



Parasitological, Haemato-biochemical and Immunological Responses in Garole Sheep Naturally Infected with Gastrointestinal Nematodes Dominant by *Haemonchus contortus*

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ABSTRACT

The experiment was conducted during June–August, 2023 at South Gurguria village and farms under the project ‘Biotech Kishan Hub’, Mohanpur, West Bengal to study gastrointestinal nematodes response in Garole sheep. Faecal samples from 70 Garole sheep aged 1–2 years of either sex were examined for three months (June–August, 2023) and total 210 animals were examined. In every month, based on FEC, 33 sheep were categorized in to 3 groups; non-infective (n=11; EPG=0), highly infective (n=11; EPG>500) and low infective (n=11; EPG<200) group and haematological, biochemical, immunological and clinical parameters were recorded in sheep of all the three groups. Uninfected sheep had significantly ($p<0.01$) higher values of haemoglobin (Hb) and packed cell volume (PCV) than both the low and highly infected groups, while peripheral eosinophil count (PEC) and total leukocyte count (TLC) were significantly higher in low infected group. Serum protein concentration including albumin and globulin levels, was significantly ($p<0.01$) reduced in both infected groups. *Haemonchus contortus* specific serum IgA concentration was significantly higher in the low infected group but, serum IgG levels did not differ significantly between the groups. The body condition score (BCS) was found to be significantly ($p<0.01$) lower in the highly infected group than in the low infected group, which had significantly lower values compared to non-infected group. The results were exactly reversed in terms of FAMACHA score in the selected sheep. Gastrointestinal nematodes cause alterations in haematobiochemical profiles and also modulate immune response of the host; hence, these parasites should be managed properly.

KEYWORDS: Garole sheep, gastrointestinal nematodes, haematobiochemical, immunoglobulin

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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1. INTRODUCTION

In India, Sheep farming plays a pivotal role in the rural economy among marginal farmers and economically weaker section of the society. Garole sheep is a small-sized breed recognized for its prolificacy and model to the saline marshy land of the Sundarban region of West Bengal, India. This breed is maintained by means of marginal farmers and landless labourers, primarily reared for mutton production. But small ruminant farming is greatly affected by the gastrointestinal nematode (GIN) infections and it is a great concern worldwide (Miller et al., 2012), the impacts of parasitism on livestock production output have been acknowledged (Charlier et al., 2014). Gastro-intestinal parasitism ranks among the most prevalent infection in livestock, clinical symptoms and after effects rely on the parasite species present and the severity of the infection in animals (Charlier et al., 2014; Mavrot et al., 2015). Gastrointestinal parasitic infections impact livestock farming by not only influencing health but also leading to significant economic losses through decreased productivity and weight, retarded growth, cost of treatment, and mortality (Nwosu et al., 2007; Singh et al., 2015; Toscan et al., 2017). Strongylid gastrointestinal nematodes significantly contribute to disease and economic losses in small ruminants, as sheep and goats become infected while grazing (Zajac and Garza, 2020). In sheep, these can vary from unnoticed weight loss to fatal conditions like anaemia, diarrhoea, and significant protein depletion (Pugh and Baird, 2012). Moreover, parasitism may lead to indirect effects on metabolism, including the mobilization of proteins for immune responses, decreased feed consumption from anorexia, or heightened vulnerability to other pathogens (Moreau and Chauvin, 2010). Small ruminants are generally infected with different types of gastrointestinal helminths but the genera that are most frequently noted and have significant economic relevance for grazing sheep worldwide include *Haemonchus*, *Teladorsagia*, *Ostertagia*, *Trichostrongylus*, *Cooperia*, *Nematodirus*, *Oesophagostomum*, and *Chabertia* (Sutherland and Scott, 2010; Abbott et al., 2012), of which *Haemonchus contortus* is the most pathogenic and predominant in India (Jas et al., 2017; Brahma et al., 2018; Hembram et al., 2024; Das et al., 2025). Evaluation of animal health issues can be done through analyzing blood using haematological and biochemical tests (Chirkena et al., 2016), while infections from gastrointestinal parasites in sheep can result in changes in haematological values and serum proteins, particularly a decrease in serum albumin levels, as well as variations in serum globulin fractions (Fernandez et al., 2006; Bordoloi et al., 2012; Tiwary et al., 2017; Nagy et al., 2020). Generally, when any parasite enters in the host body, host immune responses become stimulated against that parasite which may be helpful to eliminate the

