Research Article

High Prevalence and Severe Clinicopathological Impacts of *Raillietina* spp. in Bangladeshi Village Chickens

Md. Abdullah Al Mahmud[1], Uzzal Sarker[1], Kazi Nazmus Sakib[1], Mohammad Shah Alam[1], Md. Ataur Rahman[2], Fahima Khatun[3]*

[1]Department of Anatomy and Histology, Faculty of Veterinary Medicine and Animal Science, Gazipur Agricultural University, Gazipur 1706, Bangladesh. [2]Department of Surgery and Radiology, Faculty of Veterinary Medicine and Animal Science, Gazipur Agricultural University, Gazipur 1706, Bangladesh.[3]Department of Pathobiology, Faculty of Veterinary Medicine and Animal Science, Gazipur Agricultural University, Gazipur 1706, Bangladesh.

Abstract | The most common cestodes in non-descript village chickens (NDVCs) are *Raillietina* spp., which adversely affect poultry health and productivity. The Gazipur district is considered a major center of poultry production in Bangladesh. However, data on the prevalence, hematological impact, and intestinal pathology of *Raillietina* spp. in NDVCs in the Gazipur district remain limited. Therefore, a cross-sectional study was conducted in Gazipur district, involving the random selection of 120 free-range NDVCs. The *Raillietina* spp. were identified through morphological examination and Semichon's carmine staining after fixation of *Raillietina* spp. using alcohol formalin and glacial acetic acid. Furthermore, hematological parameters were measured using an autoanalyzer. Histopathological and histomorphometric analyses of intestinal tissues were performed using hematoxylin and eosin, and picrosirius red staining, with morphometric analysis. The results showed 91.67% overall prevalence of *Raillietina* spp. with the highest rate (100%) in Sreepur Upazila. In addition, infected NDVCs exhibited anemia, characterized by decreased Hb, RBC, and PCV levels, alongside increased WBC ($P < 0.05$). Histopathological examination revealed villus atrophy, necrosis, inflammatory cell infiltration, and mucosal erosion caused by mechanical injury due to tapeworm's scolex attaching to the intestinal wall. Along with, picrosirius red staining indicated increased deposition of type I and III collagen, suggesting chronic fibrosis. Histomorphometric analysis revealed increased crypt length and mucosal thickness, but decreased villus height in infected NDVCs. In conclusion, *Raillietina* spp. are highly prevalent in the Gazipur district, causing substantial pathological and physiological damage to NDVCs. These findings will provide baseline data for further molecular studies and will be helpful in developing a control strategy in Bangladesh.

Keywords | Collagen deposition, Hematology, Histopathology, Non-descript village chickens (NDVCs), Prevalence, *Raillietina* spp.

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***Correspondence** | Fahima Khatun, Department of Pathobiology, Faculty of Veterinary Medicine and Animal Science, Gazipur Agricultural University, Gazipur 1706, Bangladesh. **Email:** fahima@gau.edu.bd
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INTRODUCTION

Non-descript village chickens (NDVCs) rearing is one of the most common and traditional livelihood practices in rural Bangladesh (Islam and Jabbar, 2003). It also provides essential income, nutrition and food security for millions of smallholder farmers. In fact, NDVCs are well adapted to the local environment and form the background of poultry production in Bangladesh (Barua and Yoshimura, 1997). They are esteemed for their resilience, adaptability, exemplary testing and product quality (Shanta *et al*., 2017). Beyond this nutritional contribution, NDVCs' farming substantially supports rural livelihoods by generating additional income that helps families cope with financial hardship (Abdullah *et al*., 2021). In a developing country like Bangladesh, where a large proportion of the population depends on small-scale agricultural activities, NDVCs' farming serves as an accessible and sustainable means of poverty reduction and improves household food security (Ferdushy *et al*., 2016).

The NDVCs' farming has significant socioeconomic and nutritional importance, serving as a sustainable, low-input, and high-impact source of food and income (Aklilu, 2007). However, productivity in this sector remains low due to poor management, inadequate disease control and high prevalence of parasitic and infectious diseases (Kausar *et al*., 2025). Similarly, NDVC often faces challenges due to a lack of veterinary services, diagnostic facilities, and disease-prevention programs. The NDVCs roam freely in the daytime, scavenging for food, including insects, exposing them to soil-borne parasites and their own feces, which frequently contain infective ova, larvae, and oocysts (Chege et al., 2017). Among the intestinal parasites, *Raillietina* spp. are more prevalent and are transmitted via intermediate hosts in scavenging areas (Anwar *et al*., 2000). Chickens ingest these hosts during foraging, leading to infections that damage the intestinal lining and cause inflammation (Eshetu *et al*., 2001). These infections also reduce growth and egg production, severely affecting smallholder farmers who rely on NDVC farming for protein and income sources (Abdullah *et al*., 2021).

Despite the substantial health hazards of *Raillietina* spp. infections, these parasites are often neglected in rural veterinary health programs due to limited awareness and resources (Abdullah *et al*., 2021). Most existing research in Bangladesh has been focused on overall gastrointestinal helminth prevalence without examining the hematological and histopathological effects of *Raillietina* spp. Furthermore, data on the prevalence of these parasites in the Gazipur district are currently lacking. But, the incidence of *Raillietina* spp. infections appear to be increasing, and little is known about their prevalence, hematological, histopathological and histomorphometric effects on chickens, particularly in NDVCs production systems (Siddiqui *et al*., 2023).

This knowledge gap hampers the development of effective treatment, control and prevention strategies (Siddiqui *et al*., 2023). Environmental and climatic factors, along with scavenging, favor the spread and transmission of intermediate hosts, thereby increasing the risk of infection in NDVCs (Soulsby, 1982). Understanding the prevalence, hematological and histopathological changes is crucial for improving chicken health and for identifying diagnostic indicators to initiate control measures (Abbas *et al*., 2024). This study aimed to determine the prevalence and examine the hematological, histopathological and histomorphometric changes in NDVCs infected with *Raillietina* spp. The outcomes of this research will provide insights to enhance management and control, support evidence-based interventions, improve NDVCs' health, protect livelihoods, ensure food security, and promote a sustainable NDVC sector in Bangladesh.

