

PREVALENCE, EPIDEMIOLOGY AND HISTOPATHOLOGY OF *Ascaridia galli* INFECTIONS IN BACKYARD CHICKENS IN GAZIPUR DISTRICT, BANGLADESH

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➤ Supporting Information



ABSTRACT: *Ascaridia galli* is responsible for weight loss, emaciation, anemia, decreased egg production, and in severe cases, intestinal obstruction and death in chickens, resulting in significant economic losses for the poultry industry. This study aimed to assess the present status of *Ascaridia galli* in backyard chickens in Gazipur district. Additionally, the study examined histopathological changes in the intestines and livers of chickens heavily infested with this nematode. Postmortem examination, helminth collection and identification were conducted on 237 backyard chickens from March 2023 to February 2024. The overall prevalence was found to be 40.51%, with Kaliakair upazila showing the highest prevalence at 55.26%, while Sreepur upazila had the lowest at 27.08%. Factors such as season, sex, and age significantly influenced the incidence of ascariasis in backyard chickens. Adults exhibited the highest occurrence rate at 44.88%, with males (44.19%) showing a greater prevalence than females (36.22%). The highest incidence was recorded in the summer at 57.14%. In cases of severe infection, *Ascaridia galli* obstructs the small intestine, leading to inflamed, hemorrhagic intestines that produce excess mucus. The livers of the infected chickens appeared swollen, enlarged, and pale, with signs of congestion, hemorrhage, and inflammation. Histopathological analysis revealed hemorrhage and inflammatory cell infiltration in the intestines, along with damaged and lacerated intestinal tissues. The livers showed congestion, ruptured blood vessels, inflammation, hemorrhage, and necrosis. In conclusion, this study identifies potential risk factors associated with *Ascaridia galli* infection in backyard chickens in Gazipur district, offering insights that could aid in controlling and preventing such infections.

Keywords: *Ascaridia galli*, Domestic birds, Epidemiology, Histopathology, Poultry health.

INTRODUCTION

Ascaridia galli is the large nematode that parasitize the small intestine of domestic and wild birds particularly chicken (Soulsby, 1982). *Ascaridia galli* is the most prevalent (22 to 84%) parasite in backyard chickens (Kaufmann et al., 2011; Thapa et al., 2017). The adult parasite resides in the lumen of the small intestine of their host, feeding primarily on intestinal contents (Soulsby 1982; Shohana et al., 2023). *Ascaridia galli* infections causes substantial damage by reducing growth rate, weight loss, reduction in production efficiency, pathological lesions and specifically economic losses in chicken, turkey, geese and some other birds (Das et al., 2012; Bazh, 2013). Studies indicated that *Ascaridia galli* infections in laying hens can significantly reduce egg production, with severe infestations resulting in up to 30% decrease and higher mortality (Rahul et al., 2023). Direct losses in poultry occurs through intestinal obstruction, damages to the intestinal mucosa, alteration of beneficial gut microflora, suppression of the immune system and increased susceptibility to concurrent infections. *Ascaridia galli* plays a potential role in transmission of other secondary pathogens such as *Escherichia coli* (Permin et al., 2006), and *Pasteurella maltocida* (Dahl et al., 2002). This nematode can serve as a vector for transmission of pathogens such as *Salmonella enterica* (Chadfield et al., 2001), and *Histomonas meleagridis* (Jinghui and McDougald, 2003), and also impair the development of immunity following vaccination against Newcastle disease virus (Pleidrup et al., 2013).

Heavy load with *Ascaridia galli* in the intestine changes the integrity of the intestinal villi (Permin et al., 2006), thickening of the intestinal walls, intestinal lumen becomes thicken due to enteritis, and mucosal walls also become velvety. These changes can result in impaired nutrient absorption and a reduction of liver lipid storage (Das et al., 2012; Torres et al., 2019; Feyera et al., 2022). Larvae deeply embedded in the intestinal mucosa may induce hemorrhage and

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extensive epithelial damage, increased mucus secreting cells and subsequent adhesion and atrophy of the villi (Shohana et al., 2023). The larvae's migration during the tissue phase of their life cycle significantly impacts the birds that are affected, causing chronic bleeding due to migrating larvae causing gastrointestinal damage including gastritis, enteritis and ulceration of the digestive tract (Ritu et al., 2024). The severity of intestinal damage is directly related to the worm burdens in the intestine. Traumatic and toxic effects induced by the migrating larvae causes inflammatory tracts, portal vein congestion containing fibrous tissue and inflammatory cells, disarrangement of hepatic architecture, hepatocellular shrinkage and dilation of sinusoids (Feroza et al., 2019).