parasite from body of host by different immune mechanism. To develop a sustainable nematode control methods requires a solid understanding of the interaction and relationship between hosts and their parasites, especially the immune reaction (Stear et al., 2023). Increase in the serum antibody against a parasite or pathogen is characteristic feature of host immune responses which is important to become the host resistance or resilience against the pathogen (French et al., 2009; McRay et al., 2015; Escribano et al., 2019). The present study was conducted with the objectives to determine the haematobiochemical, immunological and clinical impacts of naturally occurring gastrointestinal nematodes dominated by *Haemonchus contortus* in Garole sheep maintained under semi-intensive system.

2. MATERIALS AND METHODS

2.1. Study area and animals

The experiment was conducted during the year 2023 at South Gurguria village, South 24 Paraganas (22.0866° N latitude and 88.5937° E longitude) and Farms under the project 'Biotech Kishan Hub', Mohanpur (22°56'42.88"N latitude and 88°32'0.86"E longitude), West Bengal. To study gastrointestinal response in Garole sheep, faecal samples of 70 Garole sheep of either sex in the age group of 1-2 years old, were examined for three months (June–August, 2023), with a total of 210 animals examined. The Garole sheep breed (Figure 1) was chosen for the study due to its popularity among small and marginal farmers in rural areas of West Bengal. This breed was found in the Coastal Saline Zone of West Bengal (Sunderban delta), there, the climate was humid and hot. Selected sheep of the village were reared by semi-intensive system and the animals were allowed to graze daily for 4 to 6 hours on pasture land and provided with *ad libitum* clean drinking water. Deworming practice were very negligible and irregular in that area. On



Figure 1: A flock of Garole Sheep grazing on naturally grown grass

the farm, under the Biotech Kisan-Hub, Garole sheep were also managed by semi-intensive system and deworming was performed at three to four months intervals. Throughout the study period, no deworming was practiced, and it was selectively administered only to those animals that essentially needed treatment.

2.2. Sample collection and examination

All the selected sheep were properly identified with neck tag numbers. Per-faecal faecal samples of all the sheep were collected at monthly intervals for three months, from June, 2023 to August, 2023. A part of faecal sample was examined qualitatively by sedimentation and salt flotation techniques (Soulsby, 1982), and subsequently, quantitative examination of GIN positive faecal samples was conducted by the McMaster technique to determine EPG (Soulsby, 1982). A sufficient amount of pooled samples, comprising faecal samples from all the selected sheep without any preservative added, was collected in a plastic zip lock bag for coproculture to harvest third stage infective larvae (L_3) for the identification of dominant species of nematodes. Coproculture was performed according to techniques to harvest fresh infective larvae (Anonymous, 2010), and the final harvested L_3 were washed and stored in PBS at 4°C. The larvae were identified microscopically using morphological keys (Van Wyk and Mayhew, 2013; Knoll et al., 2021). Based on faecal egg count (FEC) expressed as EPG in every month, 33 Garole sheep were selected and categorized into 3 equal groups; low infected group (EPG<200) and highly infected group (EPG>500) and non-infected group (EPG=0) for 3 occasions. Following FEC examination, 4 ml blood samples were collected from all the 33 sheep in every month and 2 ml of blood was kept in an EDTA vial while the remaining 2 ml was allowed to clot for serum separation to estimate hematological, biochemical and immunological parameters. Separated serum was transferred into a collection vial, stored at -20°C. The serum was used for ELISA and biochemical parameter studies.

