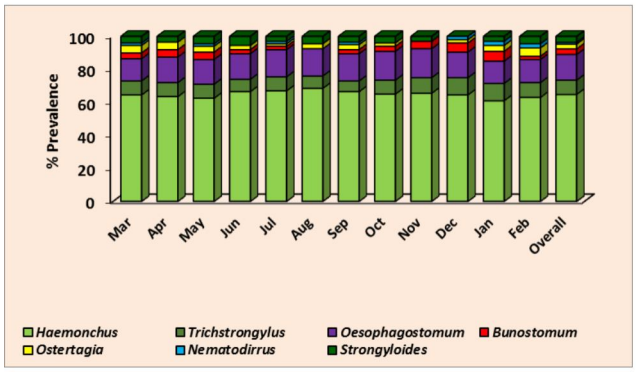

Fig. 2. Percent composition of different genera of Strongyle group of gastrointestinal nematodes in Garole sheep

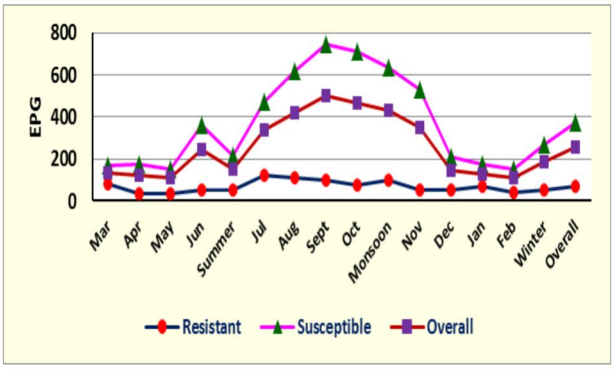

Fig. 3. Monthly and seasonal intensity of gastrointestinal nematode infection in resistant and susceptible Garole sheep

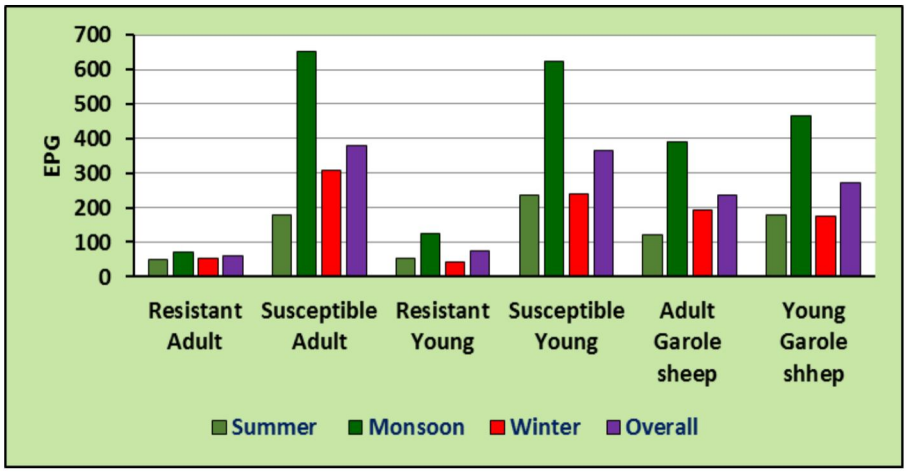

Fig. 4. Seasonal intensity of gastrointestinal nematode infection in resistant and susceptible Garole sheep of young and adult age-groups

phenotypic marker of GI nematodes resistance. In the present study, 37.78% of Garole sheep exhibited resistance to naturally occurring GI nematodes and adult sheep showed comparatively higher per cent of resistance (45%) than the young Garole sheep (32%).

DISCUSSION

The selected Garole sheep in the present study were maintained by semi-intensive system, and routine deworming practice was withheld during the study period. The overall prevalence of GI helminthoses was 63.70% in Garole sheep, and all the three types of helminth parasites including nematode, cestode and trematode were recorded. Naturally occurring GI helminthoses are common occurrence in sheep of India (Priyanka *et al*., 2020; Malathi *et al*., 2021), including Garole sheep of West Bengal (Brahma *et al*., 2015, 2018; Jas *et al*., 2017a).

In the present study, a high level of prevalence was recorded in Garole sheep which agreed with other studies conducted in Garole sheep (Brahma *et al*., 2017, 2018) and in other sheep of India (Khajuria *et al*., 2013; Singh *et al*., 2013; Jas *et al*., 2020) and in the world (Raza *et al*., 2014; Zvinorova *et al*., 2016). The environmental condition as well as the climatic condition of West Bengal is optimum all-round the year for development and survival of free-living stages of helminth parasites (Jas *et al*., 2017b).

