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Analysis of gastrointestinal parasitic impact on goats: insights into infection rates, hematological and biochemical changes, and oxidative stress responses

Varun Kumar Sarkar, Rajashekar Kamalla, Pooja Solanki, Shivansh Mehra, Ujjwal Kumar De, Devendra Prasad Pateer, Harshit Saxena and Sakshi

Varun Kumar Sarkar
Division of Medicine, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

Rajashekar Kamalla
Division of Medicine, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

Pooja Solanki
Division of Medicine, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

Shivansh Mehra
Division of Surgery, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

Ujjwal Kumar De
Division of Medicine, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

Devendra Prasad Pateer
Division of Parasitology, ICAR- Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

Harshit Saxena
Division of Medicine, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

Sakshi
Division of Medicine, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

Corresponding Author:
Varun Kumar Sarkar
Division of Medicine, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

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Abstract
This study investigates the impact of gastrointestinal parasites on the health of goats through an analysis of mixed parasite infestation rates, haematological changes, biochemical alterations, and oxidative stress indices. The research, conducted at the Indian Veterinary Research Institute in Bareilly, India, involved the examination of 112 faecal samples from goats with diarrhoea. The blood and serum sample were drawn from 25 parasitic-infected goats and from 10 healthy goats. The results revealed a significant endoparasite infection rate of 76.7%, with mixed infections accounting for 60.4%. Haematological analysis indicated a decline in red blood cell count, hemoglobin levels, and packed cell volume, suggesting potential anaemia. Biochemical profiling revealed lowered total protein and albumin levels, indicating potential malnutrition or malabsorption due to parasitic infection. Oxidative stress markers, including elevated nitrate and malondialdehyde concentrations, suggested systemic inflammation and increased lipid peroxidation in affected goats. These findings highlight the multifaceted impact of gastrointestinal parasites on goat health and provide insights into the physiological and biochemical alterations associated with parasitic infections.

Keywords: Diarrhoea, oxidative stress, gastrointestinal parasite, hematobiochemical alteration

Introduction
Diarrheal diseases in animals, caused by parasites remain a significant source of illness and death in the developing world. In young ruminants, the occurrence of diarrhoea in lambs and kids contributes substantially to both morbidity and mortality. The syndrome's causative factors encompass a range of pathogens, with protozoan and helminth parasites such as *Haemonchus contortus*, *Ostertagia circumcincta*, and *Fasciola hepatica*, as well as *Eimeria*, *Cryptosporidium*, and *Giardia*, playing particularly crucial roles (Sargison, 2004; Bhowmik *et al.*, 2020) [36, 10]. The primary protozoan infection affecting lambs and kids is coccidiosis. *Eimeria* and *Cryptosporidium* are the main agents responsible for causing coccidiosis. Although various species of *Eimeria* can infect lambs and kids, *Eimeria(E) crandallis* and *E. ovinoidalis* are recognized as the most pathogenic in sheep, while *E. ninakohlyaki-movae* holds this distinction in goats (Robertson, 2009) [33]. Strongylida nematodes, the majority of which are members of the superfamily Trichostrongyloidea, are the gastrointestinal (GI) nematodes that are most significant to small ruminants. Parasitic gastroenteritis, or PGE, is the clinical illness caused by a colony of strongylid nematodes that infects all grazing sheep and goats. The small and big bowels of sheep and goats are frequently parasitized by other nematodes from several taxonomic groups. These nematodes are *Skrjabinema* and *Trichuris* (large bowel), *Strongyloides* (small bowel), and *Aoncotheca*. These parasites relatively seldom cause illness and are not thought to be significant pathogens. Their eggs are typically regarded as accidental findings in faecal examinations, and the majority are likely to be impacted by the use of anthelmintics for those of greater importance species (Zajac, 2006) [46]. The clinical manifestation of GI parasite infection includes anaemia, weight loss, and a high death rate.

These changes impede the growth and output of the animal, resulting in financial loss for the farmer (Blackburn *et al.*, 2011) [11]. Small ruminant helminth infections of the GI tract have an indirect negative economic impact due to the expense of treating and controlling parasites, in addition to a direct negative impact on health resulting in morbidity and death (Nwosu *et al.*, 2007) [28]. Worldwide, the coexisting helminth infections seriously impair small ruminants (Perry *et al.*, 2002) [32].