2.3. Haematological parameters

Haemoglobin (Hb; gm dl⁻¹) was determined using the Cynethemoglobin method with Drabkin's solution (Pal, 2010). Packed cell volume (PCV; %) estimated by capillary microcentrifuge method (Wintrobe, 1961). Total Leukocytes Count (TLC; $\times 10^3$ mm⁻³) was estimated using haemocytometer according to standard procedures (Jain, 1993). Peripheral eosinophil count (PEC; no. μ l⁻¹) was performed in Neubauer chamber after staining with Carpentier solution as per Dawkins et al. (1989).

2.4. Biochemical parameters

Total serum protein, serum albumin, serum globulin and albumin/globulin ratio were estimated by utilizing

commercially available kits according to the kit's literature.

2.5. Immunological parameters

2.5.1. Preparation of *Haemonchus contortus* crude somatic antigen (CSAg-Hc)

Adult *Haemonchus contortus* around 300 numbers were collected from the abomasums of infected sheep slaughtered at the local abattoir in New Market, Kolkata. They were homogenized and sonicated in 10 ml of chilled 0.15M PBS (pH=7.2) by using the Ultra Turrax high performance dispenser equipped with S10N-5G IKA tissue homogenizer and sonicator (Staufen, Germany). The homogenized and sonicated materials were then centrifuged in a cold centrifuge machine (Hermile, Germany) at 4°C at 10000 rpm for 30 min. The supernatant was collected as crude somatic antigen of *Haemonchus contortus* (Jas et al., 2010). The antigens were filtered using syringe filter (0.02 μ m; Sartorius, Germany) and a protease Inhibition cocktail (Genetix, India) @ 10 μ l ml⁻¹ was added.

The protein concentration of parasite antigen was estimated (Lowry et al., 1951) and stored at -20°C until used.

2.5.2. ELISA for estimation of serum IgA and IgG

Serum concentrations of *H. contortus* specific immunoglobulins (IgG, and IgA) were measured in terms of optical density by using indirect enzyme linked immunosorbent assay (ELISA) (Bambou et al., 2008) with minor modifications against *Haemonchus contortus* crude somatic antigen (CSAg-Hc). Prior to use, the working dilutions of test serum, antigen and conjugate were determined by checkerboard titrations.

2.5.2.1. Assay procedure

Ninety-six well microtitre plates (Nunc, MaxiSorp, Denmark) were coated with 100 μ l of CSAg-Hc (5 μ g well⁻¹ in 50 mM carbonate buffer; pH 9.6) and incubated overnight at 4°C. On the next day, the wells were washed four times with a washing buffer containing PBS (0.15M, pH 7.2) with 0.05% Tween 20 (PBST). After thorough washing the uncoated sites of the wells were blocked by adding blocking buffer @ 200 μ l well⁻¹ containing PBST and 2% bovine serum albumin fraction IV (BSA). The plates were incubated at 37°C for 1.5 hr, followed by three washes with washing buffer.

For estimation of anti-*H. contortus* IgG, 100 μ l of serum sample (1:200 dilution) with blocking buffer was added to all wells for and incubated for 1.5 hours at 37°C, followed by four washes with washing buffer (PBST). After the washes, all test wells were treated with 100 μ l of 1:1000 diluted Donkey anti-sheep IgG-HRP-conjugate (Sigma Aldrich) and incubated for 1 hour at 37°C followed by four washes with PBST. Then, TMB substrate chromogen working

solution (90 μl well⁻¹) was added. The colour reaction was monitored in dark place for 10 to 15 minutes and reaction was stopped by adding 50 μl of 2 M sulfuric acid. The absorbance values were read in ELISA reader (LisaScan® EM, Emra, India) at 492 nm within 10 minutes. The test samples, positive and negative controls were also run in duplicate. The negative sample was serum collected from a one month-old lamb, and positive sample was 6 no's pooled serum sample of GINs positive sheep showing high OD value in preliminary study.

For estimation of *H. contortus* specific IgA, same procedure was followed as like IgG, except 1:100 dilution of serum sample and Rabbit anti-sheep IgA-HRP conjugate (Biorad, USA) at 1:1000 dilution were used.