MATERIALS AND METHODS

Study Area And Period

The research was conducted from April 2023 to March 2024 across five upazillas in the Gazipur district of Bangladesh (Figure 1). The Gazipur district is geographically located between latitudes 23°53' and 24°21' north and longitudes 90°09' and 92°39' east. The five sub-districts (upazillas) in Gazipur are Gazipur Sadar, Kaliakair, Kaliganj, Kapasia and Sreepur (Khatun *et al*., 2025). The villagers raised NDVCs for subsistence, and samples were collected from five upazillas of Gazipur district.

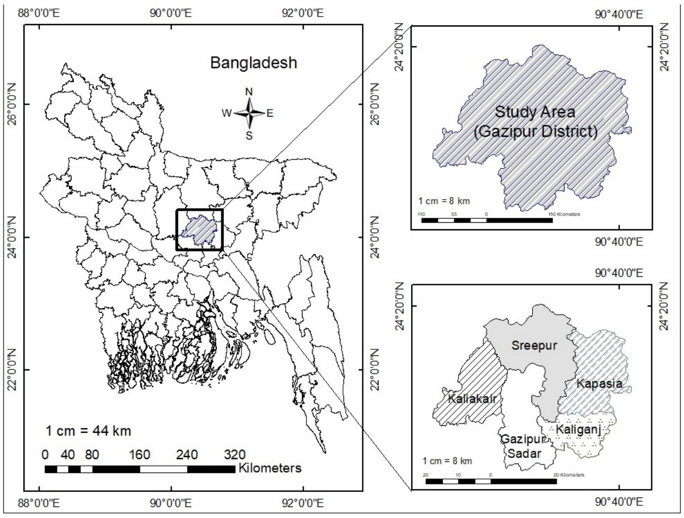

Figure 1: The map visually displays the study area (Gazipur District, Bangladesh).

Study population and sample size determination

The study population consisted of apparently healthy adult NDVCs (*Gallus gallus domesticus*) of both sexes, aged 6-12 months, with no obvious clinical abnormalities at examination, including normal posture, activity, and body condition, and no visible gastrointestinal or respiratory signs, reared under scavenging systems by individual households. A total of 120 NDVCs were randomly purchased from households in Gazipur Sadar (n= 26), Kaliganj (n= 22), Kapasia (n = 23), Kaliakair (n= 23), and Sreepur (n= 26). The NDVCs were then caged and transported to the

Anatomy Laboratory of the Department of Anatomy and Histology at Gazipur Agricultural University, Bangladesh (Khatun *et al*., 2025).

Hematological analysis

For hematological analysis, blood was drawn from the brachial veins of NDVCs (Non-infected = 10 and Infected = 10) using a sterile syringe. A volume of 1 ml of blood was placed in a test tube containing the anticoagulant ethylenediaminetetraacetic acid (EDTA). Complete Blood Count (CBC) was analyzed using the Mindray BC-3000 Plus (Mindray, China) according to the manufacturer's instructions.

Collection and identification of *Raillietina* spp.

After collection, the NDVCs were slaughtered according to halal procedures. The abdominal cavity was then opened, and the parasites were collected from the small intestine (jejunum and ileum) after longitudinal dissection of the GI tract. For the microscopic identification of *Raillietina* spp. and long-term storage, permanent slides of the parasites were prepared according to the procedures described by Thienpont *et al.* (1986). Briefly, *Raillietina* spp. were fixed with alcohol formalin and glacial acetic acid (AFA) and dehydrated using gradually increasing concentrations of ethanol (50–100%). The samples were then permeabilized using iodine and stained with Semichon's carmine solution for 5 h. Excess stain was removed with acid alcohol if necessary. Subsequently, the samples were dehydrated again with alcohol, softened using aniline oil, briefly treated with xylene, and finally mounted with Canada balsam (Figure 2). Parasites were identified based on the morphological features described by Soulsby (1982) and Wall and Shearer (1997).

Histopathological analysis

Post-sacrifice, intestinal samples (jejunum and ileum) from both non-infected (n=10) and infected (n= 10) NDVCs were randomly collected and stored in a 10% neutral buffered formalin solution for 48 h at room temperature. Subsequently, the organs were washed in PBS for 3 h, and the tissues were dehydrated in 70%, 80%, 90%, and 100% ethanol (Merck, Damstadt, Germany) for approximately 2 h each. The tissues were cleared in xylene (Merck, Damstadt, Germany) and embedded in paraffin. Using a rotary microtome (Leica, Germany), 5 µm sections of each tissue were prepared (Amin *et al*., 2016; Amin and Mahmud, 2021). The sections were stained with hematoxylin (Catalogue number: HEMH-OT-X, Biognost, Croatia) and eosin (Catalogue number: EOYA-10-OT-X, Biognost, Croatia) for histopathological examination, as described previously (Mussa *et al*., 2018; Uzzal *et al*., 2021; Rahman *et al*., 2024). To study collagen deposition and tissue remodeling, the tissue samples were stained with picrosirius red (Product Number: 24901, Polysciences, USA) according to the manufacturer's protocol. The sections were viewed using a Leica ICC50 E microscope (Leica Microsystems, Wetzlar, Germany) coupled with a digital camera, and images were captured for further studies.