In general, coprological analysis is used to estimate the prevalence of gastrointestinal helminths in animals and birds. Assessing the efficacy of this technique is limited, due to the lack of availability of adult worm burden. For accurate diagnosis, requires detailed knowledge of the parasite and precise localization within the hosts. Therefore, this study investigates the present prevalence of *Ascaridia galli* infection in backyard chickens in Gazipur district by worm count. This research also evaluated the risk factors and pathological changes associated with ascariidiosis.

MATERIALS AND METHODS

Study Area and Sampling

A cross-sectional study was conducted over one year from March 2023 to February 2024, to assess the prevalence of *Ascaridia galli* among backyard chickens in Gazipur district, Bangladesh. Gazipur district covers an area of 1806.36 square kilometers and is geographically positioned between latitudes 23°53 and 24°21 north and longitudes 90°09 and 92°39 east (Figure 1). A total of 237 apparently healthy chickens of both sexes (129 male, and 108 female) and varying ages were collected from various villages across five upazilas: Gazipur Sadar (61), Kaliganj (45), Kapasia (45), Kaliakair (38), and Sreepur (48). For sampling convenience, the chickens were categorized into two groups: growers and adults. The research was conducted over three distinct seasons: hot-dry summer (March to June), hot-wet rainy (July to October), and cool-dry winter (November to February) to account for regional climatic variations. The chickens were then caged and transported to the Parasitology Laboratory in the Department of Pathobiology at Gazipur Agricultural University, Gazipur, Bangladesh.