The OD index (IgG and IgA) for each sample was estimated by the following formula (Hassan Basri, 2019):

$$\text{OD index} = \frac{\text{Test sample-Negative control}}{\text{Positive control-Negative control}}$$

2.6. FAMACHA score

The FAMACHA® chart was a tool for monitoring anaemic animals due to parasitic infections, it involved the comparison of the colour of the eye conjunctiva, an indirect measurement of parasitic load (Burke et al., 2007). Each animal of all the three groups was graded as 1 to 5 based on the colour of their conjunctival mucous membrane. Category 1 was red for non-anaemic animals, category 2 was red-pink for non-anaemic animals, category 3 was pink for mildly anaemic animals, category 4 was pink-white for anaemic animals, and category 5 was white for severely anaemic animals. During the study period, only category 5 received anthelmintic treatment.

2.7. Body condition score

Body condition score was assessed through physical observation and by handling of the animal (Phythian et al., 2012). The body condition score was graded by observing normal activity, lack of soiling, and any physical abnormalities, along with applying firm pressure on the rib areas and running the fingers along the spine from the shoulders to tail head of the selected Garole sheep. The body condition score ranged from 1 to 4 in the selected sheep, score 1 was very lean, 2 was lean, 3 was good condition, and 4 was fat.

2.8. Statistical analysis

To obtain the value of standard error (S.E.) along with mean value all the parameters for each group were compared by Analyse-Compare means. The significance (p -value) was recorded at 5% ($p<0.05$) level and 1% ($p<0.01$) level by separate analysis of parameters using Duncan method (One-way- ANOVA). The complete statistical analyses

were performed with the help of Statistical Package for Social Scientist (SPSS), Windows Version 20.0.

3. RESULTS AND DISCUSSION

3.1. Faecal examination, coproculture and dominant strongyle larvae group

Faecal examination in study animals revealed that 77% of the animals were comparatively highly infected, with more than 500 faecal egg count (FEC) in terms of eggs gram⁻¹ (EPG) of faeces, while 23% sheep showed comparatively FEC, with EPG<200 during the study period. Coproculture studies for larval identification indicated that *H. contortus* (Figure 2) was the dominant gastrointestinal nematode, accounting for 63% larval population during the study period followed by *Trichostrongylus* spp. Brahma et al. (2022) and Hembram et al. (2024) also found that *Haemonchus contortus* was the dominant strongyle species infecting in Garole sheep. Das et al. (2025) similarly reported that *Haemonchus contortus* was the most predominant gastrointestinal nematode in Black Bengal goats in West Bengal, India.



Figure 2: Identified dominants *Haemonchus contortus* L₃ in pooled coproculture

3.2. Haematological parameters

Significant differences in haematological values among all three groups were observed (Table 1). There was reduction in Hb and PCV values in both the low and highly infected groups compared to non-infected group. Mean Hb and PCV values in low infected group were significantly ($p<0.01$) greater than the highly infected group (Table 1). In low infected group the TLC number was higher ($p<0.01$) compared to both high and non-infected groups (Table 1). Mild eosinophilia was observed in low infected group compared to highly infected group and non-infected group in the present study.

Gastrointestinal parasitic infections caused by nematode parasites have been reported to result in reduced

Table 1: Alteration in haematological values in different groups of Garole sheep infected with GI nematodes

Parameters	Highly infected group	Low infected group	Non-infected group	<i>p</i> value
Hb (g dl ⁻¹)	9.061 ^z ±0.147	10.894 ^y ±0.116	13.366 ^x ±0.314	0.000
PCV (%)	26.560 ^z ±0.328	29.575 ^y ±0.266	33.712 ^x ±0.236	0.000
TLC (x10 ³ cm m ⁻¹)	8.948 ^y ±0.100	10.951 ^x ±0.685	8.130 ^y ±0.192	0.000
PEC (No. µl ⁻¹)	39.545 ^z ±2.533	107.757 ^x ±9.112	78.636 ^y ±5.160	0.000