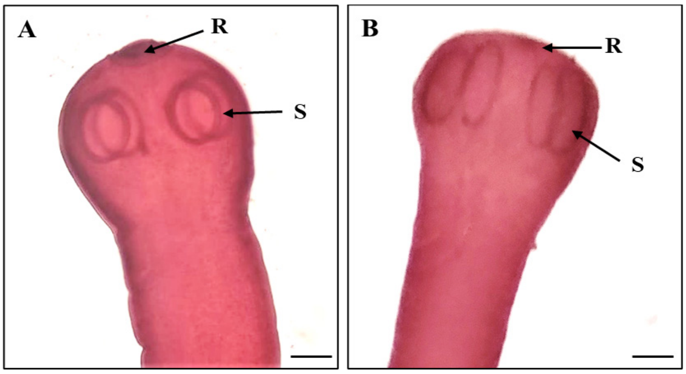

Figure 2: Microscopic identification of *Raillietina echinobothrida* (A) and *Raillietina tetragona* (B). Scolex bearing the rostellum with hooks (R) surrounded by four suckers (S). Semichon's carmine solution, scale bar 50 µm.

Intestinal histomorphometry analysis

The histomorphometric study included the jejunum and ileum of the small intestine of the chickens. The morphometric indices investigated the thickness of the muscularis externa, crypt length and width, villi height, and total mucosal length. Specific sites for the measurements were as follows: muscularis externa thickness was measured from the outer edge of the circular muscle layer to the outer edge of the longitudinal muscle layer; crypt length was measured from the base of the crypt to the junction between the crypt and the villus; crypt width was measured at the widest transverse section of the crypt; villus height was measured from the crypt–villus junction to the apex of the villus; and full mucosal length was measured from the base of the muscularis mucosae to the tip of the villus. Morphometric measurements were performed using ImageJ software (NIH, USA) on 10 randomly selected histological sections stained with hematoxylin and eosin from the jejunum and ileum of the small intestine.

Statistical analysis

The data were entered into a Microsoft Excel spreadsheet and subsequently analyzed using the Statistical Package for Social Sciences (SPSS) version 25. The prevalence of endoparasite infection was calculated as the percentage of infected NDVCs among the total number of NDVCs examined. The prevalence of *Raillietina* spp. and their distribution across different upazilas were analyzed using the chi-square test (Yasmin *et al*., 2025). Statistical analyses were conducted using post hoc independent t-tests with the Holm–Bonferroni correction to assess significant differences (Yang et al., 2024). Statistical significance was set than 0.05 ($P < 0.05$) was considered statistically significant.

RESULTS

Overall prevalence of *Raillietina* spp. in NDVCs

A total of 120 NDVCs were examined across five upazilas of Gazipur district, of which 110 were positive, resulting in an overall prevalence of 91.67% (Table 1). The prevalence remained consistently high across all study areas, with Sreepur showing infection in all examined samples (26/26, 100%). Gazipur Sadar also demonstrated a high prevalence, with 24 out of 26 samples infected (92.31%). Comparable prevalence rates were observed in Kaliakair and Kapasia, where 21 of 23 samples in each upazila were positive (91.30%). In contrast, Kaliganj recorded the lowest prevalence, with 18 infections among 22 examined samples (81.82%), although no significant differences were observed across the five upazilas ($\chi2 = 5.178$, $P > 0.05$) (Table 1). Overall, the results indicate widespread infection throughout the district, with only modest variation among the upazilas.

Table 1: Prevalence of *Raillietina* spp. in non-descript village chickens (NDVCs) in Gazipur district (N=120).

No.	Upazilas	Total No. examined	No. of infected	Prevalence (%)	χ2/ Fisher's exact	p value
1	Gazipur Sadar	26	24	92.31	5.178	0.269^{NS}
2	Kaliakair	23	21	91.30		
3	Kapasia	23	21	91.30		
4	Kaliganj	22	18	81.82		
5	Sreepur	26	26	100.00		
6	Total	120	110	91.67		

Note: Chi-Square test was performed, where NS indicates non-significance.

Hematological Data

Hematological parameters differed markedly between the non-infected and infected groups (Table 2). Infected NDVCs showed a significant reduction in hemoglobin concentration (10.63 ± 0.34 g/dl), RBC count (2.29 ± 0.17 × 10^6/µl), and packed cell volume (22.15 ± 1.11%) compared with their non-infected counterparts ($P < 0.01$). Similarly, the mean corpuscular hemoglobin (MCH) was significantly lower in the infected NDVCs (29.06±0.63 pg; $P < 0.001$). In contrast, the mean corpuscular volume (MCV) was markedly elevated in infected samples (160.68 ± 5.49 fl; $P < 0.001$), indicating pronounced alterations in the morphology of erythrocytes. A significant increase in total WBC count was also observed in infected NDVCs (3.86±0.08 × 10^3/µl) compared with non-infected NDVCs ($P < 0.001$), reflecting an active systemic response. However, the mean corpuscular hemoglobin concentration (MCHC) did not differ significantly between the two groups ($P = 0.157$). Overall, the results demonstrated substantial hematological disturbances associated with infection.

Table 2: Effect of *Raillietina* spp. on some blood parameters of infected non-descript village chickens (NDVCs).

No.	Parameters	Non-Infected (n=10) (Mean ± SEM)	Infected (n=10) (Mean±SEM)	Adj. P value
1	Hb (g/dl)	12.85 ± 0.35	10.63 ± 0.34	0.0117*
2	RBC (*10^6/µl)	3.64 ± 0.15	2.29 ± 0.17	0.0065*
3	WBC (*10^3/µl)	2.2 ± 0.21	3.86 ± 0.08	0.0000*
4	PCV (%)	29.29 ± 0.19	22.15 ± 1.11	0.0144*
5	MCH (pg)	36.66 ± 0.28	29.06 ± 0.63	0.0003*
6	MCV (fl)	87.98 ± 0.75	160.68±5.49	0.0001*
7	MCHC (g/dl)	36.09 ± 0.07	37.48 ± 0.51	0.1571^{NS}

Note: Post hoc independent *t*-tests with the Holm–Bonferroni correction were used, where *indicates significance and NS indicates non-significance.