The worms either directly infiltrate the host in clinical or subclinical helminthic infections, draw blood, cause anaemia, hypoproteinaemia and hypoglycaemia, or indirectly compete with the host for vital minerals or amino acids (Ahmed *et al.*, 2015; Moudgil *et al.*, 2017) [3, 27]. Therefore, physiological and biochemical parameters are sensitive indicators of the severity of parasite burden, and the disruption of vital metabolic activities by the liver that are necessary for the animal's optimal health and productivity. The migration of parasite larvae in hepatic and pulmonary tissues is the main source of bleeding and major tissue damage that leads to the alteration of haematological and biochemical parameters associated to GI parasites (Tariq *et al.*, 2008; Aziz and Mahmoud, 2022) [42, 7]. Stressful circumstances might increase the risk of GI parasite infections. These include weaning, dietary changes, inclement weather, travel, and gathering (Pedreira *et al.*, 2006; Al-Bayati *et al.*, 2023) [30, 5]. Oxidative stress is a disruption of equilibrium between oxidants and antioxidants (Burton and Jauniaux, 2011). Organisms are shielded from reactive oxygen species (ROS) produced during host injuries by antioxidants (Peng *et al.*, 2012) [31]. When antioxidants are lacking or ineffective, cellular damage takes place, leading to lipid peroxidation (Pentón-Rol *et al.*, 2009). ROS concentrations surged in the cells of hosts infected with different parasite species in several studies (Abd Ellah, 2010) [1]. As a result, research on oxidative stress markers, serum biochemistry, and haematology has demonstrated its importance in assessing an animal's health by revealing the degree of infection severity and host tissue damage (Otesile *et al.*, 1991) [29]. Hence, the objective of this study was to assess hematological and biochemical parameters, as well as oxidative stress indicators, in goats afflicted with intestinal protozoa and helminths linked to diarrhoea.

Materials and Methods

The research took place at the veterinary facility of the Indian Veterinary Research Institute in Bareilly, India, from July to December 2023 (coordinates 28º 24'N, 79.26'E, altitude approximately 179 m above sea level).

Fecal Sample Collection and Examination

During the study period, 112 fecal samples were gathered and analyzed from goats brought to the institute's veterinary facility due to diarrhea. The samples underwent processing using the fecal sedimentation and flotation technique to identify eggs/oocysts. Goats tested positive for coinfection were included in the study. Blood and serum samples were collected from 10 healthy goats undergoing routine health checkups and from 25 diarrheic goats positive for two or more endoparasitic species infections.

Haematological analysis

Whole blood samples were collected by venipuncture of the jugular vein, using EDTA tubes EDTA to evaluate the red blood cell (RBC) count, haemoglobin (Hb) concentration, packed cell volume (PCV), platelet count, white blood cells (WBC) count and differential leucocyte count were measured in automatic haematology analyzer (URIT 3000 vet plus, China). By venipuncturing the jugular vein, whole blood samples were collected in EDTA tubes. The following parameters were determined using an automated haematology analyzer (URIT 3000 vet plus, China): haemoglobin (Hb) concentration, red blood cell (RBC) count, packed cell volume (PCV), platelet count, total leucocyte count (TLC), and differential leukocyte count.

Biochemical profile

Commercially available kits were used to quantify total protein, albumin, aspartate aminotransferase enzyme, blood urea nitrogen, and creatinine using a semi-automatic serum biochemistry analyzer (Chem 5x, Erba Mannheim Biochemical Analyzer, Transasia BIO-medical Ltd, Mumbai, India). According to Doumas and Biggs (1972), the globulin level was computed by deducting the total protein concentration from the measured albumin concentration. Using blood total protein and albumin concentrations and Fried-Wald's equation, the A:G ratio was determined.

Analysis of oxidant/antioxidant status

Goat oxidant/antioxidant status was assessed by measuring the concentrations of malonaldehyde (MDA) and nitrate (NO) in blood. The dual boiling technique (Draper and Hadley, 1990) [17] was used to quantify MDA, an indicator of lipid peroxidation. Based on the principles of nitrate reduction with activated copper-cadmium (Cu-Cd) alloy and deproteinization with zinc sulphate, which results in colour development, the concentration of NO in serum was determined using the acidic Griess reaction. Using various potassium nitrate concentrations (0.0 to 80.0 μM), a constructed standard curve was used to calculate the NO concentration (Sastry *et al*., 2002) [37].

Statistical analysis

Results from our studies were statistically analysed using the independent sample t-test using IBM Corporation's (New York, USA) SPSS Statistics 20 software.

Results

The results of the study are presented in four parts: mixed parasite infection rate, haematological changes, biochemical changes, and oxidative stress indices.

