RESEARCH ARTICLE

Immunological Response of Black Bengal Goats to *Haemonchus contortus* Dominant Acquired Gastrointestinal Nematode Infection

Supradip Das[1]*, Ananta Hembram[1], Ruma Jas[1], Anupam Brahma[2], Shyam Sundar Kesh[3], Santanu Bera[4]

Abstract

Goat farming is severely affected by gastrointestinal nematodes (GINs) infection, which is most commonly prevalent and leads to huge production and economic losses. In the present study, total 261 Black Bengal goats, in the age group of 6 months to 2 years, were coprologically screened by flotation technique. The faecal egg count (FEC) per gram (EPG) of faeces was estimated by modified McMaster Technique and pooled faecal samples were cultured to identify dominant GINs. Based on FEC, 90 goats were categorized in to 3 groups; highly infected (EPG > 600), low infected (EPG < 200), non-infected (EPG=0) group and blood samples from selected animals were collected for estimation of haemato-biochemical and immunological parameters. Results revealed that *H. contortus* was dominant nematode from coproculture with 58% L_3 population. The highly infected group had significantly ($p<0.01$) lower values of Hb, PCV, PEC than the low infective and non-infective groups. Serum protein, albumin and globulin concentrations were reduced significantly ($p<0.01$) in both the infected groups compared to the non-infected group. FAMACHA score was found to be significantly ($p<0.01$) higher in the highly infected group than the low infected group. The *H. contortus* specific serum IgA values were comparatively higher than IgG values ($p<0.01$) in highly infected group than low infected group. Study revealed that GINs infections cause alterations in haemato-biochemical profile leading to development of pathological condition. IgG may be crucial in lowering the intensity of GIN infection in goats, but research is needed to determine the role IgA in GIN immunity in goats, as it was higher in the group with the highest infection level.

Key words: Gastrointestinal nematodes, Goats, Haemato-biochemical, *Haemonchus contortus,* Immunological.

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Introduction

In India, goat rearing is essential for the rural economy among marginal farmers and economically weaker populations. The Black Bengal goat is a popular breed among small farmers in West Bengal and equally present in neighbouring states, including Bangladesh. They are favoured for their high fertility, quick maturity, and tasty meat. These goats are resistant to diseases and can adapt to new environment easily. They graze on rice fields after harvesting and on roadside grass. However, small ruminant farming is significantly affected by Gastrointestinal nematodes (GINs) infections. GINs are most important parasites for small ruminants worldwide, causing economic issues and health concerns, by decreasing production, raising treatment expenses, slowing growth rates and posing risks of animal mortality (Miller and Horohov, 2006; Jas and Ghosh 2009).

Many farmers are unaware of the economic losses due to GINs infections and regular deworming is lacking for management of GINs in the goats. Despite the fact that goats and sheep share mostly the same species of GINs, there are less studies on goat GINs than on sheep (Baker and Gray, 2004). Physiological and pathological conditions of the animals can be evaluated through haematological and biochemical analysis (Chirkena *et al*., 2016). Still, there is limited understanding of the molecular processes involved in how goats respond to GINs infections. It is necessary to study such response in Black Bengal goats for improved management and treatment strategies. This study was

[1]Department of Veterinary Parasitology, West Bengal University of Animal and Fishery Sciences, West Bengal, Kolkata-700037, India.

[2]Department of Veterinary Parasitology, Faculty of Veterinary and Animal Sciences, RGSC, Banaras Hindu University, Mirzapur, Uttar Pradesh-231307, India

[3]Department of Veterinary Clinical Complex, West Bengal University of Animal and Fishery Sciences, West Bengal, Kolkata-700037, India

[4]Department of Animal Production Management, West Bengal University of Animal and Fishery Sciences, West Bengal, Kolkata-700037, India

Corresponding Author: Supradip Das, Department of Veterinary Parasitology, West Bengal University of Animal and Fishery Sciences, West Bengal, Kolkata-700037, India. e-mail: drdipdas88@gmail.com

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therefore aimed to investigate the host response of Black Bengal goats to naturally infected GINs by assessing various haemato-biochemical and immunological parameters.

Materials and Methods

The present study was conducted at the Department of Veterinary Parasitology, West Bengal University of Animal and Fishery Sciences, West Bengal, Kolkata, India, involving the Kishan Biotech Hub farms, Mohanpur and Joynagar, 24 South Paragonas. The goats were maintained in semi-intensive system and were allowed to graze every day for 4-6 h on pasture land throughout the study periods, and provided with limited concentrated pelleted ration on return to farm with free access to *ad libitum* clean drinking water. During the three months of the monsoon season, from July 2023 to September 2023, samples were taken from the animals once a month.