Histological study using hematoxylin and eosin staining

Histopathological examination of the small intestine revealed clear and consistent differences between non-infected and *Raillietina* spp. infected NDVCs (Figure 3). The non-infected jejunum and ileum displayed normal intestinal architecture, characterized by intact villi, well-organized crypts, and clearly demarcated mucosal and muscular layers. In contrast, infected intestines showed extensive pathological alterations affecting both the mucosal integrity and glandular structures. These changes included severe distortion, damage, fusion, and complete destruction of the villi, often accompanied by necrotic areas and calcification within the intestinal glands. The mucosa exhibited marked inflammatory responses, as evidenced by the infiltration of plasma cells, lymphocytes, and distinct eosinophils, indicating ongoing tissue injury. Structural disorganization extended to deeper layers, with hypertrophy and luminal enlargement of intestinal glands, separation of the submucosa from the muscularis externa, and accumulation of fibrous bundles within the damaged villi. In several sections, necrotic tissue was

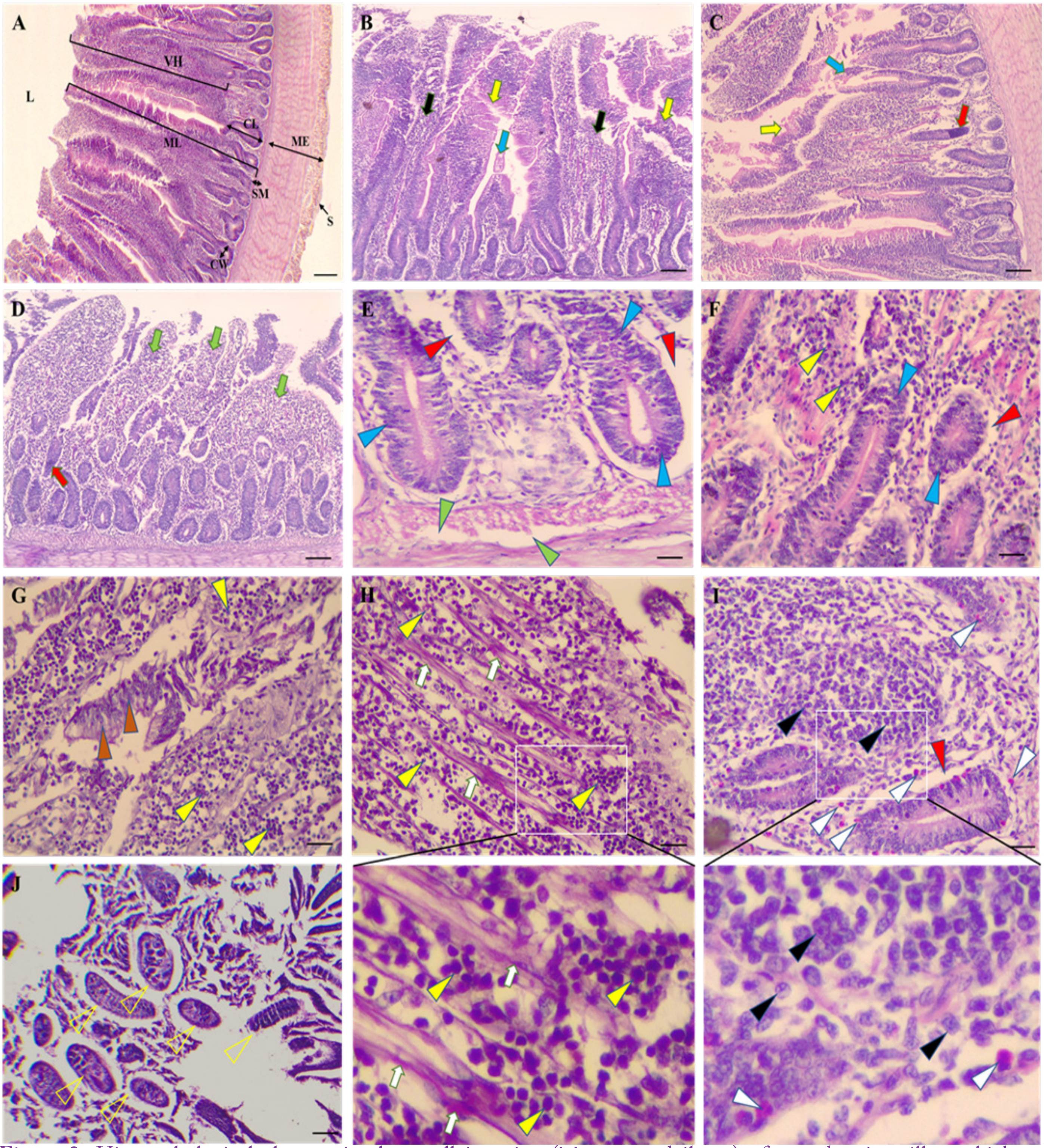

Figure 3: Histopathological changes in the small intestine (jejunum and ileum) of non-descript village chickens (NDVCs) infected with *Raillietina* spp.. Non-infected intestine appeared normal and healthy (A), whereas the infected intestine (B-J) revealed severe distortion of villi (yellow arrows), damage of villi (blue arrows), fusion of villi (black arrows), destruction of villi (green arrows), necrosis and calcification in the intestinal gland (red arrows), hypertrophy and luminal enlargement of the intestinal gland (blue arrowheads), destruction and separation of the submucosa from the muscularis externa (green arrowheads), separation of the intestinal glands from the surrounding tissue (red arrowheads), infiltration of lymphocyte cells (yellow arrowheads), necrotic tissue in damaged area of villi (orange arrowheads), bundle of fibers in damaged villi (white arrows), infiltration of plasma cells in the mucosa (black arrowheads), distinct pink colored eosinophil cells, indicating cellular injury or inflammation (white arrowheads), and Presence of *Raillietina* spp. in lumen (empty yellow arrowheads). CW, crypt width; CL, crypt length; ML, mucosal length; VH, villus height; SM, submucosa; ME, muscularis externa; S, serosa. Hematoxylin and Eosin staining, scale bar 100 µm (A-D) and 25µm (E-J).