Infection Rate

Out of the 112 screened diarrheic faecal samples, 86 samples tested positive for various intestinal parasites. The infection rate of GI parasite coinfection was 76.7%. Of these, 60.4% (52/86) were mixed infections. In this study, the overall infection rates for helminths, protozoa, and cestodes were found to be 74.41%, 66.27%, and 6.97%, respectively (Figure 1).

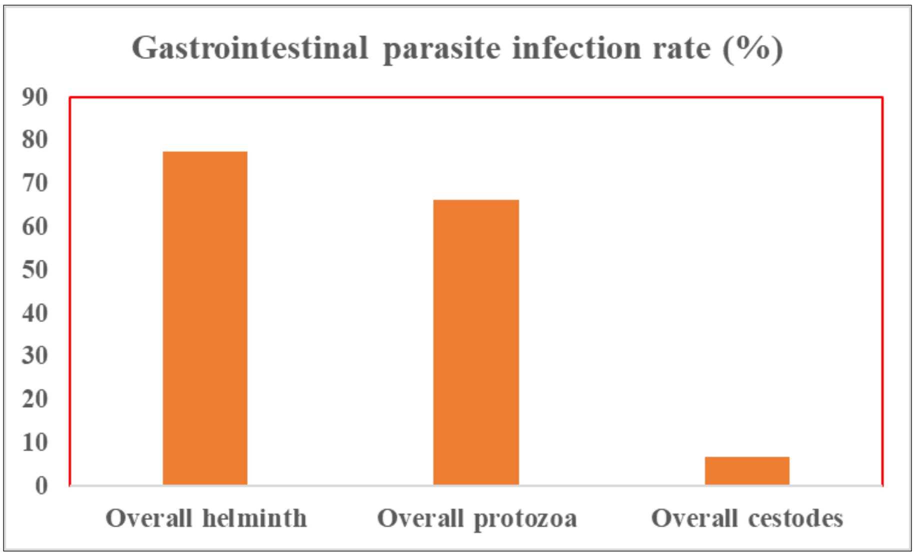

Fig 1: Overall gastrointestinal parasite infection in goat associated with diarrhoea

Haematological Changes

Table 1 presents the changes in the haemogram and leucogram in the whole blood of the affected goats and the healthy control goats. The affected goats showed significantly lower levels of Hb (7.07±0.34 g% vs 12.96±0.75 g%, $p<0.001$), TEC (4.24±0.30 ×10^6/µL vs 6.33±0.43 ×10^6/µL, p=0.001), and PCV (26.22±0.68% vs 34.88±1.29%, $p<0.001$). The TLC and absolute eosinophil count was significantly higher in the affected goats (11.28±0.65 ×10^3cell/µL vs 7.98±363.24 ×10^3 cell/µL and 0.048±0.11×10^3cell/µL vs 0.14±0.28×10^3cell/µL p=0.004 and 0.041 respectively). However, there is statistically no significant difference in absolute neutrophil count (3.60 ± 0.31×10^3 cell/µL vs 3.92 ± 0.37×10^3 cell/µL), lymphocyte count (3.99 ± 0.15×10^3 cell/µL vs 4.05 ± 0.29×10^3 cell/µL), monocyte count (0.25 ± 0.56×10^3 cell/µL vs 0.28 ± 0.35×10^3 cell/µL) and platelet count (4.27 ± 0.81×10^3 cell/µL vs3. 64 ± 0.51×10^3 cell/µL). The affected goats showed significantly lower levels of Hb, TEC and PCV indicating possible anaemia. The TLC and absolute eosinophil count were significantly higher in the affected goats, suggesting an immune response to the parasitic infection (Table 1).

Table 1: Changes in haemogram and leucogram in whole blood of gastrointestinal parasite-affected goats associated with diarrhoea and healthy control goats

Parameters	Health control (n=10)	Affected (n=25)	p value
Haemoglobin (g%)	12.9600±0.75	7.07±0.34	<0.001
Total Erythrocyte count (×10^6cell/µL)	6.33±0.43	4.24±0.30	0.001
Packed cell volume (%)	34.88±1.29	26.22±0.68	<0.001
Platelet (×10^3 cell /µL)	4.27±0.81	3.64±0.51	0.519
Total leucocyte count (×10^3 cell /µL)	7.98±363.24	11.28±0.65	0.004
Neutrophils (×10^3 cell /µL)	3.60±0.31	3.92±0.37	0.618
Lymphocyte (×10^3 cell /µL)	3.99±0.15	4.05±0.29	0.895
Monocyte (×10^3 cell /µL)	0.25±0.56	0.28±0.35	0.618
Eosinophils (×10^3 cell/µL)	0.048±0.11	0.14±0.28	0.041
Basophils (×10^3 cell /µL)	.024±0.01	0.01±0.00	0.484