Values bearing superscripts x, y in a row differs significantly (*p*<0.05)

haematological values in small ruminants (Baihaqi et al., 2020). In the present study too, Garole sheep of both the infected groups showed reduced Hb and PCV values compared to the non-infected group. The study identified *Haemonchus contortus*, a blood sucking nematode responsible for severe anaemia in infected animals (Bricarello et al., 2002). The low infected group of sheep had significantly (*p*<0.01) higher values of Hb and PCV compared to the highly infected sheep because the FEC (EPG) is indirectly related to the worm burden. Total leucocyte counts and peripheral eosinophil counts were significantly (*p*<0.01) higher in the low infected group compared to the non-infected and highly infected groups. *H. contortus* infected sheep exhibited significant reductions in hemoglobin, hematocrit, corpuscular volume, and red blood cell counts (Fernandes et al., 2022; Malede et al., 2025). In GI parasitic infections, increased value of TLC value had been recorded earlier (Aboshady et al., 2020). The increased TLC value was primarily due to immune response of the host to the GI nematodes. Eosinophils were the primary cells that combat parasites, especially non-phagocytizable organisms such as helminths (Behm and Ovington, 2020). Fernandes et al. (2022) reported increased eosinophils and platelets numbers in haemonchosis. Eosinophilia has been extensively recorded in animals infected with *H. contortus* and other nematodes, showing a strong correlation with protection (Huang and Appleton, 2016). Eosinophils were negatively correlated with faecal egg count output (Dawkins et al., 1989). A higher eosinophil count in low infected animals might be due to stronger immune response compared to the highly infected sheep in which immune response might be depressed/exhausted due to the stress of parasites.

3.3. Biochemical parameters

Total protein concentration including serum albumin and

globulin values and the albumin to globulin ratio, was higher (*p*<0.01) in non-infected group compared to both the low infected and highly infected groups in the present study (Table 2). No significant (*p*>0.05) difference in serum protein along with serum albumin and globulin, and the albumin to globulin ratio was recorded between the highly infected and low infected groups (Table 2).

In GI nematode infections, protein losing enteropathy (Soulsby, 1982) leads to loss of serum protein particularly serum albumin due to its small size in infected sheep and goats (Jas et al., 2008; Bordoloi et al., 2011). In the present study, hypoproteinaemia, along with decreased concentrations of albumin and globulin has been observed in sheep of both the infected groups. In chronic and clinical haemonchosis cases, hypoproteinemia was the most common condition (Soulsby, 1982) arising from the loss of total protein and albumin through bite wounds of *Haemonchus contortus* as well as catabolism of protein. Reductions in total protein and albumin are well documented in *Haemonchus contortus* infections due to blood loss, haemorrhagic gastritis and increased permeability of mucosa leading to protein leakage from the gastric mucosa. Reductions in total protein, albumin, globulin, and albumin-to-globulin ratio in sheep infected with *Haemonchus contortus* were reported (Alam et al., 2020; Malede et al., 2025). Haemonchosis significantly negatively impacts on serum protein concentrations, as well as macro and micro mineral levels in infected animals (Bordoloi et al., 2011). *Haemonchus contortus* was found to be the predominant nematode species in the present study; therefore, the hypoproteinaemia as detected in this present study was quite reasonable. No significant differences were observed between the two infected groups and this might be due to the fact that low infected sheep may chronically infected with low levels of helminths causing continuous

Table 2: Variation in serum biochemical parameters in different groups of garole sheep infected with GI nematodes

Parameters	Highly infected group	Low infected group	Non-infected group	<i>p</i> value
Total protein (g dl ⁻¹)	5.773 ^y ±0.091	6.007 ^y ±0.099	7.361 ^x ±0.110	0.000
Albumin (g dl ⁻¹)	2.959 ^y ±0.048	3.094 ^y ±0.051	3.967 ^x ±0.073	0.000
Globulin (g dl ⁻¹)	2.814 ^y ±0.071	2.913 ^y ±0.073	3.393 ^x ±0.096	0.000
Albumin/Globulin	1.070 ^y ±0.030	1.079 ^y ±0.029	1.214 ^x ±0.056	0.024

Values bearing superscripts x, y in a row differs significantly (*p*<0.05)

drainage of protein through the damaged gastrointestinal tract. Decreases in PCV, Hb and albumin levels, which were directly proportional to the nematode infection intensity, were reported earlier (Ahmad and Ansari, 1989).