Links Researchers

evident within severely affected villi, and adult *Raillietina* spp. were observed in the intestinal lumen of the affected villi. Overall, these histopathological findings demonstrate profound intestinal damage associated with infection, supporting morphometric evidence of altered crypt, villus, mucosal, and muscular parameters in infected NDVCs.

Histological study using picrosirius red staining

Collagen fiber remodeling in the small intestine of *Raillietina* spp. infected NDVCs showed notable differences compared to that in non-infected NDVCs (Figure 4). In healthy, non-infected intestines, collagen fibers were regularly arranged, reflecting normal gut morphology and structural integrity of the intestine. In contrast, infected tissues displayed pronounced alterations in collagen organization, characterized by increased deposition of both type I collagen (mature, dense fibers) and type III collagen (immature, fine fibers). This pattern indicates active remodeling and fibrosis, likely driven by chronic tissue injury caused by the parasite. The presence of *Raillietina* spp. within the intestinal lumen further confirms the direct association between parasitic infection and connective tissue change.

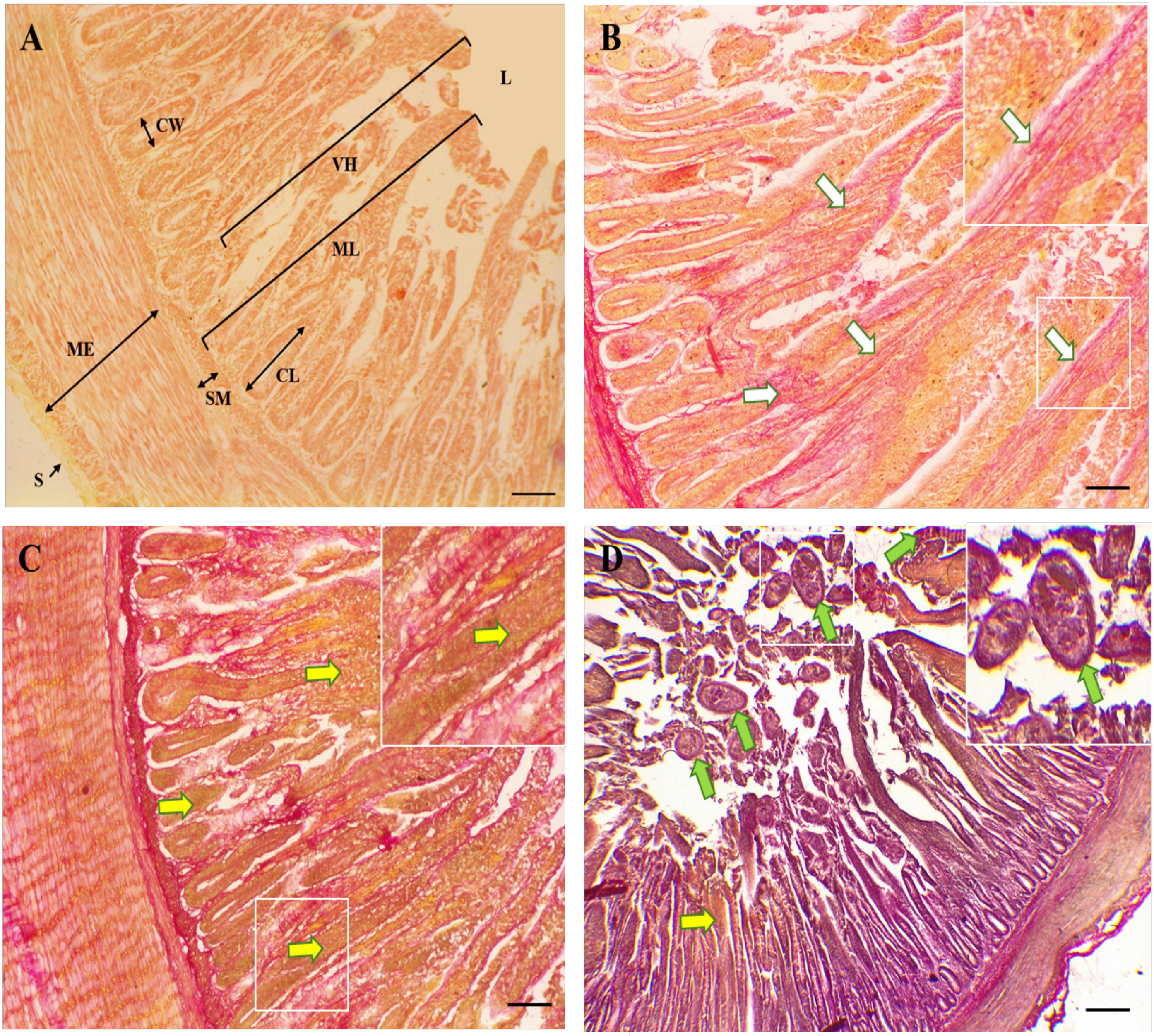

Figure 4: Remodeling of collagen fibers in the small intestine (jejunum and ileum) of *Raillietina* spp. infected non-descript village chickens (NDVCs). Non-infected intestine appeared normal and healthy gut morphology with regular arrangement of collagen fibers (A), whereas the infected intestine (C-D) revealed type I collagen fiber or mature collagen (white arrows), type III collagen fiber or immature fine collagen (yellow arrows), and presence of *Raillietina* spp. in the lumen (green arrows). CW, crypt width; CL, crypt length; ML, mucosal length; VH, villus height; SM, submucosa; ME, muscularis externa; S, serosa. Picrosirius red staining, scale bar 100 µm (A-D).