Sample Collection

All the study animals were properly identified with neck tag number. Faecal samples from all animals were collected directly from the rectum and kept in 10 % formalin in plastic vials to determine the FEC. Sufficient amount of faecal samples were pooled in plastic bag for copro-culture to enumerate the dominant infecting nematode genus. A total of 261 Black Bengal goats ranging in age from 6 months to 2 years were studied; each month, 87 new individual goats were added. Based on EPG, 30 goats were selected every month and categorized in to 3 groups; 10 animals in each group as low infected group (EPG ≤200), high infected group (EPG ≥600) and non-infected group (EPG =0) making a total of 90 in 3 months period.

4 mL blood was collected from selected animals via jugular vein puncture, 2 mL in EDTA vial for hematological parameters and 2 mL in serum vials for measuring serum immunoglobulin (IgA & IgG) and biochemical parameters.

Faecal Sample Examination

Collected faecal samples of all animals were individually examined by Flotation method with saturated salt solution for presence of nematode eggs as qualitative data and then positive samples were examined by Modified McMaster technique (Soulsby, 1982) for quantitative data of FEC in terms egg per gram (EPG) of faeces for determination of GINs burden in the infected goats.

Copro-Culture for Infective Larvae for Identification of Dominant GIN Genus

Copro-culture was performed on the positive pooled collected faeces as per standard technique to harvest fresh L_3 larvae as described by Ministry of Agriculture, Fisheries and Food of Great Britain, MAFF (1986). Every cultured recovered L_3 was washed and stored at 4°C in PBS (pH 7.2), which were then examined microscopically for identification of dominant GINs up to genus level by utilizing morphological keys of Van Wyk and Mayhew (2013).

FAMACHA Score

The FAMACHA© chart was used for monitoring parasites that involves comparing the color of the eye conjunctiva with fecal egg count (an indirect measure of parasitic load) (Kaplan *et al.,* 2004) in selected low and high infected Black Bengal goat groups. Animals were grouped into categories 1 to 5 based on the color of their eye mucus membranes.

Haemato-Biochemical Parameters

Haematological parameters included haemogloblin, packed cell volume, and peripheral eosinophils count estimated from selected goats. Haemoglobulin was determined by Cyanmethemoglobin method with Drabkin's solution (Pal, 2006). PCV was estimated by micro-centrifuge method with capillary tubes as per standard method. Peripheral eosinophils counts were determined with Carpentier's solution (Dawkins *et al*., 1989) and expressed as numbers presented per µL of blood. Biochemical parameters included serum total protein, albumin, globulin estimated by using commercially available kits (Autospan, Arkray healthcare, India) as per manufacturer's instructions.

Immunological Parameters

Preparation *H. contortus* Antigens: As copro-culture revealed that *H. contortus* was dominant pathogenic nematode in the infected goats. So*, H. contortus* specific immunoglobulin IgA and IgG in serum were estimated in low and high infective group only aganist *H. contortus* crude somatic antigens by Indirect ELISA. Adult *H. contortus* were isolated from fresh abomasum of goats collected from the local slaughter house. Approximately, 250 worms were crushed and processed in 10 mL of cold 0.15M PBS (pH-7.2) using the Ultra Turrax (S10N-5G IKA, Staufen, Germany) high performance dispenser. The homogenized mixture was kept on ice for 30 min, then centrifuged at 14331.31 x g for 30 min at 4°C in a cooling centrifuge machine (Hermile, Germany). The resulting supernatant was collected as the crude somatic antigen of *H. contortus* (Ag-Hc). Protease Inhibition cocktail (Genetix, India) was added @ 10 µL/mL in the crude somatic antigens (Ag-Hc) and kept at -20°C. The parasites' antigenic protein concentration measured using the Lawry method was found to be 2.17 mg/mL.