3.4. Immunological parameters

In this study, immunoglobulins (Ig) concentrations were measured in terms of OD values between the highly infected and low infected groups. There was no significant difference in serum IgG activity between the highly infected and low infected groups but the concentration of IgA was found to be higher ($p<0.05$) in low infected group than highly infected group (Table 3).

Table 3: Alteration in serum IgG and IgA concentration between the highly and low infective groups of sheep

Parameters	Highly infected group	Low infected group	p value
IgG	0.281±0.022	0.292±0.027	0.757
IgA	0.208 [±] 0.035	0.337 [±] 0.033	0.01

Values bearing superscripts x, y in a row differs significantly ($p<0.05$)

Gastrointestinal nematode infection can prompt plasma cells to produce higher levels of parasite-specific antibody isotypes IgG, IgA, and IgE during secondary infections compared to primary infections, occurring earlier and in larger amounts (Lacroux et al., 2006). Serum IgA concentration was found to be significantly ($p<0.05$) higher in the low infected group compared to the high infected group, which might be due to strong immune response as mentioned earlier. Several studies have demonstrated increased levels of IgA, IgE, and antigen-specific IgG in *H. contortus* infections (Schallig et al., 1994; Meeusen et al., 2005). Higher levels of serum IgA in resistant sheep were observed in gastrointestinal parasitic infections (Mackinnon et al., 2010), but conflicting results were reported by some researchers who found no differences in IgA levels (Amarante et al., 2005; Shakya et al., 2009). Increased levels of IgA and IgG were demonstrated during *H. contortus* infections in sheep (Sinski et al., 1995; Brahma et al., 2023; Lalramhluna et al., 2020). Alam et al. (2020) found that *H. contortus* infection markedly lowered the serum IgG but elevated serum IgE levels in sheep.

3.5. Clinical observations

FAMACHA score was significantly ($p<0.01$) lower in the sheep of control group than the infected sheep and the score was significantly ($p<0.01$) higher in highly infected group compared to the low infected group throughout the study period. Highly infected animals appeared depressed and somewhat lethargic with depressed body conditions. The body condition score revealed that low infected animals

had better ($p<0.01$) body condition compared to highly infected group (Table 4). In non- infected group, the body condition score was significantly ($p<0.01$) better than the two infected groups of sheep. *H. contortus* infections in sheep and goats caused microcytic normochromic anemia (Malede et al., 2025).

Table 4: Variation in clinical parameters in different groups of Garole sheep infected with GI nematodes

Para- meters	Highly infected group	Low infected group	Non- infected group	p value
FAM- ACHA score	2.939 [±] 0.130	2.272 [±] 0.089	1.333 [±] 0.083	0.000
BCS	1.818 [±] 0.091	2.303 [±] 0.092	3.181 [±] 0.080	0.000

Values bearing superscripts x, y in a row differs significantly ($p<0.05$)

In the present study, significantly low BCS was recorded in highly infected group suggesting that GIN had direct effect on body condition and growth rate. Abosse et al. (2022) reported reduction in body weight in animals infected with *H. contortus*, who were anemic (Alam et al., 2020). Body Condition Score is a subjective way to evaluate the status of a sheep flock and a key tool in the on-farm assessment and management, as well as a good indicator of animal welfare (Phythian et al., 2012; Morgan-Davies et al., 2008). There was negative energy balance in the infected group, leading to significant variation in BCS in the highly infected animals. In the present study, the FAMACHA score was directly correlated with the PCV and Hb of infected groups and animals showing high FAMACHA score were given anthelmintic drugs for GIN control.

4. CONCLUSION

GIN infection caused alterations in various physiological parameters, leading to imbalances in the haematobiochemical profile. Eosinophils and IgA reduced infection intensity. Body condition was affected due to GIN infection. FAMACHA score could be used strategically to exploit anthelmintic drugs in managing resistance.

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