Biochemical Changes

Table 2 shows the alteration in the serum biochemical profile. The total protein (5.36±0.30 g/dl vs 7.25±0.23 g/dl, p=0.001) and albumin (2.35±0.16 g/dl vs 3.52±0.13 g/dl, $p<0.001$) levels were significantly lower in the affected goats. However, the globulin level (3.73±0.27 g/dl vs3.02±0.36 g/dl), A/G ratio (1.02±0.13 vs2.61±1.10), aspartate aminotransferase (195.85±15.11vs221.08±11.22 IU/L), blood urea nitrogen (15.86±1.53mg/dl vs15.53±0.68 mg/dl), and creatinine (1.15±0.17 mg/dl vs 0.95±0.06 mg/dl) did not show significant differences. The total protein and albumin levels were significantly lower in the affected goats, possibly due to malnutrition or malabsorption caused by the parasitic infection. However, liver and kidney functions appeared to be not severely affected as indicated by the levels of globulin, A/G ratio, aspartate amino transferase, blood urea nitrogen, and creatinine (Table 2).

Table 2: Alteration in serum biochemical profile in gastrointestinal parasite-affected goats associated with diarrhoea and healthy control goats

Parameters	Health control (n=10)	Affected (n=25)	p value
Total Protein (g/dl)	7.25±0.23	5.36±0.30	0.001
Albumin (g/dl)	3.52±0.13	2.35±0.16	<0.001
Globulin (g/dl)	3.73±0.27	3.02±0.36	0.255
A/G ratio	1.02±0.13	2.61±1.10	0.375

Aspartate amino transferase (IU/L)	195.85±15.11	221.08±11.22	0.220
Blood urea nitrogen (mg/dl)	15.86±1.53	15.53±0.68	0.820
Creatinine (mg/dl)	1.15±0.17	0.95±0.06	0.179

Oxidative Stress Indices

Table 3 presents the changes in oxidative stress indices. The NO level was significantly higher in the affected goats (3.26±0.20 µM/ml vs 2.01±0.24 µM/ml, p=0.002). The MDA level, a marker of oxidative stress, was also higher in the affected goats (16.09±0.58 nMol/ml vs 13.49±0.88 nMol/ml, p=0.022). The NO level, a marker of inflammation, and MDA level, a marker of oxidative stress, were significantly higher in the affected goats (Table 3).

Table 3: Changes in oxidative stress indices in serum of gastrointestinal parasite affected goats associated with diarrhoea and healthy control goats

Parameters	Health control (n=10)	Affected (n=25)	p value
Nitric oxide(µM/ml)	2.01±0.24	3.26±0.20	0.002
Malonaldehyde (nMol/ml)	13.49±0.88	16.09±0.58	0.022

Discussion

An intestinal illness caused by protozoa and nematodes is clinically characterised by bloody and mucusy diarrhoea, inappetence and dehydration. Because they ultimately cause the death of the host, mixed infections are extremely dangerous. Additionally, it has been documented that the combined infection of *Trichuris* and *Moniezia* caused extreme malnutrition that resulted in pulmonary oedema in the Black Bengal goat kid (Maity *et al.*, 2018) [26]. Changes in the haematological, biochemical, and antioxidant markers result from a decrease in food digestibility caused by GI parasites, as well as the redirection of nutrients from production sites to tissue injury repair (Kyriazakis and Houdijk 2006; Cardia *et al.*, 2011) [25, 13].

Among the markers that might gauge an individual's state of health and reaction to stressful situations are haematological measures. The results of the current study showed a significant decrease in PCV%, Hb, and TEC; these findings are consistent with those of previous studies (Kumar *et al.*, 2013; Ahmed *et al.*, 2015; Kumar *et al.*, 2015; Moudgil *et al.*, 2017; Alam *et al.*, 2020) [23, 3, 24, 27, 4]. The ability of the parasitic infection to draw blood from the host as in heamonchosis and the extent of blood loss from leakage or haemorrhage resulting from severe damage caused by the heavy intestinal parasitic burden may be linked to the significantly lower concentrations of Hb, TEC and PCV in the infected animals (Urquhart *et al.*, 1996; Aziz and Mahmoud, 2021) [45, 7]. Additionally, our results indicated a considerable rise in the WBCs and absolute eosinophil count which were consistent with studies conducted by other researchers nationwide (Kumar *et al.*, 2015; Ahmed *et al.*, 2015; Abdel-Saeed and Salem, 2019; Aziz and Mahmoud, 2021; Al-Bayati *et al.*, 2023) [24, 3, 2, 7, 5]. This is because intestinal protozoa and nematodes can cause inflammation in the GI tract walls, and WBCs play a crucial part in the immunological response (Salem *et al.*, 2017) [34] or may be the result of eosinophils increased local immunological response (Ahmed *et al.*, 2015) [3]. Eosinophilia is linked to cell-mediated immunity and phagocytic activity, which breaks down particle matter and parasite detritus as a result of persistent infections (Awad and Ali, 2016) [6].