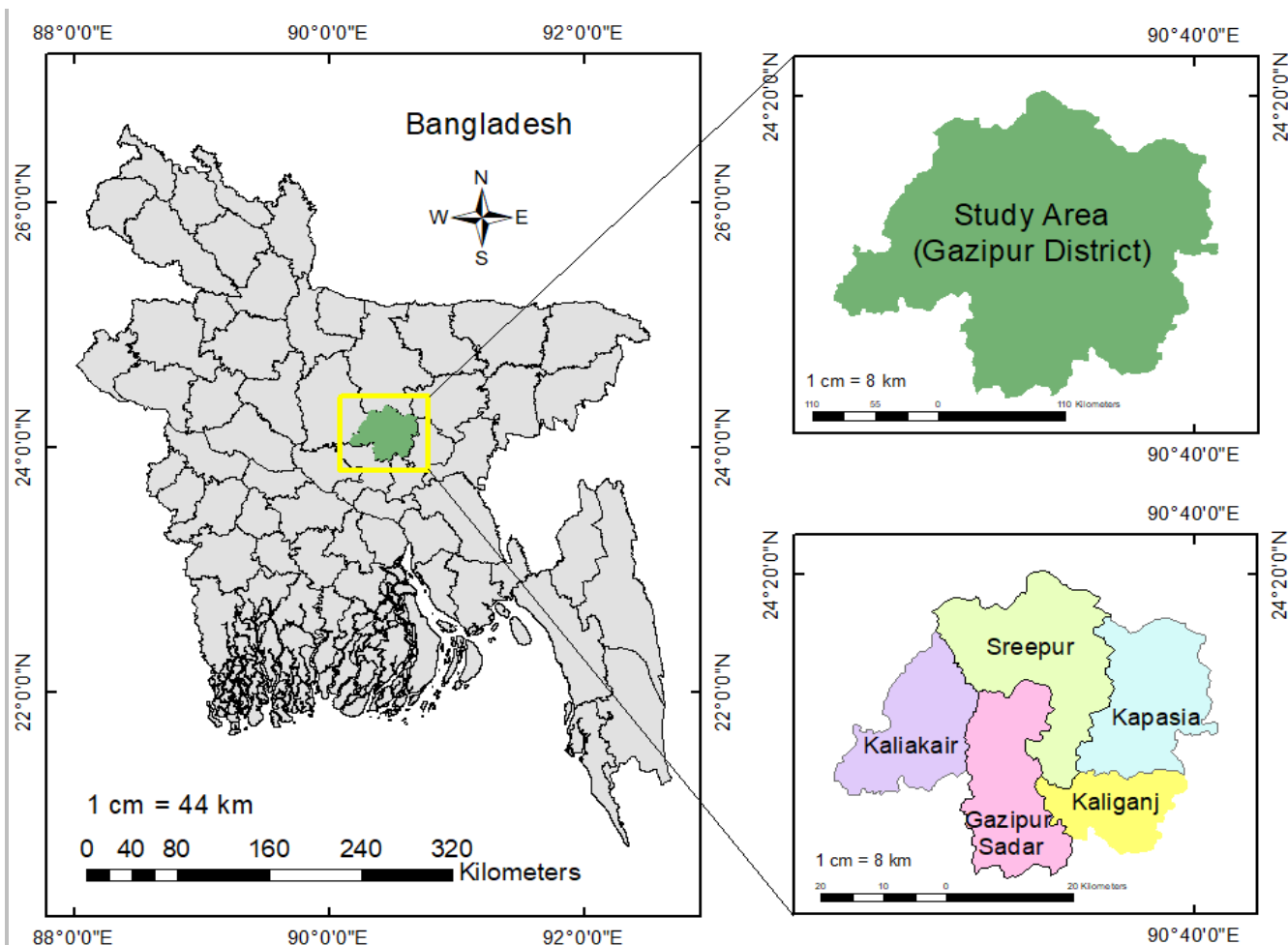


Figure 1 - Study area in Gazipur District, Bangladesh with its five upazilas.

Ethical approval

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

Post-mortem examination, helminth collection and identification

After euthanasia the birds, the small intestine was separated and incised longitudinally, and the contents were emptied into the sieves. The intestinal tracts and contents were washed in normal saline and worms were collected by curved needle or forceps. The collected helminths were examined under a light microscope after preparing temporary slide using a drop of lactophenol and identified based on taxonomic keys described by Soulsby (1982).

Histopathological examination

The mucosal surface was cautiously inspected to detect helminths particularly *Ascaridia galli* nematodes. Parasite-affected mucosal region was observed to find any pathological changes. The affected parts showing gross abnormalities were collected and preserved in 10% formalin solution for fixation. Chickens infected exclusively with *Ascaridia galli* were chosen for histopathological studies, and the affected intestine and liver tissues were collected. For studying histopathological details, tissue samples were processed routinely and the paraffin embedded tissue sections were cut at 5 µm and stained with Hematoxylin and Eosin (H&E) (Fischer et al., 2008). Control tissues taken from non-infected chickens, matched for age and sex, were used as the comparison group. At least three foci were evaluated from control and infected tissue samples, with three slides evaluated per sample in a blinded manner.

Statistical analysis

The collected data were stored in Microsoft Excel and subsequently analysed using the Statistical Package for Social Sciences (SPSS) version 25. To assess the association between *Ascaridia galli* incidence and various risk factors including age, sex, and seasonal comparison, Fisher's exact test was employed. Odds ratios (ORs) were calculated to evaluate risk within groups of identified risk factors, accompanied by 95% confidence intervals (CIs) to estimate the precision of the ORs. A p-value of less than 0.05 ($P < 0.05$) was considered statistically significant.