More than 80% of the overall prevalence of GI helminthoses was observed in sheep of Haryana (Priyanka *et al*., 2020) and on the other hand, less than 30% of Tibetan sheep were found to be infected with GI helminths in Sikkim in India (Pal *et al*., 2021) and in sheep of Egypt (Mohamed *et al*., 2023). Very high and very low prevalence of GI helminths in contrast to the present study might be due to the difference in agroclimatic conditions, rearing practices, and sheep breed in different study locations both in India as well as in the world.

The prevalence and intensity of GI helminth infections were greatest during the monsoon season. Many authors from different parts of India have reported the highest prevalence of GI helminthic infections in the monsoon than in the summer and winter seasons (Khajuria *et al*., 2013; Brahma *et al*., 2018; Priyanka *et al*., 2020). The environmental factors, especially the temperature and humidity during the rainy season in India, particularly in West Bengal, are extremely favourable for the development and survival of the free-living stages of GI nematodes and optimum for the breeding of snails, intermediate host of trematodes parasites. On the other hand, high temperature and low humidity during summer and low temperature and less humidity during the winter season are not very favourable for survival and translocation of free-living larval stages of GI nematodes and thus result in low prevalence of GI helminth infection (Brahma *et al*., 2015; Kumar *et al*., 2021)

Prevalence of Strongyle group of nematodes was higher than the other types of parasites. Out of different types of Strongyle groups of nematodes, *Haemonchus* was recorded as the predominant species infecting Garole sheep. The highest prevalence of *Haemonchus* sp. has also been recorded earlier in the sheep and goats of India (Kumar *et al*., 2021) as well as in West Bengal (Brahma *et al*., 2015; Jas *et al*., 2020). *Haemonchus* is a highly prolific worm, and infective larvae of *Haemonchus* can survive in a wide range of environmental temperatures (18°C -34°C) and humidity (50% - 100%). The prevalence of GI helminth infection was higher in young sheep than in adult sheep. Greater rate of infection in young animal might be due to lower level of immunity as a result of lesser exposure to naturally occurring GI helminths compared to the adult sheep (Raza *et al*., 2014; Zvinorova *et al*., 2016).

The intensity of infection in terms of EPG of faeces was measured every month in all the sheep to detect the resistance of Garole sheep to naturally occurring GI nematodes. About 37.78% of Garole sheep selected in the present study showed consistently low EPG or no faecal egg, and those sheep were considered resistant to GI nematode infection, while the rest of the Garole sheep showed variable EPG ranging from low FEC to very high FEC. The feeding and rearing practice was similar for all the selected sheep, and therefore, the sheep showing low EPG persistently every month were considered as resistant to naturally occurring GI nematodes.

A high percentage of Garole sheep showing individual resistance or within-breed resistance has also been recorded earlier (Bordoloi *et al*., 2012; Ghosh *et al*., 2012; Brahma *et al*., 2022b). In the present study, animals were divided into young and adult based on their age group to determine whether there was any significant difference between adult and young Garole sheep showing resistance to naturally occurring GI nematode infection.

In the present study, about 45% of adult Garole sheep (9/20) showed consistently low EPG at every month during the study period. Whereas 32% of young Garole sheep (8/25) exhibit a resistance to GI nematode infections in terms of persistent low EPG. The percentage of resistant sheep was comparatively higher in the adult age group (45%) than in the younger sheep

(32%). Greater resistance in adult sheep might be due to prior sensitization to GI nematode infection (Raza *et al*., 2014; Zvinorova *et al*., 2016) and might also be due to the presence of antibodies or memory cells of previous infection as host immune response plays an important role in host resistance to GI nematode infection (González-Garduno *et al*., 2021).

In the present study, resistant Garole sheep consisting of young and adult age groups were identified based on the faecal egg count of GI nematodes, as the FEC is the most important and practical marker for the selection of resistant and susceptible animals (Saddiqi *et al*., 2010; Bishop, 2012). From the present study, it can also be hypothesized that age might play a role in host resistance in Garole sheep against naturally occurring GI nematodes.

Gastrointestinal helminthic infections were a common occurrence in Garole sheep around the year, with the highest prevalence and intensity in the monsoon season. *Haemonchus* was recorded as the predominant GI helminth in Garole sheep. Within breed resistance to GI nematode infection was observed in Garole sheep, and the rate of resistance was higher in adult sheep compared to young sheep. The host resistance as observed in Garole sheep should be explored involving larger number of sheep and be exploited in management of anthelmintic resistance.

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Authors contribution: RJ: Conceptualization, project administration, writing of original draft; AH, SD, SSK: Methodology and investigation; SP, SB, AM: Formal analysis and data curation; SCM: Review and editing. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability statement: The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Animal ethical permission: All the animals in the present study were humanely treated with standard care guidelines established by the Institutional Animal Ethics Committee (IAEC) of West Bengal University of Animal and Fishery Sciences, (Regd No. 763/GO/Re/SL/03/CCSEA) and the project proposal (No. 763/GO/Re/SL/03/35/2022- 23) was approved by the IAEC, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata 700037.

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