Goats infected with GI parasites showed a significant drop in total protein and albumin levels in their serum biochemical examinations. The findings of our study align with the findings of Al-Bayati *et al*. (2023) [5], Kumar *et al*. (2015) [24], Moudgil *et al*. (2017) [27], Ahmed *et al*. (2015) [3], Jas *et al*. (2008) [20], and Kumar *et al*. (2005) [22]. The reduction in these parameters might be ascribed to inhibition of hunger and impairment in the absorption of nutrients from meals at the gut infection site because of surface epithelial cell loss and injury, as well as cell sloughing that results in bloody diarrhoea (Sheikh *et al.*, 2005) [38] It could be linked to disorders such as protein-losing gastroenteropathy in haemonchosis (Soulsby, 1982) [40] and impaired protein absorption from the injured intestinal mucosa in concurrent GI infections (Ahmed *et al.*, 2015) [3].

Recent research in ruminant medicine has focused on oxidative stress, which has been linked to a number of disease processes (Celi, 2011) [14]. Numerous studies have documented the existence of oxidative stress in humans and animals infected with parasites, suggesting that parasitic infections are a causal cause of oxidative stress (Upcroft and Upcroft, 2001; Celi, 2011) [44, 14]. Sheep infections with parasitic infections caused by *Trichostrongylidae*, *Fasciola*, and *Eimeria* species enhanced the generation of free radicals and oxidative stress, which in turn increased the production of lipid peroxidation (Dede *et al.*, 2000) [15]. The generation of ROS by immune effector cells is one of the host's defence mechanisms against parasites. These cells aid in the removal or death of parasites from their host, preventing the spread of infection (Ben-Smith *et al.*, 2002) [9]. Regular metabolic processes constantly produce free radicals; however, some parasite infections cause an increase in the frequency of these radicals' production (Heidarpour *et al.*, 2013) [19]. Similarly, NO is said to be a crucial part of the host's defence mechanism against parasite invasion. More recently, data supporting NO's protective effects during helminthic infections have been demonstrated NO generation is a usual finding in both protozoan and helminthic infections because most parasites produce a period of inflammation in the diseased host (Brunet, 2001) [12]. This study found that in diarrhoeic goats infected with the GI parasite, oxidative stress was indicated by a considerable rise in the lipid peroxidation biomarker (MDA) and the marker of GI tract inflammation (NO). Our findings are consistent with Kiran and colleagues (2019) [21], who noted a noteworthy rise in MDA and NO levels in the GI parasite-infected group compared with the control group. The rise in MDA level is consistent with Alam and coworker's (2020) [4] observations showed *Heamonchus contortus*-infected sheep and goats have higher MDA levels. Oxidative stress is a systemic reaction to the GI parasite and is not influenced by the parasite burden in the sheep that were parasitized (Samadieh *et al.*, 2017) [35]. An increase in ROS production is linked to systemic inflammation brought on by several infections (Sorci and Faivre, 2009) [39]. Superoxide radicals may be produced when molecular oxygen is oxidised, leading to high lipid peroxidation. This process also generates H_2O_2, which starts the peroxidation of unsaturated fatty acids in the membrane and produces MDA (Une *et al.*, 2001) [43].

Conclusion
The study underscores the substantial health implications of gastrointestinal parasitic infections in goats. The high infection rates observed, coupled with hematological and biochemical changes, point to the severity of the impact on the host. Goats affected by GI parasites exhibited signs of anemia, malnutrition, and altered oxidative stress indices. The results emphasize the need for effective parasite control strategies to mitigate economic losses for farmers and ensure optimal animal health. Additionally, the study contributes valuable insights into the physiological responses and biomarker alterations associated with parasitic infections in goats, aiding in the development of targeted interventions and preventive measures for small ruminant health management.

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