Indirect ELISA for IgA and IgG: The activities of *H. contortus* specific serum IgG and IgA were measured by an indirect enzyme linked immunosorbent assay (ELISA) as described by Bambou *et al.* (2008) with minor modification. Prior to use the working dilutions of conjugate, antigen and test plasma were determined by checker board titrations. 96 flat bottom microtitre plates (Trueline, USA) were coated with 100 µL of adult *H. contortus* Ag-Hc in 50 mM carbonate buffer (pH 9.6) at 5 µg/well and left overnight at 4°C. The following day, wells were washed four times with PBS

(Phosphate buffer saline, 0.15M, pH 7.2) containing 0.05% Tween 20. Then 200 µL of blocking buffer with 1.5 % bovine serum albumin (BSA) in PBS-T (PBS containing 0.05% Tween 20) was added to each well and incubated at 37°C for 1.5 h. Following, 3 washing, 100 µL of plasma sample, diluted 1:200 with blocking buffer, was added to each well and incubated for 1.5 h at 37°C. Then after three washes, 100 µL of 1:1000 diluted rabbit anti-goat IgG HRP-conjugate (Genei, Bangalore, India) was added to all test wells and incubated at 37°C for 2 h, then washed four times with PBS-T. Lastly, 90 µL of TMB (3,3',5,5'-Tetramethylbenzidine) substrate (Himedia, India) chromogen working solution was added, and the color reaction was observed in a dark area for 10 to 15 min. The reaction was stopped by adding 50 µL of 2 M sulfuric acid. The LisaScan® EM ELISA reader (Emra, India) was utilized to record absorbance readings at 492 nm within 10 min. During the study, a positive control was created by combining plasma from five goats with the highest IgG levels from the preliminary studies, while neonatal goat serum served as the negative control. All the samples were tested in duplicates. The method used to estimate *H. contortus* specific IgA was similar to IgG, except that it involved a plasma dilution of 1:100 and rabbit anti-sheep IgA HRP-conjugate (Biorad, USA Catalog no: AHP949P) dilution of 1:500.

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

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

Statistical Analysis

Data obtained from the study were tabulated and statistically analyzed by one way ANOVA using SPSS software version 21. The significant mean differences between groups were tested by Duncan's post-hoc analysis at $p<0.05$.

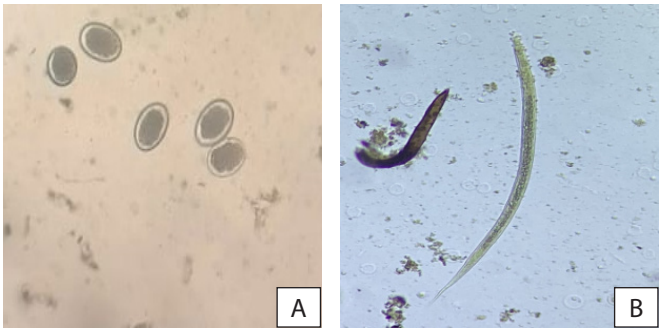

Fig. 1: (A) Strongyle eggs found in goats faecal sample; (B) *H. contortus* L_3 recovered from copro-culture.

Results and Discussion

The qualitative faecal examination revealed overall GINs prevalence (Fig.1A) as 78.16 % (204/261) and in the months of September GINs infection was highest with 81.60% (Table 1). Quantitative data in terms of FEC for indirect determination of GINs burden in the infected goats revealed that 24.50% goats were highly infective with FEC over 600 per gram of faeces, and highest FEC was 3500 per gram of faeces (that was excluded). In copro-cultured recovered L_3 (pooled) larvae *Haemonchus contortus* L_3 (Fig. 1B) was dominant nematode infecting the goats with 58% larval population.

Table 1: Prevalence of GINs in Black Bengal goats

Months	No. examined	No. positive	% positive
July	87	65	74.71
August	87	68	78.16
Sept	87	71	81.60
Overall	**261**	**204**	**78.16**

Haemato-Biochemical Parameters and FAMACHA Score

The haemato-biochemical parameters and FAMACHA score data of the study are presented in the Table 2. In high EPG group, Hb and PCV values were significantly ($p<0.001$) lower than in low EPG group. Peripheral eosinophils in low EPG group was comparatively more (55.83±1.75/µL) than in high EPG group (20.80±0.77/µL) and non-infected group (32.80 ±1.04/µL). FAMACHA score was more in high EPG group than in low EPG and non-infective groups. From the present result, it is clear that GINs infecting goats have significant effect on haematoloical parameters leading to visible change in normal physiological activity and it may be correlated with FAMACHA score as high infected group showed anaemic signs by pale conjunctiva. Due to its haematophagus nature, *H. contortus* causes haemorrhagic gastritis, blood loss, and increased permeability of the mucosa in the abomasum, which allows protein leakage from mucosa (Soulsby, 1982; Palmar *et al*., 2019). Among GINs *H. contortus* may be the responsible strongyle for low value of Hb and PCV in infected animals (Sharma *et al*., 2000). Decrease in Hb and PCV concentration in helminths infected small ruminants was reported by several authors (Bordoloi *et al*., 2012; Ahmed *et al*., 2015).