RESULTS

Overall prevalence of *Ascaridia galli* infections

Backyard chickens were collected in different rural areas of the Gazipur district. These samples were collected from the five sub-districts namely Gazipur Sadar, Kaliakair, Kapasia, Kaliganj and Sreepur. Each location has different occurrence percentage i.e. 40.98%, 55.26%, 42.22%, 40%, and 27.08%, respectively (Table 1). A total of 237 (40.51%) of the sampled chickens were infected with *Ascaridia galli*. There were no significant differences in total prevalence regarding sex in backyard chickens ($P > 0.05$). Males showed a higher prevalence of *Ascaridia galli* infection compared to females. In this study, out of 96 infected chicken males were 57 (44.19%), whereas females were 39 (36.22%) in Table 2. The current study showed a higher prevalence of *Ascaridia galli* in adult birds (44.88%, 57/127) compared to grower birds (35.45%, 39/110); however, the difference was not statistically significant ($P > 0.05$). No significant difference in infection rates was observed between grower and adult chickens ($P > 0.05$). The prevalence of parasites in chicken was found throughout the year, but the prevalence varied from season to season. This study showed a significant seasonal variation in the prevalence of *Ascaridia galli* infections in slaughtered backyard chickens, with the highest infection rates recorded during the summer season (57.14%) followed by 44.16% in the winter and 26.80% in the rainy season (Table 2).

Histopathology of *Ascaridia galli* infections in backyard chicken

The digestive organs, including the liver and intestine, were evaluated following the postmortem of the chickens. In heavy infection, the intestinal lumen is stacked with a large number of *Ascaridia galli* parasites (Figure 2). A sectional view of the lumen of the intestine may reveal a long, yellowish helminth, which is indicative of *Ascaridia galli*. It was also noted that the intestinal lesion was similar to a hemorrhagic lesion in the intestinal mucosa. The small intestine was noticeably inflamed and was secreting more mucus. The liver appeared pale, enlarged, and swollen. Additionally, there was inflammation of the liver, hemorrhage and congestion. Histopathological examination of duodenum sections revealed hemorrhage, inflammatory cell infiltration, and ruptured and lacerated tissue. The submucosal layer ruptured and showed signs of hemorrhage. There were noticeable inflammatory cells in the layer of muscularis mucosa (Figures 3A-F). In contrast to non-infected chicken characterized by no inflammatory cells, no hemorrhagic cells and no ruptured tissue in the intestinal sections (Figure 4). In the liver sections, the tissue of infected chickens had congestion, hemorrhage and inflammation of inflammatory cells. Congested sinusoids and blood vessels were present in the liver. Focal necrotic regions were visible in the liver portion. Necrotic zones had a high concentration of inflammatory cells (Figures 5A-D). In contrast, in the control chickens, there were no inflammatory cells, congestion in the central vein or any other lesions (Figure 6).

Table 1 - Prevalence of *Ascaridia galli* in Gazipur district.

Locality	No. examined	No. Infected	Overall prevalence (%)	χ^2 /Fisher's exact	P-value
Gazipur Sadar	61	25	40.98	7.09	0.13
Kaliakair	38	21	55.26		
Kapasias	45	19	42.22		
Kaliganj	45	18	40.00		
Sreepur	48	13	27.08		
Total	237	96	40.51		

Table 2 - Prevalence associated risk factors of *Ascaridia galli* infection in backyard chickens.

Factors	Categories	No. examined	No. Infected	Overall prevalence (%)	OR	95% CI	χ^2 /Fisher's exact	P-value
Age	Grower	110	39	35.45	1.48	0.877-2.504	2.17	0.1403
	Adult	127	57	44.88	1			
Sex	Male	129	57	44.19	1	0.829-2.366	1.59	0.2072
	Female	108	39	36.11	1.40			
Season	Summer (Mar-Jun)	63	36	57.14			15.22	0.0004
	Rainy (Jul-Oct)	97	26	26.80				
	Winter (Nov-Feb)	77	34	44.16				
	Total	237	96	40.51				