Table 3: Effect of *Raillietina* spp. on the histomorphometry of the small intestine (jejunum and ileum) in infected non-descript village chickens (NDVCs).

No.	Parameters	Non-Infected (n=10) (Mean ± SEM)	Infected (n=10) (Mean ± SEM)	Adj. P-Value
Jejunum	Muscularis externa, µm	271.78 ± 5.28	308.05 ± 5.68	0.0757^{NS}
	Crypt length, µm	187.71 ± 15.06	281.70 ± 4.76	0.0683^{NS}
	Crypt width, µm	77.58 ± 9.00	100.90 ± 7.14	0.2003^{NS}
	Villi height, µm	978.63 ± 39.20	912.62 ± 14.62	0.2270^{NS}
	Mucosa length, µm	1671.73 ± 16.53	1888.28 ± 17.63	0.0002*
Ileum	Muscularis externa, µm	452.91 ± 0.92	398.40 ± 2.91	0.0027*
	Crypt length, µm	217.13 ± 0.45	304.89 ± 10.43	0.0001*
	Crypt width, µm	71.32 ± 0.28	86.90 ± 3.56	0.0023*
	Villi height, µm	801.74 ± 2.80	683.42 ± 13.77	0.0001*
	Mucosa length, µm	1846.34 ± 4.05	1891.09 ± 6.64	0.0006*

Note: Post hoc independent *t*-tests with the Holm–Bonferroni correction were used, where * indicates significance and NS indicates non-significance.

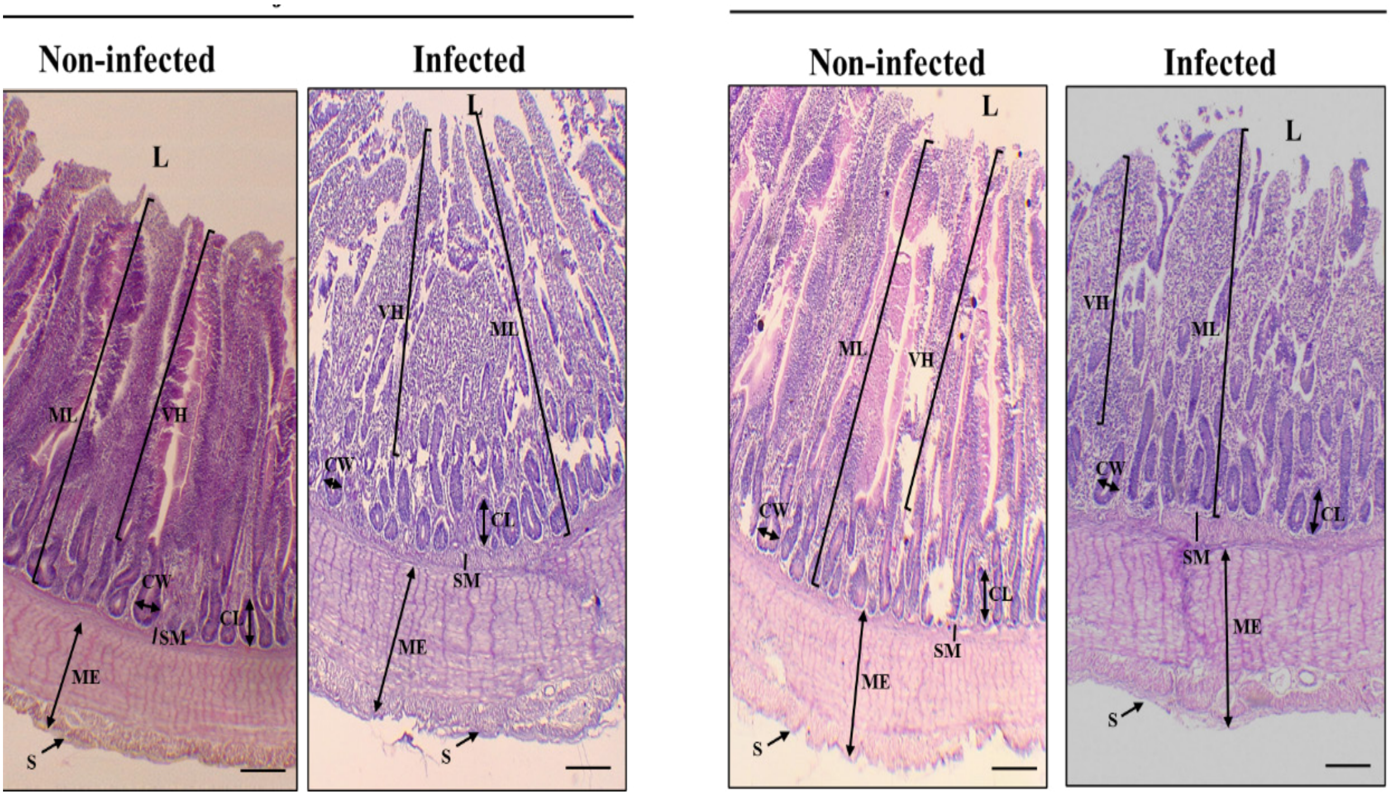

Figure 5: Effect of *Raillietina* spp. on the histomorphometry of the small intestine (jejunum and ileum) in infected non-descript village chickens (NDVCs). CW, crypt width; CL, crypt length; ML, mucosal length; VH, villus height; SM, submucosa; ME, muscularis externa; S, serosa. Picrosirius red staining, scale bar 50 µm (A-D).

Histomorphometric data

Histomorphometric analysis of the jejunum and ileum revealed distinct structural alterations between the non-infected and infected NDVCs (Table 3, Figure 5). In the jejunum, samples exhibiting infection demonstrated a non-significant alteration in the thickness of the muscularis externa, crypt length, crypt width, and villi length ($P > 0.05$). However, there was a significant increase in mucosal length ($P < 0.001$). In the ileum, infection was associated with marked histological remodeling, characterized by a significant reduction in muscularis externa thickness and villus height ($P < 0.001$), accompanied by significant increases in crypt length, width, and mucosal length ($P \leq 0.002$). Collectively, these findings indicate substantial region-specific histomorphological changes in the small intestine associated with infection, with more pronounced alterations observed in the ileum than in the jejunum.