The biochemical study showed that the total serum protein value (g/dL) was lower in high EPG group (2.90±0.05) compared to low EPG group (3.47±0.06) goats. Albumin value was also significantly ($p<0.001$) lower in high EPG group (2.93±0.06) compared to low EPG group (3.24±0.06). Serum globulin value was mildly high in high EPG group compared to low EPG group. The changes in biochemical parameters in low and high EPG groups compared to non-infected group may be due to loss of total protein and albumin through bite wounds of *Haemonchus contortus* from abomasum mucosa (Soulsby, 1982). A marked reduction in serum albumin value as well as decrease in PCV, Hb and RBC values are directly proportional to infection intensity of the nematodes (Ahmad and Ansari., 1989). From this finding, it is easy to say that animals severely infected with GINs may show clinical signs like anaemia, edema, stunted growth, loss of body weight and some time mortality.

Table 2: Mean ± SEM haemato-biochemical and FAMACHA data of GINs infected and non-infected goats

Parameters	Highly infected group (EPG >600)	Low infected group (EPG <200)	Non infected (EPG =0)	P value
Hb (g/dL)	8.77^{z}±0.17	9.88^{y}±0.19	11.68^{x}±0.21	0.000
PCV (%)	25.85^{z}±0.45	29.38^{y}±0.29	33.42^{x}±0.25	0.000
Eosinophils (/µL)	20.80^{z}±0.77	55.83^{x}±1.75	32.80^{y}±1.04	0.000
FAMACHA (1 to 5)	3.17^{x}±0.13	2.43^{y}±0.09	1.57^{z}±0.10	0.000
Total protein (g/dL)	5.84^{z}±0.10	6.71^{y}±0.10	7.55^{x}±0.10	0.000
Albumin (g/dL)	2.90^{z}±0.05	3.47^{y}±0.06	3.95^{x}±0.05	0.000
Globulin (g/dL)	2.93^{z}±0.06	3.24^{y}±0.06	3.60^{x}±0.07	0.000
A:G ratio	1.00^{z}±0.02	1.10^{y}±0.03	1.10^{x}±0.02	0.003

Immunological Parameters

In this study, IgA responses (in terms of OD value) measured in high infective and low infective groups were different against *H. contortus* antigens. In high infective group, IgA value was significantly ($p<0.001$) more (0.53±0.01) than in low infective group (0.46±0.02), while. IgG value was significantly ($p<0.001$) more in low infective (0.28±0.01) group than in high infective group (0.21±0.01). This immunological data showed that both IgG and IgA responded differently in Black Bengal goats against GINs infection.

Our findings supported some previous findings that high parasite-specific IgA responses were associated with high FEC in goats (de la Chevrotière *et al*., 2012), but in sheep strong parasite-specific IgA responses were associated with decreased faecal egg counts (FEC) (Amarante *et al*., 2005). Indeed, a high IgA response was associated with high FEC in Scottish Cashmere goats that were selectively bred for nematode resistance (McBean *et al*., 2016). Present findings of immunoglobulin responses against GINs in Black Bengal goats suggest that IgA response to GINs in Black Bengal goats is more active than IgG response with high worm burden. In this context, parasite specific IgG value is more in low EPG group compared to high EPG group suggesting that in goat parasite specific IgG is important to control the parasitic load. IgG role against GINs infection is less clear than the role of IgA and IgE in animals (Sweeney *et al*., 2016), but some IgG responses were seen in sheep infection with different GINs including *H. contortus* (Cardia *et al*., 2011; Pernthaner *et al*., 2006). This difference highlights the need for further exploration of the host-parasite interaction in goats in order to understand their immune response against gastrointestinal nematodes infection and determine their resistant status as well as control strategies against GINs.

Conclusion

It is concluded that the haemato-biochemical parameters were altered in goats with gastrointestinal nematodes; therefore, those could be used as an important diagnostic tools to assess health and disease in goats suffering from gastrointestinal nematodes. Goats with high parasitic load show better IgA response compared to low infective, but high value of IgG in low infective GINs goats suggest that IgG is responsible to control GINs in Black Bengal goats. Further investigation is necessary to examine the response of Black Bengal goats to GINs for better understanding to improve management practices and control strategies.

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