**Figure 2 - Intestinal lumen stacked with large number of *Ascaridia galli*.**

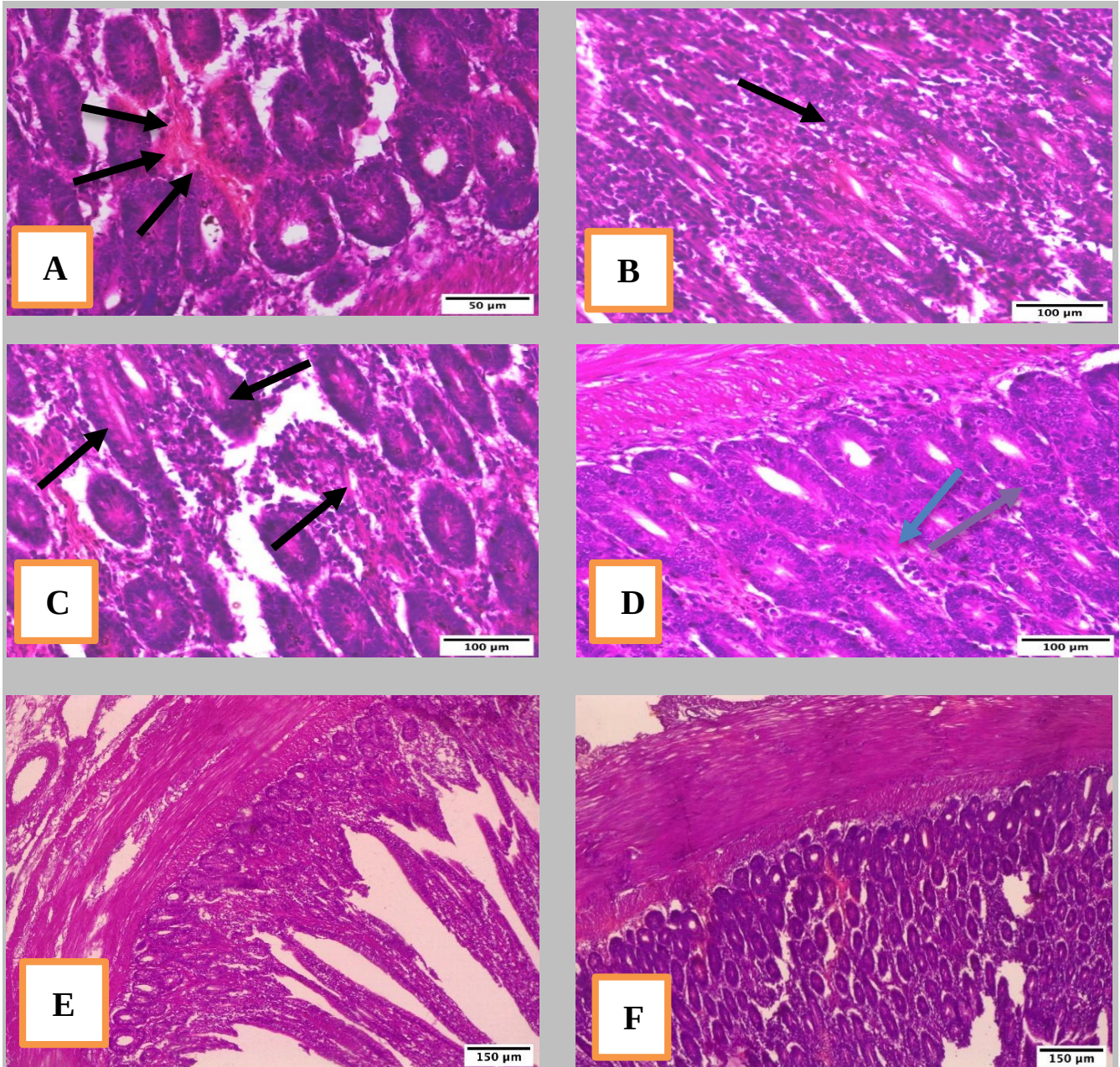


Figure 3 - Histopathology of intestine affected with *Ascaridia galli*. A) hemorrhage of the intestinal tissue (black arrow), B) inflammatory cell infiltration (black arrow), C) ruptured and lacerated tissue of intestine (black arrow) and D) hemorrhage (blue arrow) and inflammatory cells (yellow arrow), E) Desquamation of muscularis layer of intestine (black arrow) and desquamation of villi (blue arrow), F) Hemorrhage of intestine (black arrow).