DISCUSSION

The most prevalent helminths affecting poultry and other birds are *Raillietina* spp., which are distributed globally (Dakhil and Al-Musaedi, 2022), and around 66% of helminth infections worldwide are caused by Raillietina spp. (Shifaw *et al*., 2021). In this study, *R. echinobothrida* and *R. tetragona* were identified (Figure 2) by their key morphological features, such as sucker shape, hook arrangement in the rostellum, and genital pore position, following previous reports (Hofstad *et al*., 1984; Roy *et al*., 2012; Butboonchoo *et al*., 2016; Al Quraishy *et al*., 2019). The prevalence of these species (91.67%) was very high among NDVCs in the Gazipur district, indicating a persistent parasite burden. The present findings are consistent with the findings reported previously where 100.0%, 82.7%, and 72% NDVCs were found infected with *Raillietina* spp. in Bangladesh (Rabbi *et al*., 2006; Ferdushy *et al*., 2016; Siddiqui *et al*., 2023). In contrast, the surrounding areas in Bangladesh, as well as the nearby countries, have lower rates, between 18% and 44% (Yoriyo *et al*., 2008; Alam, *et al*., 2014; Rehman *et al*., 2016; Kumari *et al*., 2018; Shifaw *et al*., 2021). Among the five upazilas, Sreepur had highest infection rates (100%). This suggests that the infection spreads readily due to factors such as NDVCs roaming freely, favorable conditions for parasites, numerous hosts, and inadequate parasite control (Dey *et al*., 2020). In addition, the high infection rate may be attributable to management practices, environmental conditions, testing methods and control measures. Overall, the high infection rate in Gazipur indicates that *Raillietina* spp. is a big problem for NDVCs, underscoring the need for improved parasite control and management.

The present study revealed significant hematological alterations in NDVCs infected with *Raillietina* spp., characterized by reduced Hb, RBC, PCV, and MCH, which was supported by a previous study (Abbas *et al*., 2024). The *Raillietina* spp. penetrated the villi deeply, impairing nutritional absorption and destroying the mucosa, which ultimately caused hemorrhage in case of heavy infection (Demis *et al*., 2015; Mekibib *et al*., 2014). Due to the malabsorption, vitamin B12 deficiency, and intestinal blood loss, the concentrations of Hb, RBC, PCV, and MCH decreased (Belete *et al*., 2016; Aade and Wankhede, 2022). These results also agreed with (Taweya *et al*., 2020) in Ethiopia, (Bhure *et al*., 2011) in India and (Anah *et al*., 2017) in Al-Diwaniyah city who studied the cestode in domestic chickens and their effect on some hematological parameters. In contrast, elevated WBC and MCV, with no notable difference in MCHC, were observed in infected NDVCs. The elevated WBCs in this study may reflect an immune response to the parasite and the infectious process (Ibrahim, 2013), and this result was supported by Bhure *et al*. (2010) in India and Anah *et al*. (2017) in Al-Diwaniyah city. Elevated MCV with normal MCHC indicates macrocytic, normochromic anemia associated with regeneration or parasitic infections that affect erythropoiesis or cause nutritional deficiencies. In poultry, it's often caused by blood-sucking ectoparasites, cestodes, and hemoparasites (Aade *et al*., 2012). Such hematological alteration may exacerbate restlessness and compromise the health of infected NDVCs, highlighting the commercial and clinical imperative for routine monitoring and targeted interventions against *Raillietina* spp. to mitigate economic losses in poultry production.

Histology demonstrated that *Raillietina* spp. infection induces severe and progressive structural damage in the small intestine of NDVCs. Infected NDVCs exhibited extensive villous degeneration, fusion, and complete destruction, accompanied by epithelial sloughing, necrosis, and calcification of intestinal glands. These findings are in strong agreement with those of previous studies reporting comparable histopathological lesions in cestode-infected chickens, including villous necrosis, epithelial destruction at parasite attachment sites, glandular hypertrophy, and inflammatory cell infiltration (Al-Rubaie *et al*., 2018; Mahdi *et al*., 2018; Abbas *et al*., 2024; Oshima *et al*., 2024; Santos *et al*., 2025). Additionally, these alterations were consistently associated with pronounced inflammatory responses characterized by the infiltration of plasma cells, lymphocytes, eosinophils, and other mononuclear cells within the lamina propria and submucosa. Similar degenerative changes, including mucosal atrophy and inflammatory infiltration, have also been described by Mukaratirwa and Hove (2009), although lesion severity may vary with parasite burden and duration of infection. During histological examination, it was revealed that there was necrosis in the intestinal villi and destruction of epithelial cells where the worm's head attached. This led to the infiltration of white blood cells in response to the inflammatory reaction, and this is consistent with that reported previously (Yagoob *et al*., 2017). Such inflammatory infiltration reflects sustained host immune activation in response to chronic parasitic attachment and tissue invasion. The traumatic attachment of *Raillietina* spp. to the intestinal mucosa likely exacerbates epithelial erosion and necrosis, potentially facilitating secondary bacterial invasion and amplifying the inflammatory damage. Overall, the present histopathological evidence highlights the profound intestinal pathology associated with *Raillietina* spp. infection and underscores its role in compromising gut structure and function in NDVCs, with important implications for bird health, productivity and disease susceptibility.