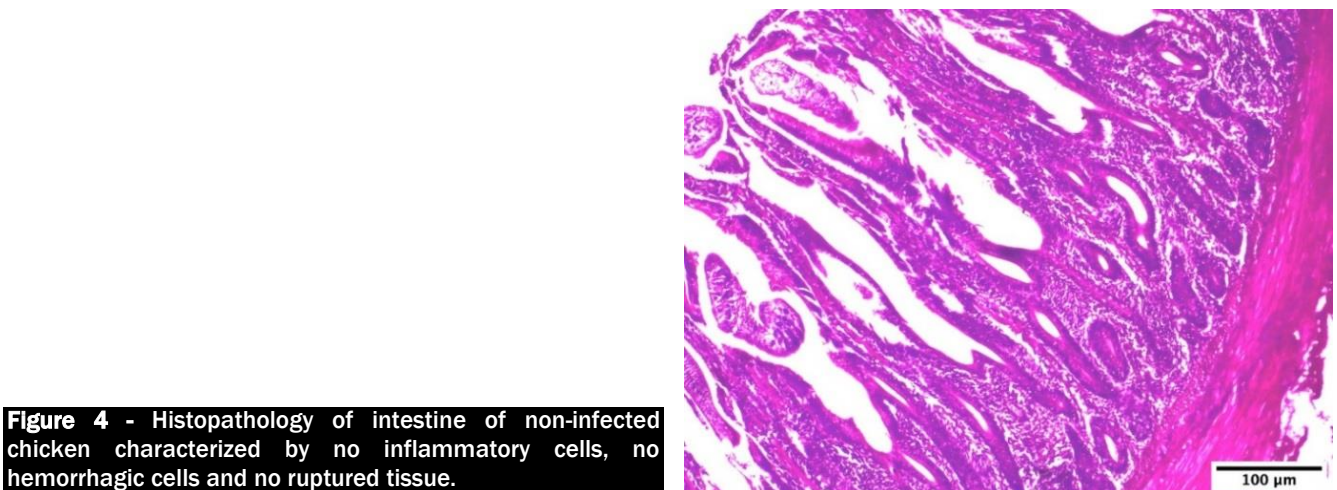


Figure 4 - Histopathology of intestine of non-infected chicken characterized by no inflammatory cells, no hemorrhagic cells and no ruptured tissue.

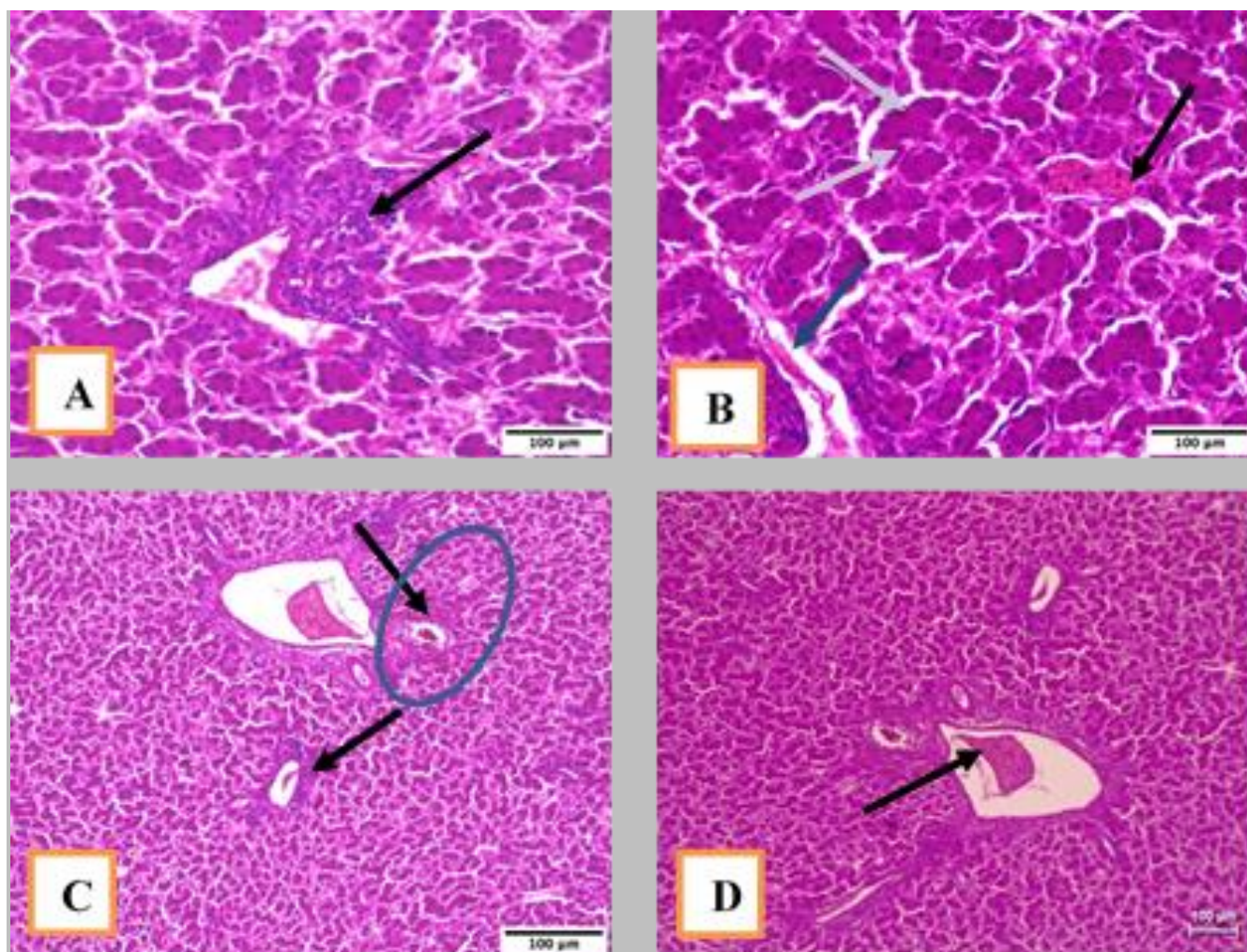


Figure 5 - Histopathological lesions in liver of affected chicken with *Ascaridia galli* A) infiltration of inflammatory cells (black arrow), B) hemorrhage (black) with congestion and ruptured tissue (yellow) and C) Necrotic zones (blue circle) concentration of inflammatory cells (black arrow), D) Congestion of tissue of liver (black arrow).

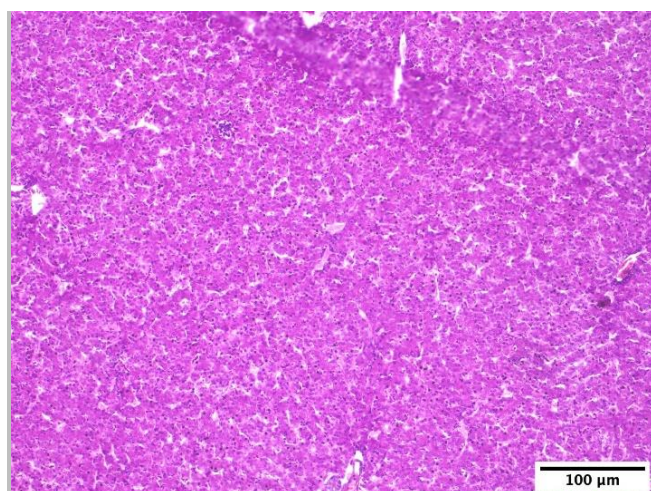


Figure 6 - Histopathology of liver of non-infected chicken characterized by no lesions of infection of *Ascaridia galli*.

DISCUSSION

The current study showed that backyard chickens naturally infected with *Ascaridia galli* reached 40.51%. This finding is consistent with another study performed in another region of Bangladesh recorded 45.6% prevalence of *Ascaridia galli* among gastrointestinal helminths in scavenging or semi-scavenging chicken (Ritu et al., 2024). However, other studies reported higher prevalence (70-85%) in Narsingdi district (Ferdushy et al., 2016), and (56%) in Dinajpur district (Trisha et al., 2021). Worldwide, the prevalence rate of *Ascaridia galli* was 32.97 % in India (Ara et al., 2021), 88% in Germany