The chronic fibrosis of intestine hampers peristalsis movement and nutrient absorption due to the damage caused by parasitic infections. In this research, the intestinal fibrosis was observed using picrosirius red staining, which revealed markedly increased deposition of both type I (thick, strongly birefringent red-orange-yellow fibers) and type III (thin, weakly birefringent green fibers) collagen in the small intestine of *Raillietina* spp. infected NDVCs. The picrosirius red-enhanced birefringence reliably distinguishes mature from immature collagen based on fiber thickness and packing (Rittié, 2017), color-based differentiation of types I and III remains contentious due to orientation-dependent artifacts (Lattouf *et al*., 2014). The possible explanation for the aberrant remodeling of collagen is the presence of luminal parasites and active fibrogenesis in response to chronic mucosal injury, which may contribute to tissue stiffening, impaired peristalsis, and compromised absorptive capacity. This appears to be the first use of this technique to evaluate parasite-induced intestinal fibrosis in NDVCs. Furthermore, the study contributes to the literature by providing new evidence emphasizing collagen changes as a key pathological feature of *Raillietina* spp. infection and the need for antiparasitic strategies to prevent long-term structural damage.

The small intestine of a chicken is considered the major site for the digestion and absorption of dietary nutrients (Svihus, 2014). The morphological and morphometric properties of the intestine reflect chickens' health status and are associated with nutrient assimilation capacity and immunological function (Nicholson *et al*., 2012). In this study, we measured gut morphometric indices (jejunum and ileum) to evaluate histological changes in *Raillietina* spp. infected NDVCs. The research demonstrated segment-specific architectural disruptions, with the ileum exhibiting elongated muscularis externa, deepened and widened crypts, extended total mucosal length, and shortened villi, while the ileum showed augmented crypt depth and width, prolonged mucosal length, and diminished muscularis externa thickness alongside reduced villus height. These quantitative alterations signify compromised epithelial barrier function, impaired nutrient absorption, and potential maldigestion due to parasitic-induced remodeling, which may exacerbate growth retardation and productivity losses in NDVCs. Consistent with these observations, prior investigations have linked cestode infections to analogous morphological shifts, including villus atrophy and crypt hyperplasia, correlating with diminished villus-to-crypt ratios and overall intestinal dysfunction (Siddiqui *et al*., 2023). In contrast, dietary interventions have been shown to ameliorate such parameters by enhancing villus height, crypt depth, and mucosal thickness across duodenal, jejunal, and ileal segments (Islam *et al*., 2024). Collectively, these findings underscore the value of histomorphometry in quantifying parasitic impacts, advocating for integrated parasite control strategies to preserve intestinal integrity and optimize NDVCs health in resource-limited settings.

Limitation and future directions

Based on the present findings, several directions for future research emerge clearly. First, longitudinal studies are needed to clarify the temporal progression of *Raillietina* spp. induced intestinal damage, linking parasite burden with sequential changes in histomorphometry, collagen remodeling, and hematological profiles. Such studies would help distinguish reversible adaptive responses from irreversible pathological remodeling. Second, integrating quantitative parasitological indices with molecular and immunohistochemical approaches could improve understanding of host–parasite interactions at the cellular and molecular levels, particularly mechanisms driving inflammation, fibrosis, and altered epithelial turnover. Third, controlled intervention trials evaluating strategic anthelmintic programs, nutritional supplementation, or integrated management practices should be prioritized to assess their effectiveness in mitigating intestinal damage and restoring hematological and morphological homeostasis in NDVCs. Additionally, future research should examine the functional consequences of the observed structural changes, including nutrient absorption efficiency, growth performance, and immune competence. Finally, expanding similar investigations across different agroecological zones and production systems would provide a broader epidemiological context and support the development of region-specific control strategies. Collectively, these research avenues would contribute to a more comprehensive understanding of *Raillietina* spp. pathobiology and inform evidence-based interventions to improve poultry health and productivity in resource-limited systems.

CONCLUSION

This study found that *Raillietina* spp. infection caused severe physiological and intestinal damage in NDVCs. Infected NDVCs showed anemia with reduced hemoglobin, red blood cell count, and packed cell volume, alongside increased white blood cell count, indicating inflammation. Histopathological analysis revealed villus atrophy, epithelial degeneration, necrosis, and inflammatory infiltration, all of which impair digestion and nutrient absorption. Picrosirius Red staining confirmed chronic fibrosis through excess Type I and III collagen deposition, whereas histomorphometry showed significant changes in villus height and crypt depth. Overall, the infection poses a major pathological and economic burden, threatening poultry health, rural livelihoods, and food security, underscoring the need for effective control measures.

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NOVELTY STATEMENT

The present study appears novel in its specific integration of prevalence data, hematological analysis, histopathological examination using both H and E, and picrosirius red staining, and histomorphometrical analysis of the ileum and jejunum. While related research exists on the prevalence and general pathology of *Raillietina* spp. infections in chickens (e.g., focusing on gross or basic histopathological changes), no directly comparable studies were found that combine all these elements, particularly the use of picrosirius red for fibrosis detection and quantitative morphometric assessments of intestinal segments. This combination could contribute new insights into the parasitic impacts on NDVCs health.

AUTHOR'S CONTRIBUTION

Md. Abdullah Al Mahmud designed the experiment, performed histopathological staining, analyzed and visualized the data, wrote the original draft, and finalized the manuscript. Uzzal Sarker and Kazi Nazmus Sakib collected field samples, stained the parasites, and assisted with drafting. Mohammad Shah Alam and Md. Ataur Rahman edited the manuscript. Fahima Khatun participated in the experimental design, drafted, and finalized the manuscript. All authors contributed to the article and approved the submitted version of the manuscript.

Ethical approval

Throughout this research, the authors adhered to all ethical guidelines approved by the Animal Research Ethics Committee of Gazipur Agricultural University, Gazipur (FVMAS/AREC/2023/20).

Generative AI and AI-assisted technology statement

Generative AI and AI-assisted technologies were used only to improve language clarity, grammar and readability of the manuscript. The authors take full responsibility for the scientific content, data interpretation and conclusion presented in this research article.

Conflicts of interest

The authors have no conflicts of interest in this study.

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