(Kaufmann et al., 2011), 77.1% in Sweden (Jansson et al., 2010), 32.3% in Tanzania (Permin et al., 2006), and 30% in Ghana (Anane et al., 2022). This difference may reflect the geographical location, sample size and management practices that play a role in the transmission dynamics of helminth infections in poultry. The current study showed that the prevalence in five sub-districts of Gazipur were comparable. The high prevalence was observed in Kaliakair (55.26%) and lower in Sreepur (27.08%). This variation might be influenced by the scavenging behaviour of the local chickens, which frequently roam freely in search of foods, scavenging various arthropods and earthworms from the superficial layer of the soil that act as the intermediate and paratenic hosts for *Ascaridia galli*, facilitating the transmission of parasitic infections (Soulsby, 1982).

The present study showed males were more susceptible to *Ascaridia galli* infection compared to females, which is agreed with the earlier studies (Sarba et al., 2019; Ritu et al., 2024). High prevalence in male chickens is probably due to reduced immune responses, hormonal differences and feeding habits (Klein, 2004). The higher percentage was found in adult birds rather than the grower might be due to the cohort effect. Nevertheless, the increase in infestation rates with increasing host age may be attributed to prolonged exposure to environmental factors, which facilitate the chances of ingestion of infective larvae through contaminated food or polluted drinks. In terms of seasonal dynamics, the highest peak of infection was observed in summer, with a comparatively lower prevalence in winter and the rainy season. The heightened occurrence of intestinal infections during summer could be attributed to the increased abundance of insects and other invertebrates, which serve as food for birds that act as hosts for the intermediate stages of these helminths in hot climates (Ara et al., 2021).

In terms of histopathological changes, heavy infection with *Ascaridia galli* resulted small in intestinal obstruction, petechial hemorrhages in the small intestine, marked inflammation and secretion of more mucus. In some cases, we found desquamation of the muscularis mucosa and loss of villi in the intestine. Previous studies found that, *Ascaridia galli* mostly causes desquamation and adhesion of mucosal villi, petechial hemorrhages in the duodenum (Ritu et al., 2024), ulcerative ventriculitis (Gopal et al., 2017), intestinal wall damage and bleeding (Skallerup et al., 2005), necrotic patches of intestinal mucosa (Rajkovic et al., 2019), petechial hemorrhage in the intestinal mucosa, nodular haemorrhagic enteritis, and inflammation of the proventriculus (Permin and Ranving, 2001).

Microscopical examination of liver showed congestion of sinusoids, fatty degeneration with coagulation necrosis and necrotic zones with a high concentration of inflammatory cells due to migration of larvae during the tissue phase of life cycle (Sharma et al., 2018). Similar finding was partially agreed with the previous study (Sharma et al., 2018; Abdel Rahman et al., 2019) who observed necrosis of liver, with mononuclear cellular infiltration, congested blood vessels and fatty degeneration with coagulation necrosis.

CONCLUSION

This study revealed a high infection rate of *Ascaridia galli* in backyard chickens in the Gazipur district. It causes subclinical infections in chickens, which may result in invisible production and economic loss. Age, sex, and season were found to have a significant impact on the prevalence of these helminth infections. Histopathological findings demonstrated that heavily infected small intestines exhibited lesions characterized by hemorrhage, inflammatory cell infiltration, villous desquamation, and necrosis of the sub-mucosal glands. Additionally, the infected livers showed severe lesions. Therefore, awareness among poultry producers should be created about the transmission of poultry parasites and the importance of proper disposal of poultry droppings for prevention and control.

DECLARATIONS

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Ethical considerations

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

Availability of data and materials

The data generated and analysed during this study are available from the corresponding author upon reasonable request.

Author's contributions

F. Khatun conceptualized the study, managed the project, conducted the experimental protocols and wrote the manuscript. I. Akter Akhi performed the prevalence and histopathological study. R. Sani Sadi contributed in histopathological study. Md A. Al Mahmud contributed to the research and data analysis. Z. Chandra Das and Md S. Islam contributed in draft preparation. Md T. Islam contributed in final manuscript writing, edited the manuscript and supervised the research work. All authors reviewed and approved the final version of the manuscript.

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Competing interests

The authors report no competing interest in this work.

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